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# Safety, antiviral activity and pharmacokinetics of JNJ-64530440, a novel capsid assembly modulator, as 4 week monotherapy in treatment-naive patients with chronic hepatitis B virus infection

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**Objectives:** We investigated JNJ-64530440 (a hepatitis B virus capsid assembly modulator) safety, antiviral activity and pharmacokinetics in patients with chronic hepatitis B (CHB) (Phase 1b, NCT03439488).

**Methods:** Twenty treatment-naive, HBeAg-positive or -negative CHB patients were randomized 4:1 to JNJ-64530440 750 mg once or twice daily, or placebo for 28 days.

**Results:** All patients (mean age 43.8 years; 85% male; 70% White; 20% HBeAg positive) completed dosing/28 day follow-up. Mild-to-moderate treatment-emergent adverse events occurred in 3/4 (placebo), 6/8 (once-daily) and 4/8 (twice-daily) patients; mostly fatigue, increased alanine aminotransferase, decreased neutrophil count, and headache. Hepatitis B virus (HBV) DNA was substantially reduced; mean (range) changes from baseline at day 29 were: −3.2 (−2.4 to −3.9) (once-daily) and −3.3 (−2.6 to −4.1) (twice-daily) $\log_{10}$ IU/mL; placebo 0.1 (0.7 to −0.6) $\log_{10}$ IU/mL. HBV DNA levels were below the lower limit of quantification (LLOQ) in 5/8 (once-daily) and 3/8 (twice-daily) patients. For patients with detectable baseline HBV RNA, mean (SE) changes versus baseline in HBV RNA at day 29 were: −2.65 (0.81) (once-daily) and −2.94 (0.33) (twice-daily) $\log_{10}$ copies/mL. HBV RNA levels were 'target not detected' in 4/6 (once-daily) and 3/7 (twice-daily) patients. JNJ-64530440 pharmacokinetics in CHB patients were comparable with those in healthy volunteers.

**Conclusions:** JNJ-64530440 750 mg once-daily or twice-daily for 28 days was well tolerated and achieved potent antiviral activity in CHB patients.

## Introduction

Current chronic hepatitis B (CHB) guidelines recommend oral antiviral therapy with a nucleos(t)ide analogue (NA).[1,2] Although NAs can maintain viral suppression and reduce liver-related complications, they must be administered life-long with potential toxicity.[1,2] Moreover, NAs rarely lead to functional cure, which is defined as the sustained loss of hepatitis B surface antigen (HBsAg) and undetectable hepatitis B virus (HBV) DNA in serum off-treatment for ≥6 months with/without HBsAg seroconversion,[3] and is regarded as a key CHB treatment goal.[1,2] Thus, novel CHB therapeutic approaches are needed.

JNJ-64530440 is a capsid assembly modulator with dual mechanisms of action (MOA), inducing the formation of 'normal' but empty non-functional HBV capsids (CAM-N), by blocking HBV replication (the 'primary' mechanism), and also inhibiting *de novo* covalently closed circular DNA (cccDNA) formation during early infection (the 'secondary' mechanism).[4] In contrast, CAM-A

compounds induce the formation of pleiomorphic non-capsid structures (A = aberrant structure), e.g. morphothiadin (GLS4)/ritonavir[5,6] and RO7049389.[7,8] JNJ-64530440 *in vitro* activity compares favourably with the *in vitro* activity of JNJ-56136379, another HBV CAM-N, in clinical development.[4,9] JNJ-64530440 showed antiviral activity *in vitro* across a panel of HBV genotype A–H isolates.[4] A Phase 1a study showed that single- (50–4000 mg) and multiple-ascending (750 mg once-daily or twice-daily, or 2000 mg once-daily) JNJ-64530440 oral doses were well tolerated in healthy volunteers.[10] We report the Phase 1b results of that study, which evaluated the safety, tolerability, antiviral activity and pharmacokinetics of JNJ-64530440 in CHB patients over 28 days.

## Patients and methods

### Study design and patients

This first-in-human, Phase 1 study (NCT03439488) was a multipart, double-blind, randomized, and placebo-controlled trial with JNJ-64530440. Single- and multiple-ascending JNJ-64530440 doses in healthy volunteers were initially assessed[10] followed by a multiple dosing phase in treatment-naive adult CHB patients (Figure S1, available as Supplementary data at *JAC* Online). CHB patients were included (see Supplementary data for the full criteria) who were hepatitis B e antigen (HBeAg) -positive (serum HBV DNA ≥20 000 IU/mL) or -negative (serum HBV DNA ≥2000 IU/mL), with ALT levels ≤5× upper limit of normal, and non-cirrhotic (Metavir stage ≤F2 based on FibroScan™ assessment <8.0 kPa[11,12]).

Key exclusion criteria included: evidence of coinfection with hepatitis A, C, D, or E, or HIV-1 infection, hepatocellular carcinoma, any prior CAM treatment and a history of cardiac arrhythmias.

### Ethics

The study protocol/amendments were approved by the appropriate Institutional Review Boards/Independent Ethics Committees at each site. This study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice and applicable regulatory requirements. All patients provided informed written consent to participate in the study.

### JNJ-64530440 dose selection and administration

Based on pharmacokinetic results in healthy volunteers, JNJ-64530440 oral doses selected for CHB patients were 750 mg once-daily and 750 mg twice-daily for 28 days. At these doses, JNJ-64530440 trough concentrations were predicted to be greater than the protein-binding-adjusted *in vitro* $EC_{90}$ ($paEC_{90}$) for both MOAs.

On day 1, 10 CHB patients per cohort were randomized 4:1 to receive JNJ-64530440 or placebo. They remained in the clinic for the first 2 days, followed by weekly visits, then stayed in the clinic for days 27 and 28 of dosing. Patients were followed up for a further 28 days without drug administration, with weekly clinic visits. Following a 14 day safety data assessment of the 750 mg once-daily cohort, the JNJ-64530440 750 mg twice-daily cohort was conducted sequentially.

JNJ-64530440 250 mg tablets with matching placebo tablets were used. Study drug intake was at approximately the same time each day, and within 30 min after a meal (once-daily dosing: breakfast only; twice-daily dosing: breakfast and dinner). For intensive pharmacokinetic sampling, study drug was administered 30 min after the start of breakfast, and lunch (∼4 h post-dose) and dinner (∼12 h post-dose), which were of the same composition for all patients.

### Safety and tolerability

Safety and tolerability were assessed continuously by monitoring adverse events (AEs), which were graded using the Division of Acquired Immunodeficiency Syndrome toxicity grading scale.[13] Patients underwent physical examination, 12-lead ECG assessment, vital signs check, and standard laboratory evaluations (clinical chemistry, haematology, urinalysis) during on-site visits.

### Antiviral assessments

HBV-DNA, HBV-RNA, HBsAg, HBeAg and hepatitis B core-related antigen (HBcrAg) levels were assessed in blood samples collected pre-dose on day 1 (baseline), and weekly during treatment and follow-up.

Plasma HBV-DNA levels were assessed using a Roche COBAS AmpliPrep/COBAS TaqMan HBV Test, v2.0 [lower limit of quantification (LLOQ) 20 IU/mL]. Serum HBV-RNA was assessed at DDL Diagnostic Laboratory (Rijswijk, Netherlands) using a validated quantitative reverse transcription PCR assay targeting the 3′ region of the genome, with a limit of detection (LOD) of 2.49 $\log_{10}$ copies/mL and an LLOQ of 4.04 $\log_{10}$ copies/mL. HBcrAg was assessed using the Lumipulse platform (Fujirebio) (LLOQ 3.0 $\log_{10}$ U/mL). Qualitative and quantitative HBsAg and HBeAg levels were assessed using Abbott Architect™ assays.[14] Anti-HBs and anti-HBe antibodies were determined using chemiluminescence immunoassays and/or enzyme-linked immunosorbent assays.

### Pharmacokinetic assessments

Blood samples were collected on day 1 (pre-dose and at 0.5, 1, 2, 4, 8, and 12 h post-dosing), pre-dose on days 2, 15 and 21, and on day 28 (pre-dose and at 0.5, 1, 2, 4, 8, and 12 h post-dosing). An additional sample was collected 24 h post-dose on day 28 only in the 750 mg once-daily cohort.

JNJ-64530440 plasma concentrations were analysed using a fully validated liquid chromatography with mass spectrometry assay (LLOQ 1.00 ng/mL) (Syneos data on file).

### HBV genome sequencing

The HBV genome was sequenced before and during treatment to monitor HBV variants. Serum samples were collected at pre-specified timepoints. HBV DNA was extracted, and the full HBV genome was sequenced using sequence read technology (Illumina). Baseline amino acid polymorphisms were defined as changes versus the universal HBV reference sequence (NCBI ID X02763; considered variant if frequency ≥15%). The analysis focused on 15 HBV core protein amino acid positions, (i.e. 23, 24, 25, 29, 30, 33, 37, 105, 106, 109, 110, 118, 124, 127, and 128) known to be associated with *in vitro* resistance to JNJ-64530440 and/or other CAMs.[15]

Selected mutations were defined as amino acid changes from patient-specific baseline sequences (frequency <1% at baseline and ≥15% post-baseline). The impact of amino acid substitutions on JNJ-64530440 *in vitro* activity was assessed in a transient replication assay using a genotype D backbone.

### Data analyses

For this exploratory study, no formal sample size calculation was performed. Demographic, baseline characteristics and safety data (including AEs) were descriptively summarized.

When HBV-DNA was 20 IU/mL (target detected) or <20 IU/mL (target not detected) values of 15 IU/mL or 5 IU/mL, respectively, were imputed for statistical analyses. HBV virological breakthrough was defined as an on-treatment HBV DNA increase by >1 $\log_{10}$ from nadir, or confirmed on-treatment HBV DNA >200 IU/mL in patients with HBV DNA <LLOQ at nadir. For HBV RNA, all values detected were considered to be quantitative

values, and samples with non-detectable levels were imputed as 5 copies/mL (0.7 $\log_{10}$ copies/mL). For HBcrAg, levels <3.0 $\log_{10}$ U/mL were imputed as 2.7 $\log_{10}$ U/mL. Mean changes from baseline for all virological parameters were calculated and presented graphically per cohort.

Plasma concentration–time data were plotted, and JNJ-64530440 pharmacokinetic parameters were calculated using non-compartmental analyses (WinNonlin Professional, Certara, Princeton, NJ, USA). Key pharmacokinetic parameters included maximum plasma concentration ($C_{max}$), plasma concentration after dosing ($C_{tau}$; i.e. at 24 h post dose for 750 mg once-daily or 12 h post dose for 750 mg twice-daily), time to $C_{max}$ ($t_{max}$), and area under the plasma concentration–time curve at the end of the dosing period ($AUC_{tau}$). Descriptive statistics were used to summarize plasma concentrations and derived pharmacokinetic parameters of JNJ-64530440. The ratio of $C_{tau}$ to $paEC_{90}$ was calculated.

## Results

### *Patient disposition and demographics*

Part 3 of this study was conducted in three sites in three countries (Moldova, $n=14$; Thailand, $n=3$; New Zealand, $n=3$) between 17 October 2018 and 17 May 2019. Overall, 56 CHB patients were screened; 31 were screen failures and 5 patients withdrew consent, so 20 patients were enrolled, and all completed dosing and the 28 day follow-up, with no major protocol deviations.

Most patients were male and white (Table 1). Two of eight patients in each JNJ-64530440 group were HBeAg positive. All four patients in the pooled placebo group were HBeAg negative. Baseline levels of HBV DNA, HBV RNA and HBsAg were higher in the JNJ-64530440 twice-daily group compared with the 750 mg once-daily and placebo groups (Table 1). HBV genotype was C or D (JNJ-64530440 750 mg once-daily), B, C, D or unknown (JNJ-64530440 twice-daily) and D or unknown (placebo).

### *Safety*

No serious AEs, discontinuations due to AEs or dose-limiting toxicities were observed (Table 2). The majority of treatment-emergent AEs were either mild (grade 1) or moderate (grade 2) in severity. The number, nature and severity of AEs did not increase with 750 mg twice-daily versus 750 mg once-daily JNJ-64530440 dosing. The only grade 3 treatment-emergent AEs were two isolated ALT elevations (one in each JNJ-64530440 cohort) that occurred on days 2 (750 mg once-daily) and 8 (750 mg twice-daily) and resolved over 7 and 28 days, respectively, and were considered possibly treatment related (750 mg once-daily) and probably treatment related (750 mg twice-daily); treatment of these patients continued unchanged. JNJ-64530440 dose was not adjusted and no concomitant therapy was initiated. Overall, AEs reported with JNJ-64530440 were similar to those in patients receiving placebo. The most common treatment-emergent AEs in the JNJ-64530440 cohorts (occurring in ≥2 volunteers) were fatigue, increased ALT, decreased neutrophil count, and headache (Table 2). No AEs were recorded during follow-up.

The majority of the laboratory abnormalities were grade 1. The most common ≥grade 2 abnormalities in either JNJ-64530440 cohort were grade 2 increased cholesterol and grade 2/3 increased LDL-cholesterol (Table S1). In the 750 mg once and twice-daily arm all patients had an eGFR Grade of 2 or less at baseline and remained that way through the treatment period and follow up [Figure S7; estimated glomerular filtration rate (eGFR) assessed by the Modification in Diet in Renal Disease study equation]. There were no corresponding increases in creatinine. One placebo patient had a Grade 3 eGFR at baseline, while having Grade 2 at screening, and remained Grade 3 during the treatment period.

No clinically significant changes in physical examination results, vital signs, ECGs, haematology and urinalysis results were recorded (data not shown).

### *Antiviral activity*

JNJ-64530440 treatment substantially reduced HBV DNA levels in CHB patients versus placebo (Figure 1). On day 29, mean (minimum; maximum) change in HBV DNA from baseline was 0.1 (0.7; −0.6; placebo), −3.2 (−2.4; −3.9; JNJ-64530440 750 mg once-daily), and −3.3 (−2.6; −4.1; JNJ-64530440 750 mg twice-daily) $\log_{10}$ IU/mL (Figure 1; Table 3 and Table S2). The proportion of patients with HBV DNA levels <LLOQ at the end of treatment was 0/4, 5/8 (63%) and 3/8 (38%), respectively (Table 3). There were no virological breakthroughs in either JNJ-64530440 cohort during dosing. During the 28 day off-treatment follow-up, HBV DNA levels increased in both JNJ-64530440 groups, and almost reached baseline levels after 4 weeks (Figure S2). Individual HBV DNA reductions from baseline did not differ between HBeAg-positive and -negative patients (Figure S3). The HBV DNA response to JNJ-64530440 was similar across all viral genotypes evaluated.

At baseline, 3/4 (placebo), 2/8 (JNJ-64530440 once-daily) and 1/8 (JNJ-64530440 twice-daily) patients had undetectable HBV RNA levels (all HBeAg-negative; Table 3 and Table S2). In patients with detectable HBV RNA, HBV RNA levels were decreased with JNJ-64530440 versus placebo (Figure 2a). On day 29, the mean (SE) change in HBV RNA from baseline was –0.21 (not applicable, $n=1$; placebo), −2.65 (0.81, $n=6$, JNJ-64530440 once-daily), and –2.94 (0.33, $n=7$; JNJ-64530440 twice-daily) $\log_{10}$-copies/mL (Table 3 and Table S2). The proportion of patients with detectable HBV RNA at baseline and HBV RNA target not detected at end of treatment was 0/1, 4/6 and 3/7, respectively (Table 3 and Table S2). During follow-up, HBV RNA levels increased in both JNJ-64530440 groups (Figure S4).

In patients with detectable HBcrAg levels at baseline, on day 29, mean (SE) change in HBcrAg from baseline was −0.35 (0.35, $n=4$; placebo), −0.52 (0.19, $n=6$; JNJ-64530440 once-daily), and −0.62 (0.17, $n=6$; JNJ-64530440 twice-daily) $\log_{10}$ IU/mL (Figure 2b); decreases in HBcrAg appeared smaller for HBeAg-positive versus HBeAg-negative patients (Figure S5). The proportion of patients with HBcrAg ≥LLOQ at baseline and HBcrAg levels <LLOQ at the end of treatment was 0/2, 2/6 and 0/6, respectively.

Changes in HBeAg were assessed in the four HBeAg-positive patients in the study ($n=2$ JNJ-64530440 once-daily; $n=2$ JNJ-64530440 twice-daily). Limited HBeAg declines were observed in all four JNJ-64530440-treated patients and ranged between 0.1 and 0.3 $\log_{10}$ IU/mL. During follow-up, HBeAg levels increased to baseline levels in three out of four patients (Figure S6).

No relevant changes in HBsAg levels were observed in any cohort over 28 days.

**Table 1.** Baseline patient demographics, characteristics and virological parameters

| Characteristic | JNJ-64530440 dosage | | | Pooled placebo (*N* = 4) | All patients (*N* = 20) |
|---|---|---|---|---|---|
| | 750 mg once-daily (*N* = 8) | 750 mg twice-daily (*N* = 8) | Both arms (*N* = 16) | | |
| Demographics | | | | | |
| age, years, mean (SD) | 44.6 (15.5) | 42.6 (8.5) | 43.6 (12.1) | 44.3 (18.6) | 43.8 (13.1) |
| male, *n* (%) | 5 (63) | 8 (100) | 13 (81) | 4 (100) | 75 (85) |
| BMI, kg/m$^2$, median (range) | 24.5 (22–35) | 27.7 (24–33) | 25.2 (22–35) | 29.8 (27–32) | 27.1 (22–35) |
| race, *n* (%) | | | | | |
| White | 6 (75) | 4 (50) | 10 (63) | 4 (100) | 14 (70) |
| Asian | 2 (25) | 3 (38) | 5 (31) | 0 | 5 (25) |
| native Hawaiian or other | 0 | 1 (13) | 1 (6) | 0 | 1 (5) |
| Disease characteristics | | | | | |
| ALT, *n* (%) | | | | | |
| $<1.25 \times$ ULN | 6 (75) | 3 (38) | 9 (56) | 3 (75) | 12 (60) |
| $1.25 \times$ ULN to $<2.50 \times$ ULN | 0 | 3 (38) | 3 (19) | 0 | 3 (15) |
| $2.50 \times$ ULN to $<5.00 \times$ ULN | 2 (25) | 2 (25) | 4 (25) | 1 (25) | 5 (25) |
| metavir fibrosis stage, *n* (%)[a] | | | | | |
| F0 | 3 (38) | 3 (38) | 6 (38) | 1 (25) | 7 (35) |
| F1 | 2 (25) | 3 (38) | 5 (31) | 1 (25) | 6 (30) |
| F2 | 3 (38) | 2 (25) | 5 (31) | 2 (50) | 7 (35) |
| Virological parameters (unless stated otherwise, values are median (range)/mean (SD) | | | | | |
| HBeAg positive, *n* (%) | 2 (25) | 2 (25) | 4 (25) | 0 | 4 (20) |
| HBV DNA, $\log_{10}$ IU/mL | 4.83 (3.58–9.03)/5.49 (2.04) | 6.06 (3.79–8.86)/6.16 (1.87) | 5.44 (3.58–9.03)/5.82 (1.92) | 4.51 (2.91–6.11)/4.51 (1.68) | 5.44 (2.91–9.03)/5.56 (1.91) |
| HBV RNA, $\log_{10}$ copies/mL[b] | 3.36 (0.70–7.33)/3.68 (2.50) | 5.16 (0.70–8.27)/4.90 (2.57) | 3.87 (0.70–8.27)/4.29 (2.53) | 0.70 (0.70–4.30)/1.60 (1.80) | 3.78 (0.70–8.27)/3.75 (2.60) |
| HBsAg, $\log_{10}$ IU/mL | 4.04 (4.07–7.83)/3.56 (1.32) | 3.95 (6.30–8.01)/3.95 (0.57) | 3.95 (4.07–8.01)/3.76 (1.00) | 3.81 (5.33–8.01)/3.57 (0.88) | 3.95 (4.07–8.01)/3.72 (0.96) |
| HBcrAg, $\log_{10}$ U/mL[c] | 3.6 (2.70–8.70)/4.8 (2.52) | 5.5 (2.70–8.80)/5.6 (2.29) | 4.8 (2.70–8.80)/5.2 (2.36) | 3.6 (2.70–5.10)/3.8 (1.24) | 4.6 (2.70–8.80)/4.9 (2.23) |
| HBeAg, $\log_{10}$ IU/mL | 3.00 (5.82–6.18)/3.00 (0.25)[d] | 2.91 (5.65–6.18)/2.91 (0.37)[d] | 3.00 (5.65–6.18)/2.96 (0.26)[e] | –[f] | 3.00 (5.65–6.18)/2.96 (0.26)[e] |
| HBV genotype, *n* (%)[g] | | | | | |
| B | 0 | 2 (25) | 2 (13) | 0 | 2 (10) |
| C | 2 (25) | 2 (25) | 4 (25) | 0 | 4 (20) |
| D | 6 (75) | 3 (38) | 9 (56) | 3 (75) | 12 (60) |
| unknown | 0 | 1 (13) | 1 (6) | 1 (25) | 2 (10) |

ALT, alanine aminotransferase; BMI, body mass index; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IU, international units; SD, standard deviation; ULN, upper limit of normal.

[a]Metavir fibrosis stage was assessed by elastography.

[b]Exploratory test; at baseline, 2/8 (750 mg once-daily), 1/8 (750 mg twice-daily) and 3/4 (placebo) patients had undetectable HBV RNA levels.

[c]At baseline, 2/8 (750 mg once-daily), 2/8 (750 mg twice-daily) and 2/4 (placebo) patients had HBcrAg levels below LLOQ.

[d]Based on measurements in two subjects.

[e]Based on measurements in four subjects.

[f]Not measured in any subjects.

[g]Genotype data were determined using LiPA and/or sequence-based genotype assays.

**Table 2.** Summary of treatment-emergent AEs following administration of JNJ-64530440 for 4 weeks

| Characteristic | JNJ-64530440 dosage | | | | |
|---|---|---|---|---|---|
| | 750 mg once-daily (*N* = 8) | 750 mg twice-daily (*N* = 8) | Both arms (*N* = 16) | Pooled placebo (*N* = 4) | All patients (*N* = 20) |
| At least one AE | 6 (75) | 4 (50) | 10 (63) | 3 (75) | 13 (65) |
| At least one SAE | 0 | 0 | 0 | 0 | 0 |
| At least one fatal AE | 0 | 0 | 0 | 0 | 0 |
| At least one AE leading to treatment discontinuation | 0 | 0 | 0 | 0 | 0 |
| Worst reported AE grade | | | | | |
| Grade 1 | 5 (63) | 2 (25) | 7 (44) | 2 (50) | 9 (45) |
| Grade 2 | 0 | 1 (13) | 1 (6) | 1 (25) | 2 (10) |
| Grade 3[a] | 1 (13) | 1 (13) | 2 (13) | 0 | 2 (10) |
| Grade 4 | 0 | 0 | 0 | 0 | 0 |
| Specific on-treatment AEs ($n \geq 2$ participants in any one group) | | | | | |
| fatigue | 2 (25) | 0 | 2 (13) | 0 | 2 (10) |
| ALT increase | 1 (13) | 1 (13) | 2 (13) | 0 | 2 (10) |
| blood potassium increase | 0 | 0 | 0 | 2 (50) | 2 (10) |
| neutrophil count decrease | 2 (25) | 0 | 2 (13) | 0 | 2 (10) |
| headache | 2 (25) | 0 | 2 (13) | 2 (50) | 4 (20) |
| nasal congestion | 1 (13) | 0 | 1 (6) | 1 (25) | 2 (10) |

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SAE, serious AE.
[a]The two Grade 3 AEs were both isolated ALT elevations.

### *JNJ-64530440 pharmacokinetics*

Figure 3 shows JNJ-64530440 plasma concentration–time curves. On day 1, mean (SD) JNJ-64530440 $C_{max}$ values were 6193 (2128) ng/mL with twice-daily dosing versus 5795 (2137) ng/mL with 750 mg once-daily dosing (Table S3). The corresponding $C_{max}$ values on day 28 were 8863 (2834) and 6794 (2608) ng/mL, respectively. The mean $C_{tau}$:paEC$_{90}$ (for the secondary MOA) ratios on day 28 were 2.9 and 11.1 for 750 mg once-daily and 750 mg twice-daily dosing, respectively. Day 28 mean (SD) $AUC_{tau}$ values were similar between 750 mg once-daily and 750 mg twice-daily dosing, i.e. 89 709 (38 061) and 81 647 (15 859), respectively (Table S3). Median $t_{max}$ values were similar between dosing regimens (3–4 h) (Table S3). The accumulation of JNJ-64530440 $C_{max}$ and $AUC_{tau}$ between days 1 and 28 was 1.2- and 1.3-fold for 750 mg once-daily, and 1.6- and 1.9-fold for 750 mg twice-daily dosing, respectively.

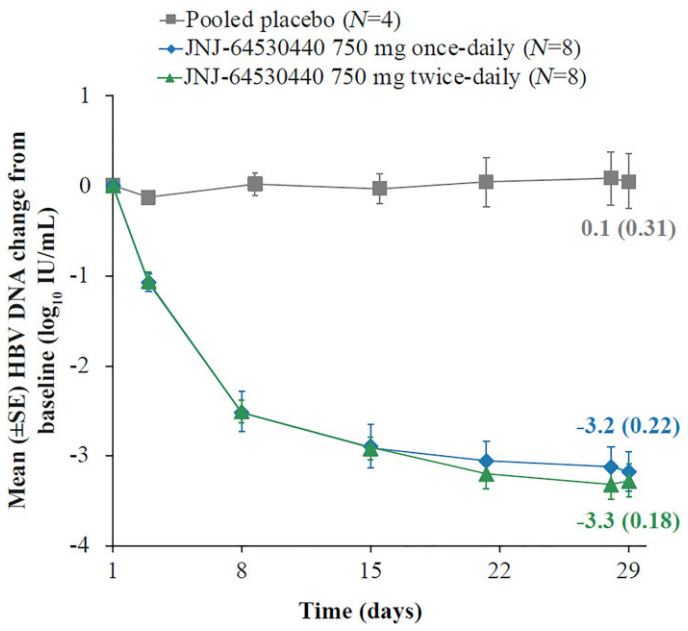


**Figure 1.** Mean (SE) changes in HBV DNA from baseline up to day 29 during once-daily or twice-daily dosing of JNJ-64530440 in CHB patients. CHB, chronic hepatitis B; HBV, hepatitis B virus; IU, international units; SE, standard error of the mean.

### *HBV genome sequencing*

Baseline sequence data was available for 16/20 (80%) patients overall, and 5/16 (31%) patients with baseline sequence data had ≥1 polymorphism at HBV core protein amino acid positions of interest 24 (F24Y), 29 (D29S), 105 (I105L/T/V), and/or 109 (T109M). *In vitro* JNJ-64530440 resistance data were only available for F24Y, I105L and T109M and showed that none of these reduce JNJ-64530440 *in vitro* activity (fold change in 50% effective concentration <2.0). All four JNJ-64530440-treated patients (one JNJ-64530440 once-daily and three JNJ-64530440 twice-daily treated) with polymorphisms showed pronounced declines in HBV DNA of 2.61 to 4.08 $\log_{10}$ IU/mL from baseline to levels <LLOQ from day 15 or day 21 onwards until the end of treatment (Table S4).

Paired baseline and post-baseline sequences were available for 12/16 (75%) patients treated with JNJ-64530440 and 2/4 patients receiving placebo. No patient showed emergence of variants at one or more of the HBV core protein positions of interest.

**Table 3.** Antiviral activity of JNJ-64530440 in CHB patients

| Parameter | JNJ-64530440 dose for 28 days | | Pooled placebo |
|---|---|---|---|
| | 750 mg once-daily (*N* = 8) | 750 mg twice-daily (*N* = 8) | (*N* = 4) |
| HBV DNA | | | |
| mean (SE) at baseline, $\log_{10}$ IU/mL | 5.49 (0.72) | 6.16 (0.66) | 4.51 (0.84) |
| mean (SE) change from baseline, $\log_{10}$ IU/mL | | | |
| day 15 | −2.91 (0.24) | −2.93 (0.12) | −0.04 (0.17) |
| day 29 | −3.2 (0.22) | −3.3 (0.18) | 0.05 (0.31) |
| Patients with HBV DNA <LLOQ, *n* (%) | | | |
| baseline | 0 | 0 | 0 |
| day 15 | 3 (38) | 2 (25) | 0 |
| day 29 | 5 (63) | 3 (38) | 0 |
| HBV RNA | | | |
| mean (SE) at baseline, $\log_{10}$ copies/mL | 3.68 (0.88) | 4.90 (0.91) | 1.60 (0.90) |
| mean (SE) change from baseline when detectable at baseline, $\log_{10}$ IU/mL | | | |
| day 15 | −2.61 (0.18) | −2.28 (0.14) | 0.22 (NA) |
| day 29 | −2.65 (0.18) | −2.94 (0.33) | −0.21 (NA) |
| HBV RNA at baseline | | | |
| undetectable, *n* | 2 | 1 | 3 |
| detectable, *n* | 6 | 7 | 1 |
| Undetectable HBV RNA when detectable at baseline, *n/N* | | | |
| day 15 | 4/6 | 1/7 | 0/1 |
| day 29 | 4/6 | 3/7 | 0/1 |

CHB, chronic hepatitis B; HBV, hepatitis B virus; IU, international unit; LLOQ, lower limit of quantification; NA, not applicable; SE, standard error.

## Discussion

In this study, JNJ-64530440 750 mg once-daily or twice-daily administered orally for 28 days was well tolerated and demonstrated substantial HBV DNA and RNA reductions in treatment-naive patients with CHB.

No serious AEs, treatment discontinuations or dose-limiting toxicities were observed with JNJ-64530440 at 750 mg once-daily or 750 mg twice-daily doses for 28 days in CHB patients, consistent with findings of the preceding Phase 1a study in healthy volunteers.[10] JNJ-64530440 750 mg twice-daily dosing did not increase the frequency, nature or severity of AEs in CHB patients versus once-daily dosing.

The most common ≥grade 2 abnormalities with JNJ-64530440 were decreased eGFR, and increased total cholesterol and LDL-cholesterol. Grade 2 decreases in eGFR from baseline were mainly observed in the JNJ-64530440 750 mg twice-daily arm and were not accompanied by clinically relevant changes in creatinine.

JNJ-64530440 at 750 mg once-daily and 750 mg twice-daily dosing for 28 days resulted in substantial HBV DNA decreases from baseline (−3.2 versus −3.3 $\log_{10}$ IU/mL, respectively) and a high proportion of patients achieved HBV DNA levels ≤LLOQ from as early as day 15 of dosing. This rapid and immediate antiviral effect of JNJ-64530440 coincides with the rapid increases in JNJ-64530440 plasma concentrations post-dosing. Keeping the limitations of cross-study comparisons in mind, these HBV DNA decreases compared favourably to those observed for other CAMs after 4 weeks of dosing in Phase 1b studies e.g. vebicorvir (ABI-H0731; CAM-N) (mean maximum decrease up to 2.8 $\log_{10}$ IU/mL[16]), JNJ-56136379 (mean decrease up to 2.92 $\log_{10}$ IU/mL[14]), NVR 3-778 (CAM-N) (mean decrease 1.97 $\log_{10}$ IU/mL[17]), and RO7049389 (CAM-A) (median decrease up to 3.0 $\log_{10}$ IU/mL[8]).

JNJ-64530440, as do other CAMs, directly affects capsid formation and thereby reduce release of HBV-RNA-containing particles.[14,16–19] Consistent with this MOA, substantial declines in HBV RNA levels were achieved in all CHB patients with detectable HBV RNA at baseline and dosed with JNJ-64530440 for 28 days, with a high proportion of patients having undetectable HBV RNA levels by the end of dosing. After cessation of JNJ-64530440 treatment and during the follow-up, both HBV DNA and RNA levels increased, consistent with the $t_{½}$ of JNJ-64530440.

In contrast to JNJ-64530440, HBV NAs inhibit viral replication and production of infectious DNA-containing Dane particles, but they do not prevent release of HBV-RNA-containing particles.[18] Long-term NA treatment can lead to cccDNA reduction,[20] but does not completely block HBV replication, allowing replenishment of the cccDNA pool.[21] In addition to interfering with capsid assembly and thereby inhibiting the formation of DNA- and RNA-containing particles, CAM-Ns, such as JNJ-64530440 also prevent *de novo* cccDNA formation (secondary MOA).[18,19]

Following 28 days of JNJ-64530440 treatment, reductions (≥0.3 log) in HBcrAg levels were seen in the majority (7/8) of HBeAg-negative patients with detectable HBcrAg levels at baseline, with 2/6 patients in the JNJ-64530440 750 mg once-daily group achieving HBcrAg levels <LLOQ at the end of treatment.

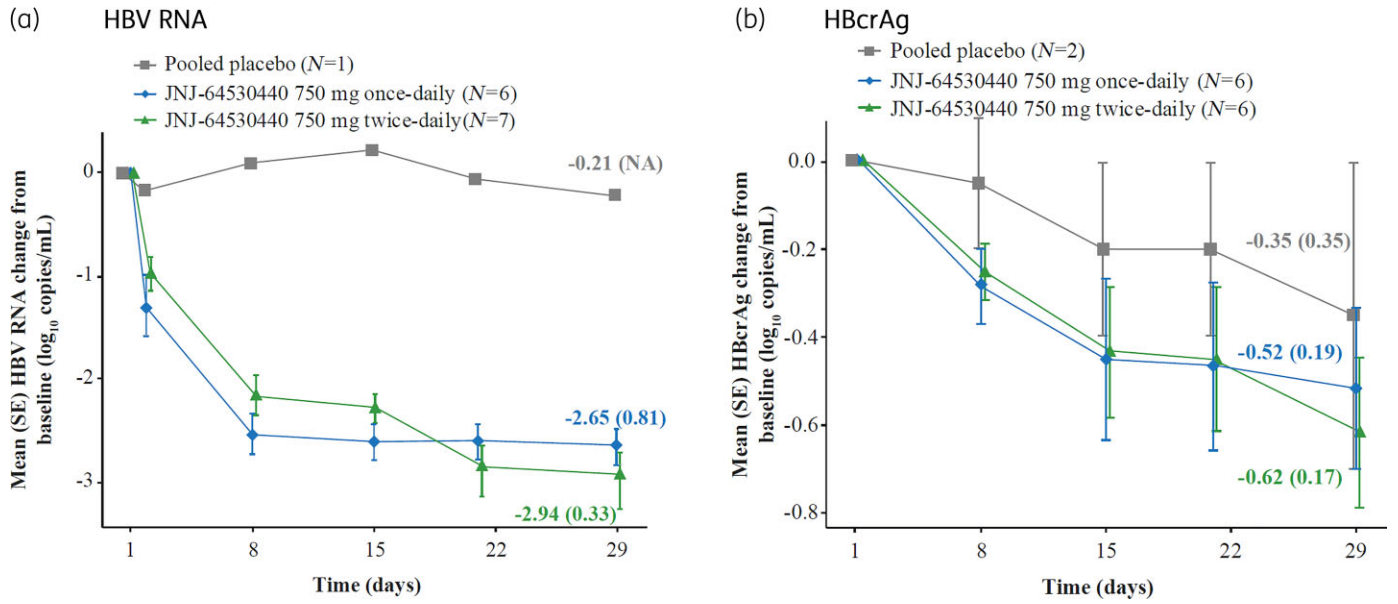


**Figure 2.** Mean (SE) changes in (a) HBV RNA and (b) HBcrAg levels from baseline up to day 29 during once-daily or twice-daily dosing of JNJ-64530440 in CHB patients in patients with detectable levels at baseline. CHB, chronic hepatitis B; HBcrAg, hepatitis B core-related antigen; HBV, hepatitis B virus; IU, international unit; NA, not applicable; SE, standard error of the mean.

Although HBcrAg, which measures a composite of HBeAg (main component measured in HBeAg positive patients with this assay), HBV core protein (HBcAg) and PreCore-related antigen (PreC or 'p22cr'), is considered a surrogate marker for cccDNA activity,[22,23] the reductions seen in this study might be at least partially due to the directly inhibition of the release of the HBV core protein.[14] In the four HBeAg-positive patients, reductions in HBeAg levels were observed during treatment with JNJ-64530440 with maximal decline of 0.3 $\log_{10}$ IU/mL in two out of four HBeAg-positive JNJ-64530440-treated patients, and a partial return towards baseline levels after treatment cessation. However, data should be interpreted with caution given the small sample size and short treatment duration. No declines in HBsAg levels were seen with JNJ-64530440 treatment, which is consistent with other short-term CAM-N Phase 1b studies.[14,16] The impact of CAM add-on treatment on viral replication and antigen levels will need to be examined further in studies exploring longer-term CAM dosing in combination with standard of care.

Five of 16 patients overall, with sequence data available, carried polymorphisms at HBV core positions described to confer

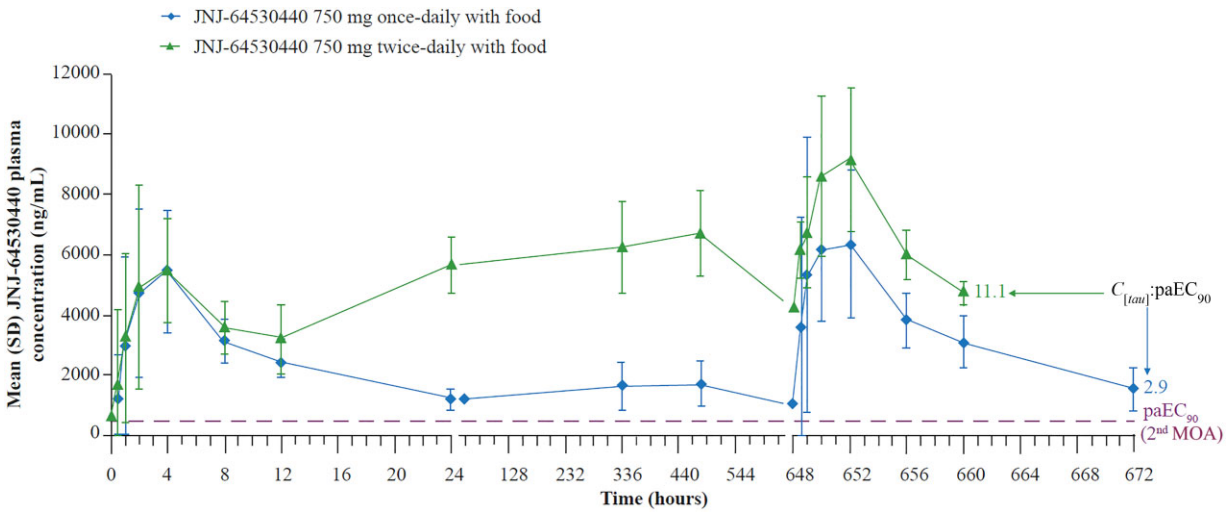


**Figure 3.** Plasma concentrations of JNJ-64530440 with time following once-daily or twice-daily dosing in chronic hepatitis B patients. $C_{tau}$, plasma concentration over the dosing interval; MOA, mechanism of action; $paEC_{90}$, protein binding-adjusted 90% effective concentration; SD, standard deviation.

resistance to CAMs, including one JNJ-64530440-treated patient with I105T polymorphism and one placebo-treated patient with T109M polymorphism, previously linked with reduced response with AB-506[24] and vebicorvir.[16] Importantly, these polymorphisms did not reduce JNJ-64530440 *in vitro* activity and had no impact on JNJ-64530440-induced HBV DNA decline in this study. No emergence of HBV core resistance variants was noted.

In CHB patients, pharmacokinetic parameters with 750 mg JNJ-64530440 once-daily and 750 mg twice-daily dosing were comparable to those reported in healthy volunteers.[10] JNJ-64530440 750 mg twice-daily resulted in higher exposures in CHB patients versus once-daily dosing. An important finding in CHB patients is the achievement of high JNJ-64530440 plasma concentrations, even at trough levels. In *in vitro* studies in primary human hepatocytes, the $EC_{50}$/$EC_{90}$ of JNJ-64530440 for blocking HBV replication (primary MOA) were 22 nM/103 nM, and for inhibition of *de novo* cccDNA formation (secondary MOA) were 136 nM/373 nM, respectively.[4] The mean ratios of JNJ-64530440 $C_{tau}$:paEC$_{90}$ (secondary MOA i.e. requiring much higher concentrations than the primary MOA) on day 28 were 2.9 and 11.1 for once-daily and twice-daily JNJ-64530440 dosing, respectively, indicating the achievement of plasma concentrations far in excess of those required for both MOAs. While the twice-daily dosing regimen had an improved $C_{min}$:$EC_{90}$ ratio and was safe and well tolerated, it needs to be balanced against the convenience of a once-daily regimen.

Limitations of this study include the predominantly male and White population, relatively small sample size of CHB patients in each dosing cohort, particularly of HBeAg-positive patients and Asian patients. Furthermore, the study duration was only 28 days, which may be too short for the secondary MOA to contribute an effect on HBsAg levels.

In conclusion, JNJ-64530440 at 750 mg once-daily or twice-daily over 28 days was generally well tolerated in CHB patients, and resulted in substantial HBV DNA and HBV RNA reductions from baseline. JNJ-64530440 pharmacokinetic parameters were comparable to those previously observed in healthy volunteers.

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## Transparency declarations

E.J.G. has been an advisor and/or speaker for AbbVie, Aligos, Gilead, Janssen, Novartis, and Roche. C.S. has provided advice to Johnson & Johnson and Vir Biotechnology. E.B., A.J., N.G. and P.T. have no disclosures; T.V., O.L., W.T., T.N.K., M.P., L.D. and P.V.R. are employees of Janssen Pharmaceuticals and may be Johnson & Johnson stockholders. C.W. and J.Z.Y. are former employees of Janssen Pharmaceuticals and are now employees of Aligos Therapeutics and may be stockholders.

## Data availability

The data sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at https://www.jansen.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.

## Supplementary data

Inclusion and exclusion criteria, Tables S1 to S4 and Figures S1 to S7 are available as Supplementary data at *JAC* Online.

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