Research Article
Viral Hepatitis

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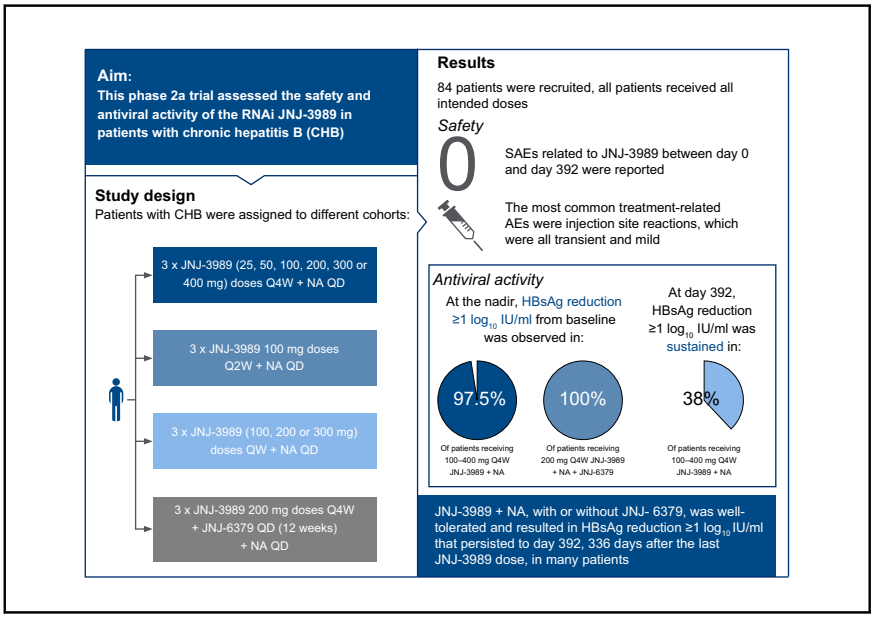
Combination treatments including the small-interfering RNA JNJ-3989 induce rapid and sometimes prolonged viral responses in patients with CHB

Graphical abstract



Highlights

- Small-interfering RNA (siRNA) lowers HBsAg levels in patients with CHB.
- Safety and efficacy of 3 siRNA JNJ-3989 doses + a nucleos(t)ide analogue was studied.
- All JNJ-3989 doses were well tolerated in patients with CHB in this study.
- Many patients experienced HBsAg declines ≥1 $\log_{10}$ IU/ml from baseline.
- HBsAg reduction persisted 336 days after the last JNJ-3989 dose in many patients.

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Lay summary

Hepatitis B virus affects people's livers and produces particles called hepatitis B surface antigen (HBsAg) that damage a person's liver and can help the virus infect a person for a long time, known as chronic hepatitis B (CHB). In this study, a new treatment called JNJ-3989 was assessed (in combination with normal treatment known as nucleos(t)ide analogues), for its safety and effectiveness in reducing the number of HBsAg particles in people with CHB. The results of this study showed that treatment with JNJ-3989 could be safe for people with CHB, lowered their HBsAg levels, and kept HBsAg levels lowered for 336 days in 38% of patients after receiving their last dose of JNJ-3989.

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Combination treatments including the small-interfering RNA JNJ-3989 induce rapid and sometimes prolonged viral responses in patients with CHB

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Background & Aims: RNA interference therapy has been shown to reduce hepatitis B surface antigen (HBsAg) levels in preclinical models, which could confer functional cure in patients with chronic hepatitis B. This phase IIa trial (ClinicalTrials.gov Identifier: NCT03365947) assessed the safety and efficacy of the small-interfering RNA JNJ-73763989 (JNJ-3989) plus a nucleos(t)ide analogue (NA), with/without the capsid assembly modulator JNJ-56136379 (JNJ-6379) in patients with chronic hepatitis B.

Methods: Treatment-naïve and NA-suppressed patients received 3 subcutaneous JNJ-3989 doses every week (QW; 100, 200, or 300 mg), 2 weeks (Q2W; 100 mg) or 4 weeks (Q4W; 25, 50, 100, 200, 300, or 400 mg), or JNJ-3989 Q4W (200 mg) plus oral JNJ-6379 250 mg daily for 12 weeks. Patients received NAs throughout.

Results: Eighty-four patients were recruited. All treatments were well tolerated, with all 5 serious adverse events considered unrelated to study drugs. JNJ-3989 100 to 400 mg Q4W resulted in HBsAg reductions ≥1 $\log_{10}$ IU/ml from baseline in 39/40 (97.5%) patients at the nadir. All patients receiving the triple combination (n = 12) had HBsAg reductions ≥1 $\log_{10}$ IU/ml from baseline at the nadir. HBsAg reductions were similar for HBeAg-positive (n = 21) and HBeAg-negative (n = 47) patients in all JNJ-3989 Q4W treatment arms, including the triple combination (n = 68). Smaller HBsAg reductions were seen with 25 mg (n = 8) and 50 mg (n = 8) than with 100 to 400 mg (n = 40). Shorter dosing intervals (QW [n = 12] and Q2W [n = 4]) did not improve response *vs.* Q4W dosing. HBsAg reductions ≥1 $\log_{10}$ IU/ml from baseline persisted in 38% of patients 336 days after the last JNJ-3989 dose.

Conclusions: JNJ-3989 plus an NA, with/without JNJ-6379, was well tolerated and resulted in HBsAg reductions up to 336 days after the last JNJ-3989 Q4W dose.

Clinical trial number: NCT03365947.

Lay summary: Hepatitis B virus affects people's livers and produces particles called hepatitis B surface antigen (HBsAg) that damage a person's liver and can help the virus infect a person for a long time, known as chronic hepatitis B (CHB). In this study, a new treatment called JNJ-3989 was assessed (in combination with normal treatment known as nucleos(t)ide analogues), for its safety and effectiveness in reducing the number of HBsAg particles in people with CHB. The results of this study showed that treatment with JNJ-3989 could be safe for people with CHB, lowered their HBsAg levels, and kept HBsAg levels lowered for 336 days in 38% of patients after receiving their last dose of JNJ-3989.

Introduction

Approximately 292 million people are chronically infected with HBV globally.[1] Expression of high levels of viral antigens, especially hepatitis B surface antigen (HBsAg), contributes to chronic HBV infection by suppressing innate and adaptive immune responses.[2–4] Daily oral nucleos(t)ide analogues (NAs) are currently the standard of care for chronic hepatitis B (CHB) treatment.[5,6] Although NA treatments are well tolerated and suppress HBV DNA, their effect on HBV RNA and viral antigen levels is limited. Functional cure, defined as off-treatment HBsAg loss and undetectable HBV DNA levels with or without anti-HBs seroconversion,[6,7] is currently the ideal treatment outcome for

Keywords: Hepatitis B virus; chronic hepatitis B; hepatitis B surface antigen; antiviral therapy; RNA interference; JNJ-3989.
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patients with CHB and is associated with improved long-term clinical outcomes;[8] however, functional cure is achieved in <3% of patients over 48 to 53 weeks of NA therapy,[9] meaning most patients require chronic treatment. Patients who do not achieve functional cure have an elevated risk of hepatocellular carcinoma compared with those who do.[10]

JNJ-73763989 (JNJ-3989, formerly ARO-HBV) contains 2 short-interfering RNA (siRNA) triggers targeting HBV RNAs expressed from both covalently closed circular DNA (cccDNA) and HBV DNA integrated into the host genome. In preclinical models, JNJ-3989 monotherapy or in combination with entecavir resulted in reductions in pregenomic RNA (pgRNA), HBsAg, hepatitis B e antigen (HBeAg) and HBV DNA.[11] Single JNJ-3989 doses had a good safety profile in healthy volunteers.[12] JNJ-56136379 (JNJ-6379), a class-N capsid assembly modulator (CAM-N) currently in phase II trials in patients with CHB (ClinicalTrials.gov Identifier: NCT03361956),[13] interferes with capsid assembly, thereby inhibiting pgRNA encapsidation and the *de novo* formation of cccDNA and, consequently, viral replication.[14] Phase I and II data showed JNJ-6379 therapy for up to 24 weeks was well tolerated, and resulted in HBV DNA and RNA suppression, but did not result in clinically meaningful HBsAg or HBeAg reductions.[13,15] The purpose of this study was to determine whether a combination therapy comprising siRNA, NA, and CAM-N might lead to functional cure by direct suppression of viral replication and/or by immune restoration induced by a reduction in viral antigen levels.[3]

The primary objective of this phase IIa, open-label trial (NCT03365947, AROHBV1001) was to determine the safety and tolerability of JNJ-3989 using multiple ascending doses in patients with CHB. A secondary objective was to determine the reduction in HBsAg from Day 0 to post-dose nadir. Exploratory objectives included: measuring the reduction from Day 0 to post-dose nadir of HBV RNA, hepatitis B core-related antigen (HBcrAg), and HBeAg; determining the antiviral activity of JNJ-3989 plus an NA up to Day 392, or up to Day 168 in patients also receiving JNJ-6379; evaluating the optimal dosing interval for JNJ-3989.

Patients and methods

Patient population

Patients with CHB aged 18 to 65 years, with a BMI between 19 to 38 kg/m^2 and with circulating HBsAg ≥5 IU/ml at screening were recruited. Full inclusion and exclusion criteria are listed in Table S1. Patients were screened from Days -60 to -1 prior to treatment initiation.

Study design

Eligible patients were assigned to cohorts 1b to 12 (4–12 patients per cohort), to receive 3 subcutaneous JNJ-3989 doses (Fig. 1).

JNJ-3989 Q4W + NA QD	JNJ-3989 dose	Cohort patient population	Total number of patients enrolled	Number of NA-naïve patients enrolled	Number of NA-experienced patients enrolled
Cohort 1b	25 mg	All eligible patients with CHB	n = 8	n = 4	n = 4
Cohort 1c	50 mg	All eligible patients with CHB	n = 8	n = 1	n = 7
Cohort 2b	100 mg	All eligible patients with CHB	n = 8	n = 2	n = 6
Cohort 3b	200 mg	All eligible patients with CHB	n = 8	n = 0	n = 8
Cohort 4b	300 mg	All eligible patients with CHB	n = 8	n = 0	n = 8
Cohort 5b	400 mg	All eligible patients with CHB	n = 8	n = 1	n = 7
Cohort 8	300 mg	NA-naïve patients with HBeAg-positive CHB	n = 4	n = 4	n = 0
Cohort 9	300 mg	NA-suppressed patients with HBeAg-positive CHB	n = 4	n = 0	n = 4
JNJ-3989 Q2W + NA QD					
Cohort 6	100 mg	All eligible patients with CHB	n = 4	n = 0	n = 4
JNJ-3989 QW + NA QD					
Cohort 7	100 mg	All eligible patients with CHB	n = 4	n = 1	n = 3
Cohort 10	200 mg	All eligible patients with CHB	n = 4	n = 2	n = 2
Cohort 11	300 mg	All eligible patients with CHB	n = 4	n = 1	n = 3
JNJ-3989 Q4W + NA QD + JNJ-6379 250 mg QD					
Cohort 12	200 mg	All eligible patients with CHB	n = 12	n = 5	n = 7

Fig. 1. Study design. CHB, chronic hepatitis B; JNJ-3989, JNJ-73763989; JNJ-6379, JNJ-56136379; NA, nucleos(t)ide analogue; QD, once daily; Q2W, every 2 weeks; Q4W, every 4 weeks; QW, every week.

Table 1. Baseline demographics and disease characteristics.

JNJ-3989 dosing schedule	Q4W									Q2W	QW			
	1b n = 8	1c n = 8	2b n = 8	3b n = 8	4b n = 8	8* n = 4	9** n = 4	5b n = 8	12† n = 12	6 n = 4	7 n = 4	10 n = 4	11 n = 4	All patients
Cohort	25 mg	50 mg	100 mg	200 mg	300 mg	300 mg	300 mg	400 mg	200 mg	100 mg	100 mg	200 mg	300 mg	N = 84
Age - yr-median (range)	44.3 (31-52)	49.6 (36-58)	52.7 (32-66)‡	46.6 (41-57)	51.8 (40-63)	29.8 (25-42)	36.2 (30-42)	41.3 (29-61)	44.3 (27-57)	48.2 (40-59)	40.3 (37-48)	53.9 (45-59)	49.0 (39-66)‡	45.5 (25-66)
Male - n (%)	5 (62.5)	6 (75.0)	6 (75.0)	5 (62.5)	8 (100.0)	2 (50.0)	2 (50.0)	6 (75.0)	8 (66.7)	3 (75.0)	3(75.0)	1 (25.0)	3 (75.0)	58 (69.0)
Race - n (%)														
Asian	6 (75.0)	6 (75.0)	8 (100.0)	8 (100.0)	5 (62.5)	3 (75.0)	4 (100.0)	6 (75.0)	12 (100.0)	3 (75.0)	1 (25.0)	4 (100.0)	3 (75.0)	69 (82.1)
Caucasian	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)
Other	2 (25.0)	2 (25.0)	0 (0.0)	0 (0.0)	2 (25.0)	1 (25.0)	0 (0.0)	2 (25.0)	0 (0.0)	1 (25.0)	3 (75.0)	0 (0.0)	1 (25.0)	14 (16.7)
BMI - kg/m^2 median (range)	24.8 (20.3-43.5)	24.3 (18.0-40.1)	24.0 (21.8-28.8)	24.8 (18.8-31.9)	26.6 (21.3-35.6)	23.9 (18.9-29.8)	25.1 (21.8-32.5)	23.3 (20.5-36.2)	24.6 (20.1-36.4)	24.6 (22.5-31.0)	28.7 (26.8-31.9)	23.9 (21.1-26.6)	23.6 (20.2-25.4)	24.6 (18.0-43.5)
HBeAg-positive/ negative - n (%)	2 (25.0)/ 6 (75.0)	1 (12.5)/ 7 (75.0)	1 (12.5)/ 7 (87.5)	1 (12.5)/ 7 (87.5)	3 (37.5)/ 5 (62.5)	4 (100.0)/ 0 (0.0)	4 (100.0)/ 0 (0.0)	1 (12.5)/ 7 (87.5)	4 (33.3)/ 8 (66.7)	0 (0.0)/ 4 (100.0)	0 (0.0)/ 4 (100.0)	1 (25.0)/ 3 (75.0)	0 (0.0)/ 4 (100.0)	22 (26.2)/ 62 (73.8)
HBeAg at baseline (log_{10} PEIU/ml) -mean (SD)§	1.17 (2.47)	-0.23 (-)	1.94 (-)	2.74 (-)	0.86 (0.60)	2.77 (0.49)	1.55 (1.31)	0.11 (-)	0.65 (1.19)	-	-	3.00 (-)	-	1.47 (1.32)
NA suppressed - n (%)	4 (50.0)	7 (87.5)	6 (75.0)	8 (100.0)	8 (100.0)	0 (0.0)	4 (100.0)	7 (87.5)	7 (58.3)	4 (100.0)	3 (75.0)	2 (50.0)	3 (75.0)	65 (77.4)
HBsAg at baseline (log_{10} IU/ml) - mean (SD)	3.13 (1.26)	3.33 (0.66)	2.93 (0.96)	2.50 (1.32)	3.04 (0.85)	4.81 (0.65)	3.76 (0.37)	3.18 (0.80)	3.04 (0.79)	2.72 (0.60)	3.15 (0.24)	3.00 (1.07)	3.24 (0.68)	3.14 (0.95)
HBcrAg at baseline (log_{10} kU/ml) - mean (SD)‖	2.34 (1.87)	1.70 (0.91)	3.02 (2.63)	1.64 (2.45)	2.82 (2.00)	5.98 (0.041)	4.21 (1.25)	1.96 (1.12)	2.34 (1.73)	0.86 (0.71)	1.12 (1.33)	2.62 (2.80)	-	2.56 (1.96)
HBV DNA at baseline (log_{10} IU/ml) - mean (SD)‖	5.79 (2.61)	3.79 (3.00)	5.76 (3.07)	-	4.82 (2.42)	8.56 (0.55)	2.30 (-)	2.28 (0.85)	4.53 (1.85)	-	-	3.95 (1.73)	4.27 (-)	5.06 (2.43)
HBV RNA at baseline (log_{10} U/ml) - mean (SD)‖	4.46 (1.79)	2.92 (1.21)	3.22 (1.41)	3.47 (2.48)	5.25 (2.16)	7.72 (0.98)	5.40 (1.64)	2.97 (1.06)	3.39 (1.84)	2.16 (0.16)	2.27 (0.16)	5.92 (3.30)	2.17 (0.62)	3.94 (2.08)
ALT at baseline (U/L) - mean (SD)	46.5 (26.6)	24.4 (8.9)	36.4 (25.5)	30.4 (23.4)	33.4 (20.3)	39.8 (5.7)	28.5 (11.7)	29.4 (13.7)	25.5 (11.2)	30.0 (11.8)	30.5 (18.9)	26.0 (7.4)	25.5 (10.6)	31.3 (17.5)

All patients received a daily oral dose of an NA.
ALT, alanine transaminase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LLoQ, lower limit of quantification; NA, nucleos(t)ide analogue; QW, every week; Q2W, every 2 weeks; Q4W, every 4 weeks.
*Patients in cohort 8 were all HBeAg-positive and NA-naïve at baseline.
**Patients in cohort 9 were all HBeAg-positive and NA-experienced at baseline.
†Patients in cohort 12 received a triple combination of JNJ-3989 200 mg, an NA, and JNJ-6379 250 mg.
‡Two patients aged 66 years old had their 66th birthday between screening and the first dose and therefore qualified for the study.
§Includes only patients who are HBeAg positive at baseline.
‖Includes only patients with baseline values >LLoQ.

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Table 2. Summary of most common TEAEs regardless of causality from Day 0 to Day 112.

JNJ-3989 dosing schedule	Q4W								Q2W	QW			
Cohort	1b n = 8	1c n = 8	2b n = 8	3b n = 8	4b n = 8	5 n = 8	8* n = 4	9** n = 4	12† n = 12	6 n = 4	7 n = 4	10 n = 4	11 n = 4	Total n = 84
JNJ-3989 dose - mg	25	50	100	200	300	400	300	300	200	100	100	200	300	
SAE‡/related to JNJ-3989	0	0	1/0	0	0	2/0	0	0	0	0	0	0	0/0	3/0
AE leading to discontinuation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Patients reporting AEs	6 (75.0)	4 (50.0)	4 (50.0)	3 (37.5)	6 (75.0)	7 (87.5)	2 (50.0)	2 (50.0)	2 (16.7)	2 (50.0)	4 (100.0)	3 (75.0)	2 (50.0)	47 (56.0)
Most common TEAEs (reported in >2 participants) by preferred term														
Injection site reaction‡‡	1 (12.5)	0	0	0	2 (25.0)	2 (25.0)	2 (50.0)	1 (25.0)	0	0	2 (50.0)	0	2 (50.0)	12 (14.3)
Upper respiratory tract infection	0	1 (12.5)	0	1 (12.5)	1 (12.5)	3 (37.5)	1 (25.0)	1 (25.0)	1 (8.3)	0	0	2 (50.0)	0	11 (13.7)
Headache	0	1 (12.5)	0	0	2 (25.0)	1 (12.5)	0	0	0	0	0	1 (25.0)	1 (25.0)	6 (7.1)
Blood CK increase	1 (12.5)	0	0	0	1 (12.5)	0	1 (25.0)	0	0	0	1 (25.0)	0	0	4 (4.8)
Back pain	0	1 (12.5)	0	0	1 (12.5)	0	1 (25.0)	0	0	0	1 (25.0)	0	0	4 (4.8)
Fatigue	1 (12.5)	1 (12.5)	1 (12.5)	0	0	1 (12.5)	0	0	0	0	0	0	0	4 (4.8)
Oropharyngeal pain	0	1 (12.5)	1 (12.5)	0	1 (12.5)	0	0	0	0	0	0	0	1 (25.0)	4 (4.8)
Pruritus/pruritus generalized	1 (12.5)	0	1 (12.5)	0	0	0	0	0	0	0	0	1 (25.0)	1 (25.0)	4 (4.8)
Naso-pharyngitis	0	0	0	0	0	0	0	0	0	0	1 (25.0)	1 (25.0)	1 (25.0)	3 (3.6)
Pain in extremity	0	0	0	0	0	0	1 (25.0)	0	0	0	1 (25.0)	0	1 (25.0)	3 (3.6)
Rash	1 (12.5)	0	0	0	0	0	0	1 (25.0)	0	0	0	0	1 (25.0)	3 (3.6)

AE, adverse event; CK, creatine kinase; HBeAg, hepatitis B e antigen; NA, nucleos(t)ide analogue; QW, every week; Q2W, every 2 weeks; Q4W, every 4 weeks; SAE, serious adverse event; TEAE, treatment-emergent adverse event.
*Patients in cohort 8 were all HBeAg-positive and NA-naïve at baseline.
**Patients in cohort 9 were all HBeAg-positive and NA-experienced at baseline.
†Patients in cohort 12 received a triple combination of JNJ-3989 200 mg, an NA, and JNJ-6379 250 mg.
‡SAEs included 2 events of anxiety/depression reported in 1 patient; and menorrhagia.
‡‡Injection-site reactions included 4 reports of erythema, 3 reports of bruising, 2 reports of hematoma, 1 report of discolouration, 1 report of pain, and 1 report of rash.

Patients in cohort 1b to 5b, 8, and 9 received JNJ-3989 (25 mg, 50 mg, 100 mg, 200 mg, 300 mg, or 400 mg) every 4 weeks (Q4W). Patients in cohort 6 received JNJ-3989 (100 mg) every 2 weeks (Q2W). Patients in cohorts 7, 10, and 11 received JNJ-3989 (100 mg, 200 mg, or 300 mg, respectively) every week (QW). Patients in cohort 12 received JNJ-3989 200 mg Q4W and oral JNJ-6379 250 mg once daily (QD) for 12 weeks. All patients received NA treatment throughout the study. NA-suppressed patients continued their current therapy (entecavir or tenofovir), NA-naïve patients received entecavir or tenofovir (selection between the 2 was made at the investigator's discretion). Patients visited the clinic for JNJ-3989 dose administration or safety and efficacy measurements on Days 0, 7, 14, 28, 42, 56, 70, 84, and 112 (end of study). Patients who gave consent for additional follow-up visited the clinic on Days 168, 224, 280, 336, and 392.

This study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and applicable regulatory requirements. The protocol was reviewed by Independent Ethics Committees or Institutional Review Boards and modifications were approved by the Health and Disabilities Committee or the local equivalent. Patients provided informed written consent.

Safety assessments

Safety assessments, including physical examination, vital signs, electrocardiograms, and clinical laboratory tests were performed during study visits. All adverse events (AEs) were recorded from Day 0 to end of follow-up and classified by severity as Grade 1, 2, 3, or 4. The incidence and frequency of AEs and serious AEs (SAEs), their relationship to study drug, including those leading to patient withdrawal, dose modification, or treatment discontinuation were categorized by dose and treatment group. Treatment-emergent AEs (TEAEs) were summarized using the latest version of the Medical Dictionary for Regulatory Activities, and severity was classified as mild, moderate, or severe.

Virology assessments

Blood samples collected during clinic visits underwent quantitative assays for HBsAg (Roche Elecsys, lower limit of quantification [LLoQ] = 0.05 IU/ml),[16] HBeAg (Diasorin Liaison, LLoQ = 0.01 Paul-Ehrlich-Institute units [PEIU]/ml),[17] HBcrAg (Fujirebio Lumipulse, LLoQ = 1 kU/ml),[18] HBV RNA (Abbott m2000, LLoQ = 1.65 $\log_{10}$ U/ml),[19] and HBV DNA (Roche Cobas, LLoQ = 20 IU/ml).[20]

Statistical analysis

As this study was a proof-of-principle study, no formal sample size was calculated. Safety analyses were summarized by cohort and treatment group. We present quantitative $\log_{10}$ changes in HBsAg, (and when >LLoQ at Day 0), HBeAg, HBcrAg, HBV DNA, and HBV RNA from baseline to Day 112, and nadir to Day 168 (end-of-follow-up for triple therapy) and to Day 392 (end-of-follow-up for dual therapy HBsAg response). Imputation rules for censored values are detailed in Table S2. Sustained response was defined as HBsAg reduction of ≥1.0 $\log_{10}$ from Day 0 at Day 392. All other viral parameters were assessed based on HBsAg response.

Results

Patient demographics and disposition

Overall, 105 patients were screened and 84 were recruited (Fig. S1). Baseline demographics and disease characteristics are

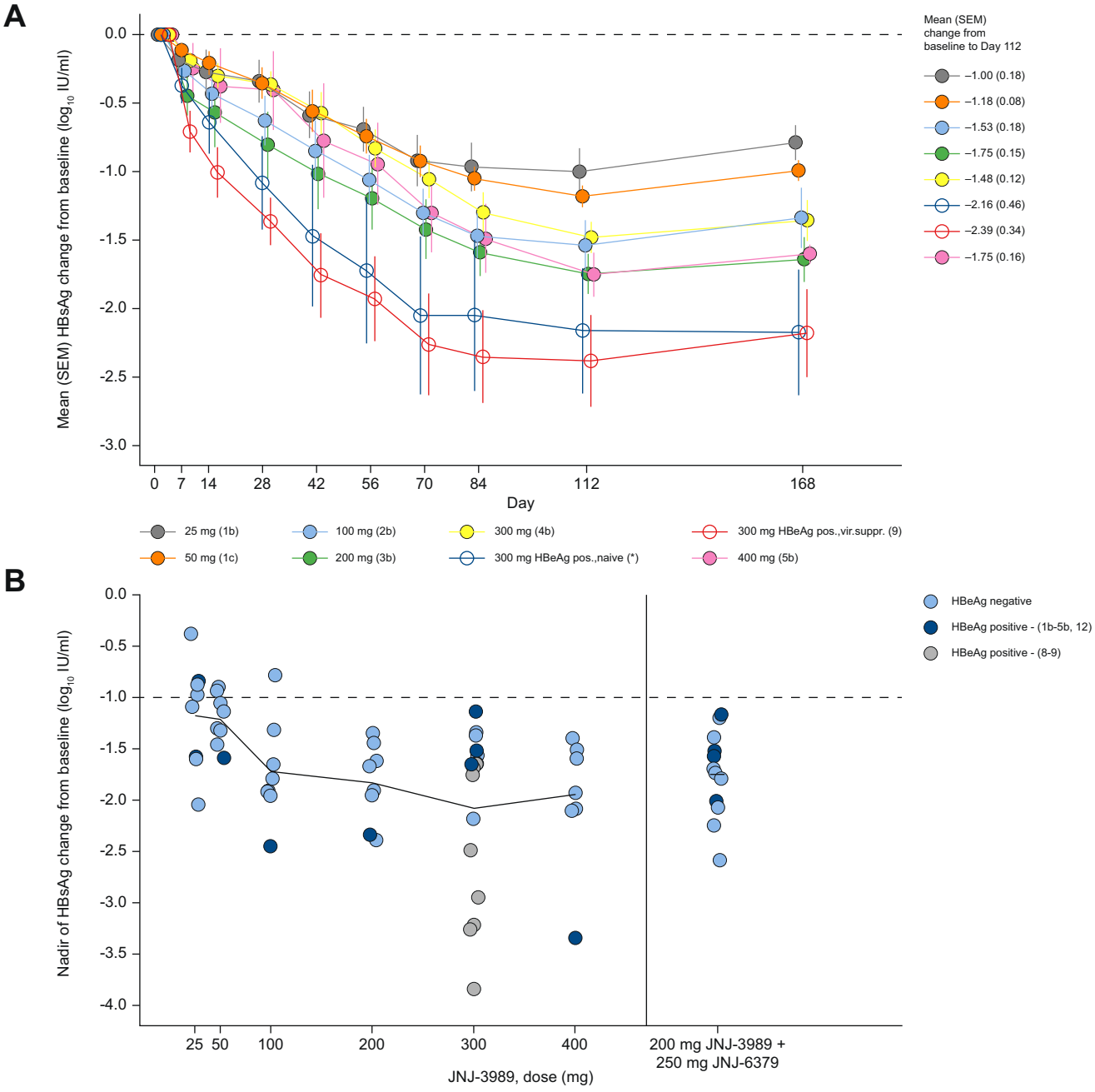

Fig. 2. Mean and individual changes in HBsAg. (A) Mean change in HBsAg from Day 0 to Day 168 in JNJ-3989 dual combination Q4W dose cohorts. (B) Individual nadir of HBsAg change from Day 0 to end of follow-up in the dual combination Q4W dose cohorts *vs.* dose (Day 392) and the triple combination therapy cohort (Day 168). Solid black line in Fig. 2B connects the mean nadir of each dose. HBsAg, hepatitis B surface antigen; pos, positive; Q4W, every 4 weeks; vir, virologically; suppr, suppressed.

shown in Table 1. Most patients were male (69.0%), Asian (82.1%), receiving NA treatment (77.4%), and HBeAg-negative (73.8%), with a median age of 45.5 years (range, 25–66). All patients received all intended doses without dosing interruptions or reductions.

Safety and tolerability

Three JNJ-3989 doses plus an NA were generally well tolerated through Day 112. Three SAEs through Day 112 were reported in 2 patients; all considered unrelated to study drug (Table 2). The most common AEs possibly related to JNJ-3989 were AEs at the injection site including discolouration, erythema, bruising, and rash, which were all transient and mild. One AE of mild, Stage 1, acute kidney injury was reported as related to JNJ-3989. This patient, in cohort 5b (receiving JNJ-3989 400 mg), developed an increase of serum creatinine from 1.10 mg/dl at baseline to 1.55 mg/dl on Day 7 in the context of creatine supplementation. Serum creatinine levels returned to 1.06 mg/dl on Day 14 following a creatine supplementation dose reduction. No AEs reported between Days 112 to 392 were considered related to

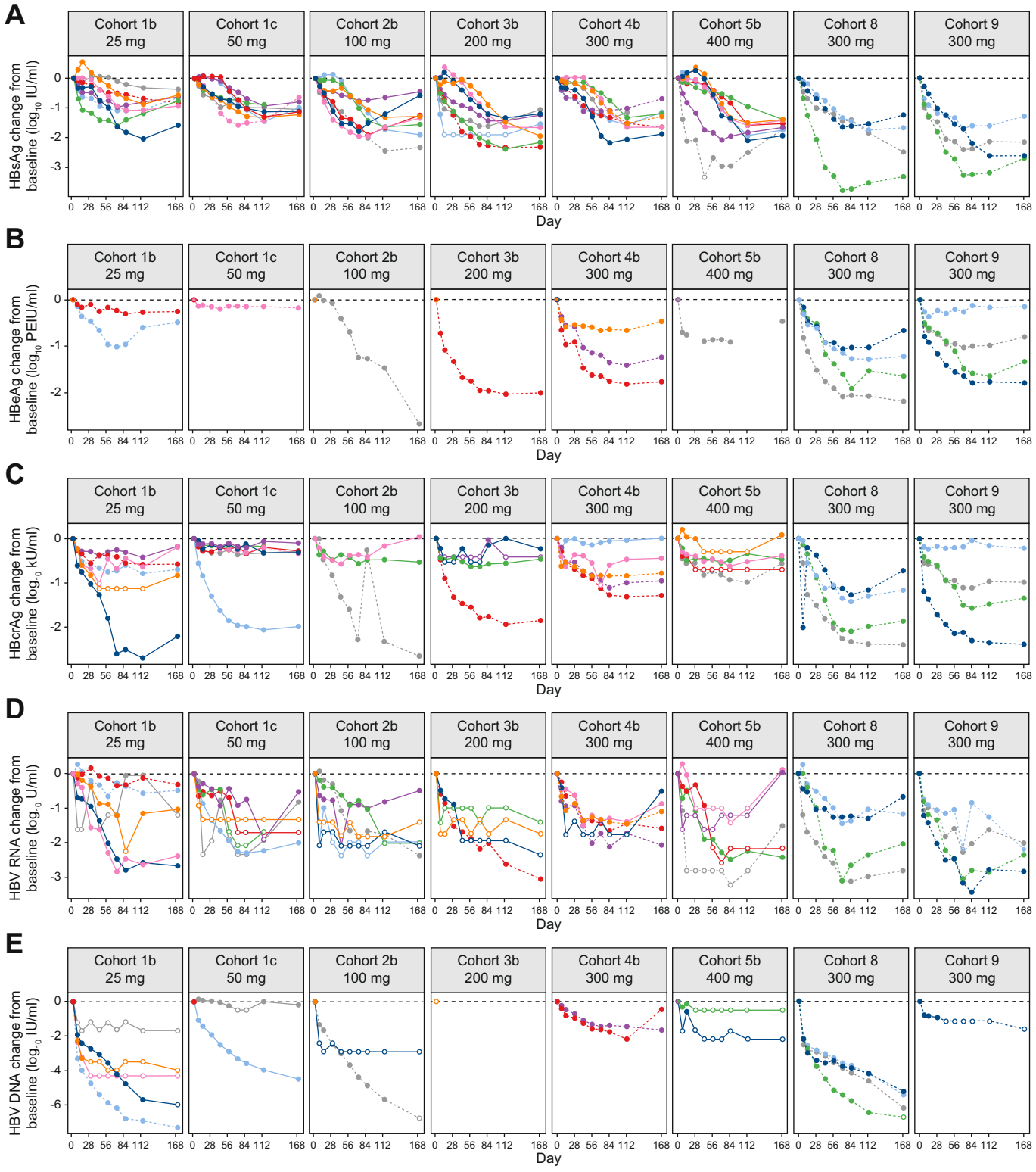

Fig. 3. Individual changes in serologic markers of HBV infection from Day 0 to Day 168 by JNJ-3989 Q4W cohort. (A) HBsAg, (B) HBeAg, (C) HBcrAg, (D) HBV RNA, and (E) HBV DNA. For HBeAg, HBcrAg, HBV RNA, and HBV DNA, only patients with Day 0 levels above LLoQ were included. Empty circles represent values below LLoQ. HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LLoQ, lower limit of quantification; PEIU, Paul-Ehrlich-Institute units; Q4W, every 4 weeks.

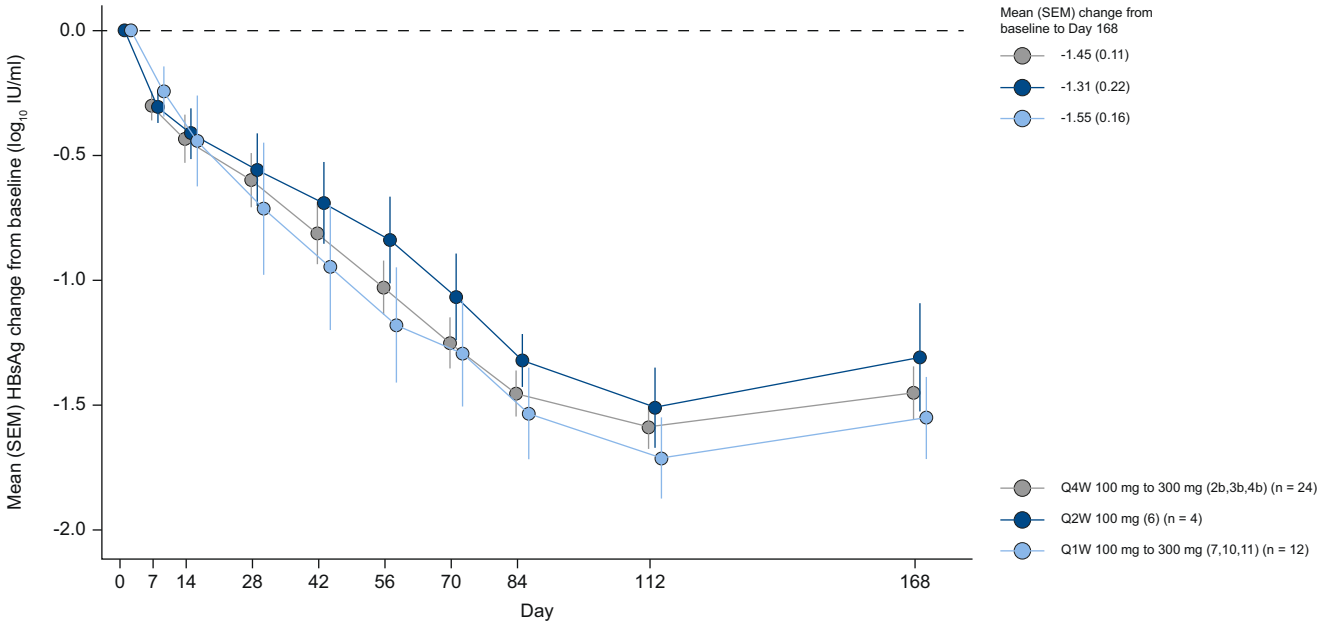

Fig. 4. Mean HBsAg decline from Day 0 to Day 168 in the different dosing regimen groups. Pooled JNJ-3989 100 to 300 mg 3x Q4W, 100 mg 3x Q2W, and pooled 100 to 300 mg 3x QW. HBsAg, hepatitis B surface antigen; QW, every week; Q2W, every 2 weeks; Q4W, every 4 weeks.

JNJ-3989. One SAE of hyperthyroidism in a patient in cohort 11 and 1 SAE of retroperitoneal mass (histology: benign Schwannoma) in a patient in cohort 12 were reported after Day 112, both SAEs were considered unrelated to JNJ-3989.

There were no reports of alanine transaminase (ALT) elevations >3x upper limit of normal and total bilirubin >2x upper limit of normal. Two patients (2.4%) developed mild hyperbilirubinemia on the first day of study treatment, which lasted for 5 and 7 days and were considered possibly and probably related to JNJ-3989, respectively, and were not associated with other clinically relevant laboratory findings (Table 2). Five mild ALT elevations were reported across cohorts 3b, 4b, 5b, and 8, 2 of which were reported as AEs: 1 asymptomatic non-drug-related peak ALT at 130 U/L on Day 85 in cohort 3b and 1 possibly drug-related, abnormal serum ALT, which peaked at ALT 136 U/L on Day 69 in cohort 4b, representing the highest treatment-emergent ALT elevations through Day 112.

Three JNJ-3989 doses combined with an NA plus JNJ-6379 (cohort 12) were well tolerated. In cohort 12, no discontinuations were reported. Grade 1, transient isolated ALT elevations occurred in 5 patients (57–122 U/L) and resolved during continued dosing.

Antiviral activity

Treatment with JNJ-3989 plus an NA resulted in HBsAg reductions from Day 0 to Day 112 in all Q4W dose cohorts (Fig. 2, Fig. 3A, and Table S3). Smaller mean HBsAg reductions were seen with 25 mg (1.00 $\log_{10}$ IU/ml) and 50 mg (1.18 $\log_{10}$ IU/ml) *vs.* 100 to 400 mg (1.48 to 2.39 $\log_{10}$ IU/ml) JNJ-3989 dosing. HBsAg responses in patients receiving 100 to 400 mg of JNJ-3989 were similar (Fig. 2A), suggesting the dose-response plateaued above 100 mg. The mean reduction of HBsAg from Day 0 to nadir was also greater in patients receiving JNJ-3989 100 to 400 mg Q4W than JNJ-3989 25 to 50 mg Q4W (mean [SEM] = 1.93 [0.10] $\log_{10}$ IU/ml *vs.* 1.19 [0.10] $\log_{10}$ IU/ml, respectively). Of patients who received 100 to 400 mg of JNJ-3989 Q4W, 39/40 (97.5%) achieved a ≥1 $\log_{10}$ IU/ml reduction in HBsAg from Day 0 to the post-dose nadir (Fig. 2B), and 30/40 patients (75%) had HBsAg <100 IU/ml at Day 112.

In cohorts with Q4W dosing, no significant difference in the extent of HBsAg reductions were seen with respect to HBeAg status or treatment history, though interpretation should consider the limited number of HBeAg-positive patients (Fig. 2B and Fig. S2). Similar HBsAg declines were observed in HBeAg-positive patients who were NA-naïve (cohort 8) or NA-suppressed (cohort 9) prior to study initiation. QW and Q2W JNJ-3989 dosing regimens showed comparable HBsAg reductions to Q4W dosing (Fig. 4).

HBeAg, HBcrAg, and HBV RNA declines from Day 0 to Day 112 were observed in most patients who had quantifiable baseline values of these parameters. In patients with baseline levels >1 log above LLoQ for the respective marker, the mean HBeAg reduction was 1.47 $\log_{10}$ PEIU/ml compared with 2.12 $\log_{10}$ IU/ml for HBsAg in the same patients, the mean HBcrAg reduction was 1.20 $\log_{10}$ kU/ml compared with 1.97 $\log_{10}$ IU/ml for HBsAg, and the mean HBV RNA reduction was 1.93 $\log_{10}$ U/ml compared with 1.86 $\log_{10}$ IU/ml for HBsAg (Fig. 3, Fig. 5, and Table S3). While the mean declines in HBeAg and HBcrAg were less pronounced than those of HBsAg and HBV RNA, in some patients HBeAg and/or HBcrAg declines were similar or even more pronounced than HBsAg and/or HBV RNA declines (Fig. S3 and Fig. S4).

HBV DNA reductions from Day 0 to Day 112 were seen across all cohorts in patients with quantifiable levels at baseline, all of whom received concurrent NA treatment.

The mean (SEM) reductions from Day 0 to the nadir in HBeAg (1.75 [0.27] $\log_{10}$ PEIU/ml *vs.* 0.51 [0.25] $\log_{10}$ PEIU/ml), HBcrAg (1.35 [0.22] $\log_{10}$ kU/ml *vs.* 0.86 [0.23] $\log_{10}$ kU/ml) and HBV RNA (2.34 [0.18] $\log_{10}$ IU/ml *vs.* 1.90 [0.22] $\log_{10}$ U/ml) were also greater in patients receiving JNJ-3989 100 to 400 mg Q4W *vs.* JNJ-3989 25 to 50 mg Q4W, respectively.

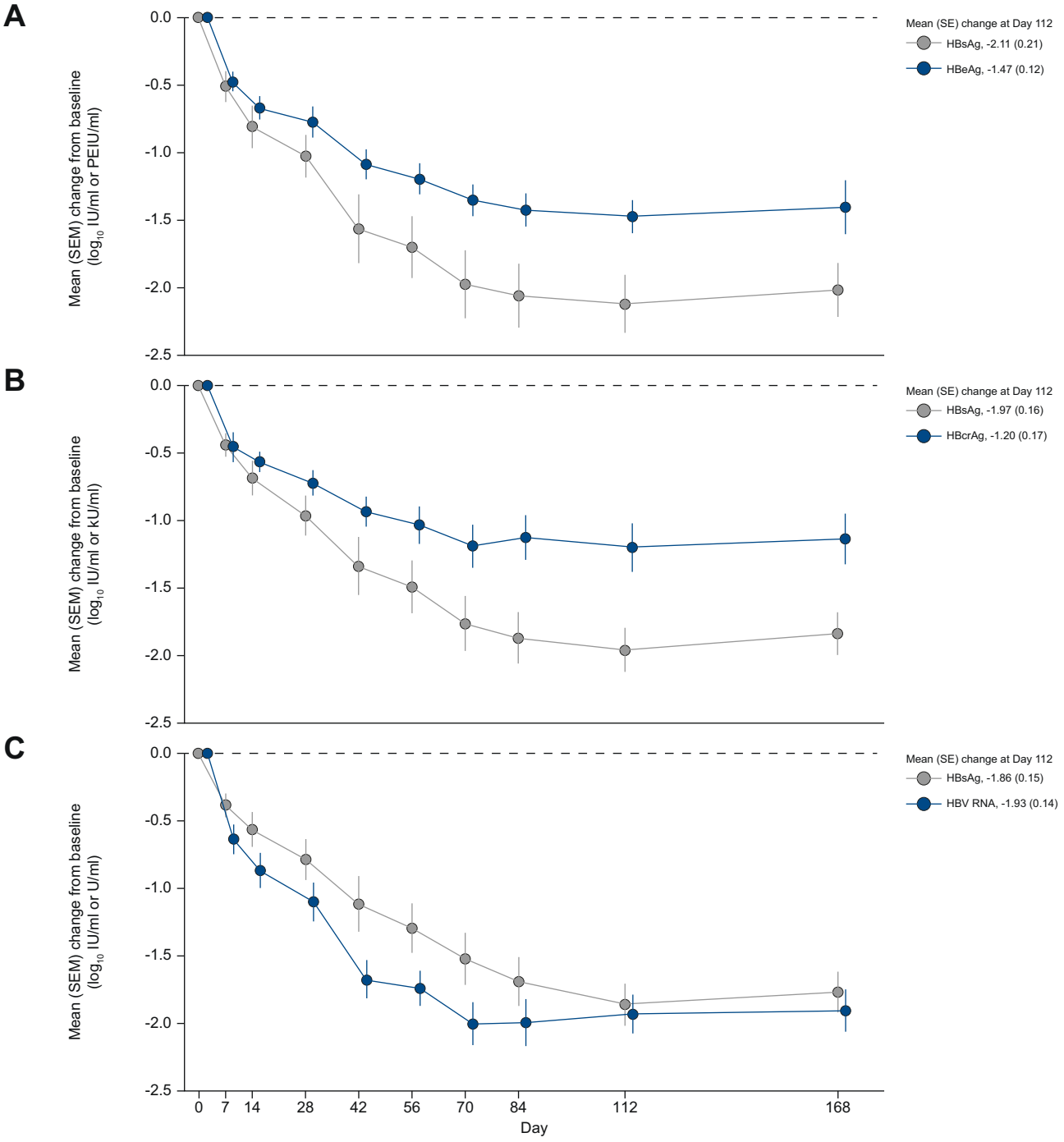

Fig. 5. Mean changes in serologic markers of HBV infection from Day 0 to Day 168 in dual combination Q4W 100 to 400 mg dose cohort patients with baseline levels >1 log_{10} above LLoQ of the respective assay. (A) HBsAg and HBeAg change in patients with baseline HBeAg >1.11 PEIU/ml (n = 13). (B) HBsAg and HBcrAg change in patients with baseline HBcrAg >10 kU/ml (n = 18). (C) HBsAg and HBV RNA change in patients with baseline HBV RNA >2.65 log_{10} U/ml (n = 21). HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LLoQ, lower limit of quantification; PEIU, Paul-Ehrlich-Institute units; Q4W, every 4 weeks.

Of the 40 patients receiving JNJ-3989 100 to 400 mg Q4W, 39 had follow-up data until day 392. Fifteen of the 39 patients (38.0%) were sustained HBsAg responders defined as maintaining ≥1 log_{10} IU/ml HBsAg reductions to Day 392, 336 days after the last JNJ-3989 dose. The mean [SEM] HBsAg reductions were 1.96 (0.20) log_{10} IU/ml in sustained responders and 0.63 (0.05) log_{10} IU/ml in non-sustained responders (Fig. 6 A). Fewer ALT elevations were seen in HBsAg-sustained responders *vs.* non-sustained responders. All ALT elevations were relatively minor (Fig. S5).

In patients receiving JNJ-3989 100 to 400 mg Q4W, mean (SEM) reductions at Day 392 were more pronounced in HBsAg-sustained responders *vs.* non-responders for HBeAg (1.71 [0.45]

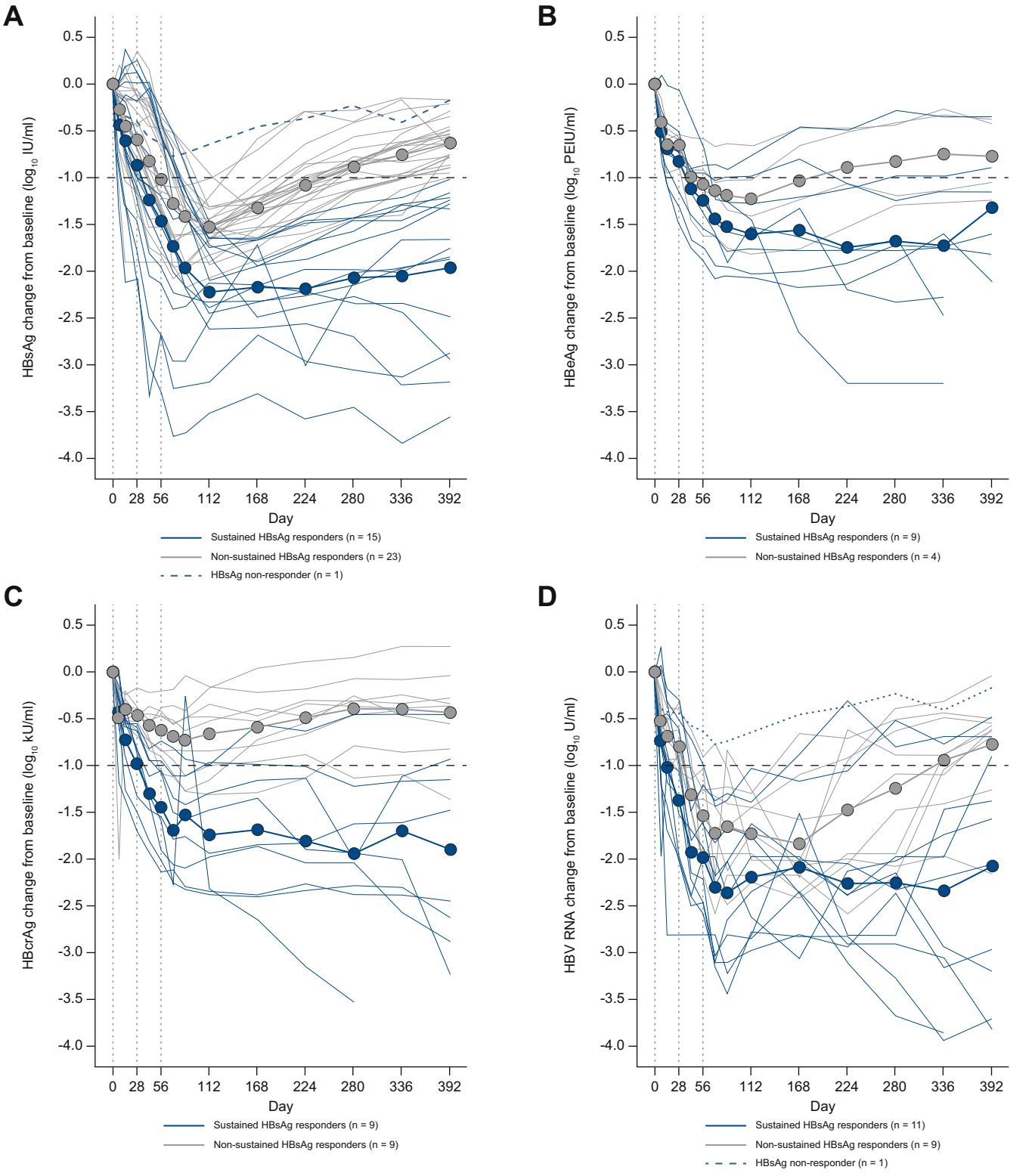

Fig. 6. Mean and individual changes in serologic markers of HBV infection from Day 0 to Day 392 by HBsAg responder status in Q4W 100 to 400 mg dose cohorts. (A) HBsAg, (B) HBeAg, (C) HBcrAg, and (D) HBV RNA. Sustained HBsAg responders defined as patients with ≥1 $\log_{10}$ HBsAg from Day 0 to Day 392. Only patients with HBeAg, HBcrAg, and HBV RNA >1 $\log_{10}$ of LLoQ were included in panels (B), (C), and (D) respectively. Circles represent mean. HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LLoQ, lower limit of quantification; PEIU, Paul-Ehrlich-Institute units; Q4W, every 4 weeks.

log_{10} PEIU/ml *vs.* 0.77 [0.22] log_{10} PEIU/ml), HBcrAg (2.20 [0.43] log_{10} kU/ml *vs.* 0.43 [0.15] log_{10} kU/ml), and HBV RNA (2.36 [0.46] log_{10} U/ml *vs.* 0.78 [0.20] log_{10} U/ml), respectively (Fig. 6B-D). Mean reductions across all 4 serologic parameters were similar for patients with the same HBsAg responder status.

All 12 patients receiving triple combination therapy achieved HBsAg decline of ≥1 log_{10} IU/ml at the nadir (mean [SEM] HBsAg log_{10} IU/ml reduction, 1.75 [0.12]) (Fig. 2B). In the limited number of patients with quantifiable parameters at Day 0, reductions from Day 0 to post-dose nadir in HBeAg (0.81 [0.30] log_{10} PEIU/ml, n = 4) and HBcrAg (0.78 [0.16] log_{10} kU/ml, n = 8) were less pronounced than for HBsAg or HBV RNA (2.6 [0.38] log_{10} U/ml, n = 9) (Fig. 6A-D and Fig. S6A-D). An increase in HBV RNA was seen in some patients after the last JNJ-6379 dose, consistent with other studies using CAMs.[15,21,22] Patients with quantifiable HBV DNA levels at Day 0 (n = 7) had robust reductions through Day 168 (Fig. S6E and Fig. S7E).

Discussion

In this phase IIa study, 3 doses of JNJ-3989 siRNA therapy plus a NA, with/without the CAM-N JNJ-6379, were well tolerated in patients with CHB and resulted in robust HBsAg, HBV RNA, HBeAg, and HBcrAg reductions. Both HBsAg reductions ≥1 log_{10} from baseline and HBsAg levels <100 IU/ml in patients treated with NAs have been associated with an increased incidence of off-treatment HBsAg seroclearance.[13] In the present study, JNJ-3989 Q4W 100 to 400 mg dosing resulted in ≥1 log_{10} HBsAg reductions in all but 1 patient, and 30/40 patients (75%) had HBsAg <100 IU/ml at Day 112. Dosing QW or Q2W did not result in faster or greater HBsAg reductions *vs.* Q4W dosing. This may be consistent with preclinical findings with other N-acetylgalactosamine-conjugated siRNAs targeted to asialoglycoprotein receptors on hepatocytes, which accumulate in acidic intracellular compartments and release slowly into the cytoplasm.[23] Here, they are loaded into the RNA-induced silencing complex to degrade all viral RNA, resulting in HBV protein knockdown including HBsAg decline, decreased HBV replication due to pgRNA decline, and decreased HBx expression that might lead to lower cccDNA levels and/or transcription.

No SAEs related to the study drug were reported through Day 392. In combination with an NA, all JNJ-3989 doses had similar safety profiles. ALT elevations were infrequent, mild, self-resolving, and not correlated with the extent of HBsAg reductions.

The kinetics of HBsAg decline varied among patients. Greater reductions in HBsAg *vs.* HBeAg and HBcrAg were generally seen, while HBV RNA reductions were similar to HBsAg in the dual combination therapy cohorts. Causes of such variability are not yet understood; however, they may be due to HBsAg production from cccDNA and integrated DNA, while other viral proteins are only produced by cccDNA.[24–27] Alternatively, RNA species may differ in their susceptibility to the siRNA mechanism due to the secondary structure of the RNA.

JNJ-3989 treatment resulted in HBsAg reductions with no significant difference regarding HBeAg status or antiviral treatment status, indicating that JNJ-3989 is pharmacologically active in the major CHB subpopulations. Transient HBsAg seroclearance (HBsAg <0.05 IU/ml) was observed in 2 patients (data not shown).

With slow hepatic siRNA trigger clearance and corresponding persistence of pharmacodynamic activity, a gradual return to baseline is commonly observed with N-acetylgalactosamine-siRNAs against endogenous liver targets.[28] However, sustained reduction of all viral markers under continued NA treatment was seen in a sub-group of patients through Day 392, 336 days after the last JNJ-3989 dose. Whether the profound HBsAg reductions induced by this siRNA can lead to sustained host control of HBV remains to be examined in future studies.

A cohort exploring the combination of JNJ-3989, an NA, and JNJ-6379 was introduced to assess the safety and tolerability of this combination. The safety profile of the triple combination seen in this study is aligned with that reported previously for JNJ-6379 and CAMs.[13,15,29,30] All ALT elevations reported in cohort 12 were isolated, transient, and mild and no new AEs of concern were noted in this 12-patient cohort. The antiviral activity in cohort 12 was comparable to other cohorts with Q4W dosing of JNJ-3989 in this study. However, characterizing the antiviral effect of JNJ-6379 in this regimen was difficult due to the short treatment duration, as loss of cccDNA results from hepatic cell turnover, which occurs over a longer time frame. Large reductions in HBV RNA were observed that were likely due to direct inhibition of HBV RNA release by JNJ-6379.[31]

As this was a phase IIa proof-of-principle study, it was not double-blinded or placebo-controlled and relatively few patients were recruited. The study population was predominantly Asian, which may limit the generalizability of these results to non-Asians. Phase II studies including REEF-1 (NCT03982186) and REEF-2 (NCT04129554) are currently underway to investigate the safety and efficacy of therapies comprising JNJ-3989 in combination with NAs, with or without JNJ-6379, in multi-ethnic cohorts of patients with CHB.

In conclusion, there were no safety concerns associated with the use of the dual and triple combination regimens. Three JNJ-3989 doses in combination with an NA, with or without JNJ-6379, resulted in pronounced HBsAg, HBeAg, HBcrAg, HBV DNA, and HBV RNA declines that persisted in many patients, supporting further investigation of longer duration regimens of JNJ-3989 in combination with an NA and JNJ-6379 in larger, multi-ethnic populations of patients with CHB.

Abbreviations

AE, adverse event; ALT, alanine aminotransferase; CAM-N, class-N capsid assembly modulator; cccDNA, covalently closed circular; CHB, chronic hepatitis B; CK, creatine kinase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; JNJ-3989, JNJ-73763989; JNJ-6379, JNJ-56136379; LLoQ, lower limit of quantification; NA, nucleos(t)ide analogue; PEI, Paul-Ehrlich-Institute; pgRNA, pregenomic RNA; pos, positive; Q2W, every 2 weeks; Q4W, every 4 weeks; QD, once daily; QW, every week; RNAi, RNA interference; SAE, serious adverse event; siRNA, short-interfering RNA; suppr, suppressed; TEAE, treatment-emergent adverse event; vir, virologically.

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Conflicts of interest

MFY serves as advisor/consultant for AbbVie, Arbutus Biopharma, Allovir International, Aligos Therapeutics, Clear B Therapeutics,

Dicerna Pharmaceuticals, Finch Therapeutics, GlaxoSmithKline, Gilead Sciences, Janssen, Merck Sharp and Dohme, and Roche and received grant/research support from Assembly Biosciences, Arrowhead Pharmaceuticals, Dicerna Pharmaceuticals, Gilead Sciences, Merck Sharp and Dohme, Roche, and Vir Biotechnology. SS received honoraria for advisory boards or speaker fees from AbbVie, AstraZeneca, Bayer, BMS, Chiesi, CSL Behring, Dr Falk, Eisai, Gilead, Ipsen, MSD, Pfizer, and Roche Products. WS received payments from Janssen for providing a clinical site for the current study. AJT served on advisory boards for Janssen, Gilead, AbbVie, Merck, BMS, Roche, Bayer, Eisai, Roche Diagnostics, and Assembly Biosciences; received speaker fees from Gilead, AbbVie, Merck, Roche, and BMS; received institutional research grants from NHMRC/MRFF, Gilead Sciences, and Roche Diagnostics; and serves as a board member and director of the Gastroenterological Society of Australia. BG, TS, and JH are current or former employees of Arrowhead and may be Arrowhead shareholders. MB, RK, MB, OL, and FDR are current or former employees of Janssen Pharmaceuticals and may be Johnson & Johnson stockholders. GC is an Abbott employee and shareholder. KJ receives funding from SpringBank Pharmaceuticals. CLL discloses sponsored lectures for Gilead Sciences. RGG is/has been a consultant and/or advisor to Abbot, AbbVie, Access Biologicals, Alexion, Antios, Arrowhead, Bayer AG, Bristol Myers Squibb, Eiger, Eisai, Enyo, eStudySite, Forty-Seven Inc, Gilead Sciences, HepaTX, HepQuant, Intercept, Ionis Pharmaceuticals, Janssen, Laboratory for Advanced Medicine, Lilly, Merck, Salix, Shionogi, and Trimaran and Viking Therapeutics; provides consulting for ADMA Biologics, AEC Partners, Arena Pharmaceuticals Inc, Arterys Inc, Cirina, Consumer Health Products Assoc, DRG Abacus, Intellia, Iqvia, KannaLife, Labyrinth Holdings, Organovo, Patient Connect and Spring Bank; is on scientific or clinical advisory boards for Abbott, AbbVie, Merck, Arrowhead, Bayer, Dova Pharmaceuticals, Eiger, Enyo, Hatch Biofund, HepQuant, Intercept, Janssen, and Medimmune; is an advisory consultant for Biocollections, Fujifilm/Wako, and Quest; is chair of the clinical advisory board for Prodigy, is on the data safety monitoring board for Altimmune, Arrowhead, CymaBay Therapeutics, and Durect; has speaker contracts with AbbVie, Bayer, Bristol Myers Squibb, Dova Pharmaceuticals, Eisai, Genentech, Gilead, Intercept, Salix, and Shionogi; is a minor stock shareholder in Athenex, Cocrystal, RiboSciences, and Triact; and holds stock options in Athenex, Eiger, HepaTx, and HepQuant; over 80% of income from Pharma received by RGG is directed to research, education, public policy, and/or donated to charities. EG has been an advisor and/or speaker for AbbVie, Arrowhead, Assembly, Gilead, GSK, Janssen, Merck, Novartis, Roche, and Vir Biotechnology. SL, THL, WC, CS, and DKHW have no disclosures to report.

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

Authors' contributions

All authors were involved in the critical revisions of the manuscript and review for important content and were accountable for all aspects of the work (accuracy and integrity), and approved the final manuscript submitted.

Data availability statement

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 (YODA) Project site at http://yoda.yale.edu.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.07.010.

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