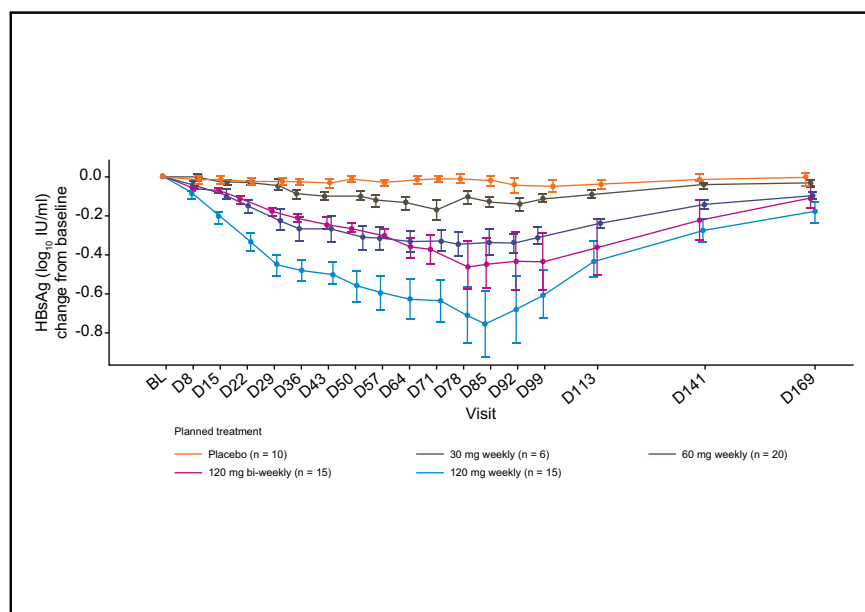


Phase IIa, randomised, double-blind study of GSK3389404 in patients with chronic hepatitis B on stable nucleos(t)ide therapy

Graphical abstract



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Lay summary

Hepatitis B virus (HBV) can result in chronic HBV infection, which may ultimately lead to chronic liver disease, primary liver cancer and death; HBV proteins may prevent the immune system from successfully controlling the virus. GSK3389404 is an investigational agent that targets HBV RNA, resulting in reduced viral protein production. This study assessed the safety of GSK3389404 and its ability to reduce the viral proteins in patients with chronic HBV infection. GSK3389404 showed dose-dependent reduction in hepatitis B surface antigen, with an acceptable safety profile. While no clear optimal dose was identified, the findings from this study may help in the development of improved treatment options for patients with chronic HBV infections.

Highlights

- GSK3389404 is a GalNAc-conjugated antisense oligonucleotide targeting HBV pregenomic and mRNA transcripts.
- GSK3389404 treatment for 12 weeks led to a dose-dependent reduction of HBsAg in patients with chronic HBV infection.
- HBsAg reduction occurred in both HBeAg+/- patients, indicating the target site is away from the integration hotspot.
- Only 3 of 56 patients had a >1.5 log IU/ml reduction in HBsAg and no HBsAg seroclearance was achieved.
- GSK3389404 had an acceptable safety profile with no unexpected safety signals.



Phase IIa, randomised, double-blind study of GSK3389404 in patients with chronic hepatitis B on stable nucleos(t)ide therapy

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Background & Aims: Bepirovirsen, an antisense oligonucleotide targeting pregenomic and mRNA transcripts of HBV, has been conjugated to *N*-acetyl galactosamine (GSK3389404) to enhance hepatocyte delivery. This dose-finding study was the first to assess GSK3389404 for chronic HBV infection.

Methods: This phase IIa, randomised, double-blind, placebo-controlled, 2-part study was conducted in 22 centres in Asia (NCT03020745). Pharmacokinetic findings from Part 1 informed Part 2 dosing. In Part 2, patients with chronic hepatitis B on nucleos(t)ide analogue therapy were randomised 11:2 to

GSK3389404 (30, 60, 120 mg weekly or 120 mg bi-weekly) or placebo until Day 85. Coprimary endpoints included HBsAg response ($\geq 1.5 \log_{10}$ IU/ml reduction from baseline) rate, safety and pharmacokinetics.

Results: Parts 1 and 2 included 12 (9 GSK3389404, 3 placebo) and 66 patients (56 GSK3389404, 10 placebo), respectively. In Part 2, one patient each in the 60 mg weekly, 120 mg weekly and 120 mg bi-weekly arms achieved a HBsAg response. HBsAg reductions were dose-dependent (Day 85: mean 0.34 [60 mg weekly] to 0.75 \log_{10} IU/ml [120 mg weekly]) and occurred in hepatitis B e antigen-positive and -negative patients. No patient achieved HBsAg seroclearance. 43/56 (77%) GSK3389404- and 9/10 (90%) placebo-treated patients reported adverse events. No deaths were reported. Alanine aminotransferase flares ($>2x$ upper limit of normal) occurred in 2 GSK3389404-treated patients (120 mg weekly, 120 mg bi-weekly); both were associated with decreased HBsAg, but neither was considered a responder. GSK3389404 plasma concentrations peaked 2–4 hours post dose; mean plasma half-life was 3–5 hours.

Keywords: chronic hepatitis B; antisense oligonucleotide; GSK3389404; HBsAg; HBeAg; virological response; safety; pharmacokinetics.

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Conclusions: GSK3389404 showed an acceptable safety profile and target engagement, with dose-dependent reductions in HBsAg. However, no efficacious dosing regimen was identified.

Clinical trial number: NCT03020745.

Lay summary: Hepatitis B virus (HBV) can result in chronic HBV infection, which may ultimately lead to chronic liver disease, primary liver cancer and death; HBV proteins may prevent the immune system from successfully controlling the virus. GSK3389404 is an investigational agent that targets HBV RNA, resulting in reduced viral protein production. This study assessed the safety of GSK3389404 and its ability to reduce the viral proteins in patients with chronic HBV infection. GSK3389404 showed dose-dependent reduction in hepatitis B surface antigen, with an acceptable safety profile. While no clear optimal dose was identified, the findings from this study may help in the development of improved treatment options for patients with chronic HBV infections.

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Introduction

Worldwide, an estimated 257 million people were living with chronic HBV infection in 2015.¹ Several phases of chronic HBV infection have been identified, characterised by hepatitis B e antigen (HBeAg) status, levels of serum HBV DNA and alanine aminotransferase (ALT), with or without results of liver biopsies indicating fibrosis.^{2,3} These phases dictate the treatment strategy in patients with chronic HBV infection.^{2–4}

The current treatment goal is to achieve a functional cure, defined as sustained loss of serum hepatitis B surface antigen (HBsAg) with undetectable HBV DNA.⁵ Functional cure is associated with improved clinical outcomes and reduced risk of hepatocellular carcinoma, particularly if achieved prior to 50 years of age.^{6,7} It is also associated with a lower chance of disease reactivation, allowing patients to be off-therapy with minimal risk of relapse.⁶ Nucleos(t)ide analogues (NAs) and pegylated interferons (PEG-IFNs) are recommended therapies for chronic hepatitis B. However, neither therapy eradicates chronic HBV infection, and NAs require lifelong use in most patients.⁴ While PEG-IFNs provide disease control in some patients, their poor tolerability and variable effect limit their use.^{2,4} The inability of current antiviral treatment options to induce function cure highlights the need for novel therapies.²

Antisense oligonucleotides (ASOs) and small-interfering RNAs (siRNAs) are being developed to target HBV RNA transcripts.⁸ Bepirovirsen (GSK3228836, ISIS 505358), a second-generation 2'-O-methoxyethyl (MOE) modified antisense phosphorothioate oligonucleotide targets all HBV-derived RNA transcripts.⁹ GSK3389404 comprises bepirovirsen covalently bonded to 3 N-acetyl galactosamines (GalNAcs) to enhance delivery to hepatocytes. Following entry into target cells, the GalNAc-GSK3228836 conjugate is metabolised to release bepirovirsen, which is complementary to sequences present in all HBV-derived RNA transcripts. Bepirovirsen binding to HBV RNA results in ribonuclease H-mediated RNA degradation. Inhibition of HBsAg via this RNA degradation transcript could allow for host immune response recovery, facilitating sustained suppression of HBV and functional cure.¹⁰

GSK3389404 has been shown to reduce HBsAg in a transgenic HBV mouse model.¹¹ Doses up to 120 mg/week administered subcutaneously for 4 weeks have been tested in healthy volunteers. Dose-proportional plasma exposure with no indication of GSK3389404 accumulation was reported, and no safety concerns were identified.¹² The current study is the first clinical trial assessing the effects of GSK3389404 in patients with chronic HBV infection. The objectives were to assess the safety, tolerability and pharmacokinetics (PK) of multiple doses of GSK3389404 and identify efficacious doses and dosing regimens.

Patients and methods

Study design

This was a phase IIa, multicentre, randomised, double-blind, placebo-controlled, 2-part study in patients with chronic HBV infection (NCT03020745) (Fig. 1). Part 1 was a single ascending dose study with 3 sequential cohorts, with patients within each cohort randomised (3:1) to receive GSK3389404 or placebo. The first cohort received 60 mg GSK3389404, and the second and third cohorts received the same dose of 120 mg. PK findings from Part 1 informed doses used in Part 2.

Part 2 was a multiple-dose, dose-ranging study in patients with chronic HBV infection on stable NA treatment from 22 sites in the Asia-Pacific region: China, Hong Kong, Japan, Republic of Korea, Philippines and Singapore. Patients were randomised (11:2) to GSK3389404 or matching placebo. Following a screening period (up to 45 days), GSK3389404 was administered at 3 dosing regimens during a 12-week treatment period (Days 1–85): 60 mg weekly (12 doses overall); 120 mg weekly (12 doses overall); or 120 mg bi-weekly (6 doses overall). Treatment withdrawal/stopping criteria are reported in the [supplementary methods](#). Patients were followed until Day 169. An optional extended post-treatment follow-up period was offered with study visits on Days 270, 360 and 450.

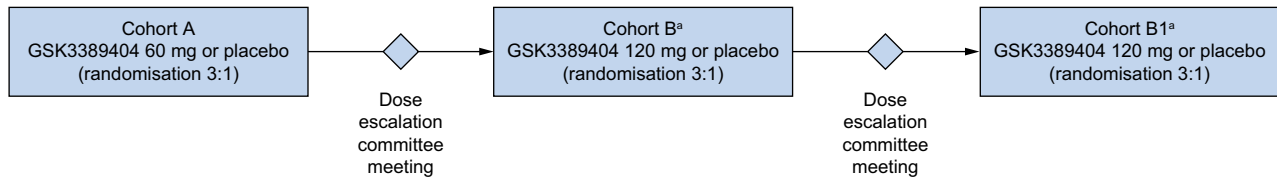
A sentinel group was randomised (1:1) to GSK3389404 or placebo for each dosing regimen. After safety data from the sentinel group were reviewed, the remaining patients were randomised (10:1) to GSK3389404 or placebo. A PK substudy in Japanese patients enrolled an additional cohort to assess GSK3389404 30 mg weekly in addition to the 3 doses assessed in the main study (Fig. 1). Randomisation schedules are detailed in the [supplementary methods](#).

The study protocol, amendments, informed consent, and other information that required pre-approval were reviewed and approved by a national, regional, or investigational center ethics committee or institutional review board (Table S1). The study was conducted in accordance with the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice, applicable country-specific requirements, all applicable patient privacy requirements and the ethical principles outlined in the Declaration of Helsinki. All patients provided informed consent. The full protocol and statistical analysis plan for the study are available online: <https://www.gsk-studyregister.com/en/trial-details/?id=205670>.

Patient population

Full eligibility criteria for both parts of the study (including protocol amendments) are provided in the [supplementary methods](#). Briefly, adults (18–70 years at time of informed consent), with documented HBV infection ≥ 6 months prior to screening were eligible. Both HBeAg-positive and -negative patients were permitted. Patients

Part 1: Single ascending dose



Part 2: Multiple dose, dose ranging

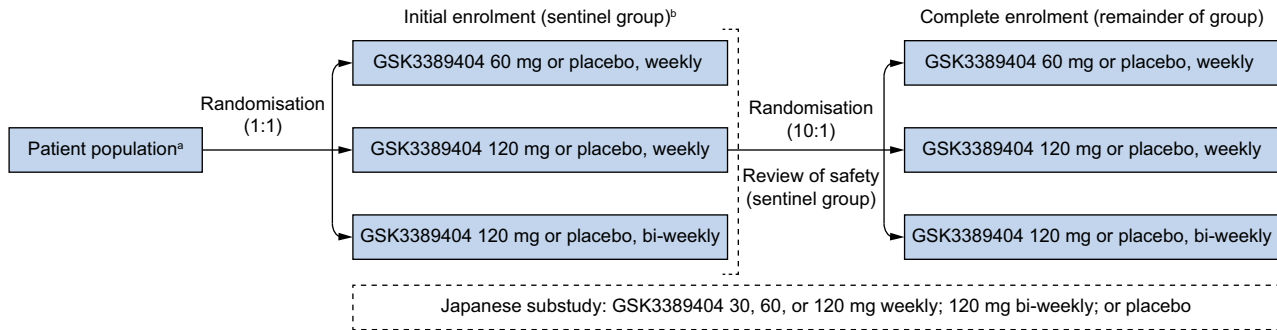


Fig. 1. Study design. ^aHBeAg-positive and HBeAg-negative patients were enrolled. ^bThe sentinel group consisted of one active and one placebo patient per dosing regimen. HBeAg, hepatitis B e antigen.

receiving NAs were required to have HBV DNA concentrations below the lower limit of quantification (20 IU/ml), plasma or serum HBsAg concentration >50 IU/ml, ALT concentration ≤2x upper limit of normal (ULN) for patients on stable NA therapy or ALT <5x ULN for treatment-naïve patients and for patients who were not receiving treatment at the time of eligibility assessment. Patients were excluded from Part 2 if they were diagnosed with, or there was evidence of, cirrhosis within 12 months of screening.

Part 1 of the study included treatment-naïve patients and those receiving active treatment. For Part 2, patients were required to have been receiving stable NA therapy (no changes to the planned NA regimen for ≥6 months prior to screening and no planned changes over the study duration). Other concomitant medications were administered as medically necessary throughout the study. Traditional Chinese medicine and/or acupuncture related to chronic HBV therapy, PEG-IFN or other immunomodulating therapies, immunosuppressive medication (e.g. prednisone) for >2 weeks, and other oligonucleotide or siRNA therapy, were not permitted.

Endpoints

Efficacy, safety and PK of GSK3389404 were coprimary objectives. The primary efficacy endpoint was HBsAg response rate at each dose level (proportion of patients with ≥1.5 log₁₀ IU/ml reduction in HBsAg levels from baseline at any time point), to identify efficacious doses and dosing regimens of GSK3389404. Safety was assessed through adverse event (AE) monitoring, clinical laboratory tests, vital signs, electrocardiogram (ECG) and physical examinations. Derived GSK3389404 plasma PK parameters included area under the concentration-time curve, maximum observed concentration, time of maximum observed concentration, half-life and apparent subcutaneous plasma clearance. Although not predefined endpoints, additional efficacy outcomes were also assessed: change in HBsAg and HBeAg over time, development of antibodies against HBsAg (anti-HBs), and ALT flares (defined as >2x ULN).

Secondary endpoints were the correlation between GSK3389404 PK parameters and pharmacodynamic parameters, as well as PK parameters of bepirovirsen. Exploratory endpoints were

to assess and determine correlations between pharmacodynamic parameters and HBV biomarkers (including HBV DNA, HBV RNA, HBsAg, HBeAg and/or hepatitis B core-related antigen [HBcrAg]). Data for secondary and exploratory endpoints will be reported elsewhere.

Assessments

Patients were assessed at various time points throughout the study including Day 1, 29 (4 weeks of treatment), 57 (8 weeks of treatment), 85 (12 weeks of treatment), 113 (12 weeks of treatment, 4 weeks of follow-up), 169 (12 weeks of treatment, 12 weeks of follow-up) and 270 (12 weeks of treatment, 26 weeks of follow-up). Virology assessments are detailed in the [supplementary methods](#).

AEs were graded for severity according to Division of Acquired Immune Deficiency Syndrome (DAIDS) criteria. AEs were monitored continuously throughout the study. ECG was performed at screening and on Days 1, 29, 57, 85 and 169. Vital signs and injection-site reactions were assessed, and samples collected for laboratory assessments (haematology, chemistry, urinalysis), complement, prothrombin time and activated partial thromboplastin time (aPTT) at screening and each dosing visit, and during post-treatment follow-up until Day 169.

In Part 1 (single dose): PK samples were collected pre-dose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 48 hours after the dose, and on Day 8 and Day 30 after the dose. In Part 2 main study (multiple dose): PK samples were collected pre-dose and at 1, 2 and 3 hours post dose on Days 1, 29 and 57; one PK sample was collected on Day 169. In the Japanese substudy, PK samples were collected pre-dose and at 1, 2, 3, 4, 6, 8, 12 and 24 hours post dose on Day 1, and pre-dose and at 1, 2 and 3 hours post dose on Days 29 and 57; one PK sample was collected on Day 169. Plasma concentrations were measured using the analytical methods previously described.¹²

Sample size and statistical analysis

The actual number of patients to be enrolled was determined by the number of dose levels selected for Part 2 of the study, to allow for

approximately 10 evaluable patients in each of the active treatment arms and approximately 12 patients in the placebo arm. This total sample size was calculated to provide enough power using a Bayesian model-based approach for the primary efficacy analysis, sharing common degrees of freedom across treatment arms. The Japanese substudy planned to enrol 22 patients.

Efficacy analyses were performed in the intent-to-treat population, comprising all randomised patients, based on the allocated treatment. Safety analyses used the safety population, which included all patients who received ≥ 1 dose of study treatment, based on the actual treatment received. Non-compartmental PK analysis was performed in the PK population, comprising all patients in the safety population for whom ≥ 1 evaluable PK sample was obtained and analysed. PK parameters were derived from Day 1 data from the Japanese substudy only.

Data for response rate were analysed using a dose-response model; a Bayesian logistic regression model was used to identify an efficacious dose. A treatment was declared efficacious if the posterior probability that the difference in the response rates between that treatment and placebo was at least 90% positive. Safety and PK parameters were summarised using descriptive statistics.

No interim analyses were planned or performed.

Results

Patient population

This study was conducted between 14 February 2017 and 28 January 2019. Twelve and 66 patients were enrolled in Part 1 and Part 2, respectively. The intent-to-treat and safety populations comprising 66 patients included 25 patients enrolled in the Japanese substudy. Sixty-five of the 66 patients were included in the PK population. Overall, 64 (97%) patients completed the last follow-up visit on Day 169. One patient withdrew due to an AE, and one did not attend the Day 169 visit (Fig. 2).

The 12 patients enrolled in Part 1 had a mean age of 49.3 years, 8 (67%) were male and 10 (83%) were HBeAg-negative. Baseline demographics and clinical characteristics of patients enrolled in Part 2 were generally comparable across treatment arms (Table 1). Consistent with patients in Part 2

receiving stable NA treatment, HBV DNA viral loads were <20 IU/ml or target not detected for all patients at baseline with one exception (22 IU/ml; 60 mg weekly treatment arm, who returned to <20 IU/ml levels after Day 8).

Efficacy: Part 1

No significant change in HBsAg, HBeAg or HBV viral load was observed after a single dose of GSK3389404 (data not shown).

Efficacy: Part 2

Response rate

Three patients (one per active treatment arm in the main study) achieved a ≥ 1.5 log₁₀ IU/ml decrease in HBsAg level. The maximum reductions for these patients were 1.54 log₁₀ reduction at Day 85 (60 mg weekly), 2.72 log₁₀ reduction at Day 86 (120 mg weekly) and 2.37 log₁₀ reduction at Day 93 (120 mg bi-weekly). The Day 1 level of HBsAg of these 3 patients was 100.7, 1,455 and 274.6 IU/ml, respectively. None of these patients experienced an ALT flare ($>2 \times$ ULN).

HBsAg and HBeAg change over time

Reductions in HBsAg levels were dose dependent and observed in both HBeAg-positive and -negative patients (Fig. 3). Mean level of HBsAg declined during treatment and started to return to baseline values approximately 2 weeks after the end of GSK3389404 treatment (Day 85). Mean (SD) HBsAg declines at Day 85 in the placebo, 30 mg weekly, 60 mg weekly, 120 mg weekly and 120 mg bi-weekly arms were 0.02 (0.08), 0.13 (0.07), 0.34 (0.32), 0.75 (0.65) and 0.44 (0.50) log₁₀ IU/ml, respectively (Table S2). No patient achieved HBsAg seroclearance. The observed dose-dependent HBsAg decline was similar regardless of the level of HBsAg at baseline, i.e. for patients with low ($<1,000$ IU/ml) and higher baseline HBsAg ($>1,000$ IU/ml) levels (data not shown).

Analysis of changes in HBeAg was limited by the low number of HBeAg-positive patients (n = 18); however, no overall reductions in HBeAg levels were observed (Fig. S1).

Anti-HBs

Two patients in the 120 mg weekly arm and one in the placebo arm who had HBV surface antibodies below the reference range

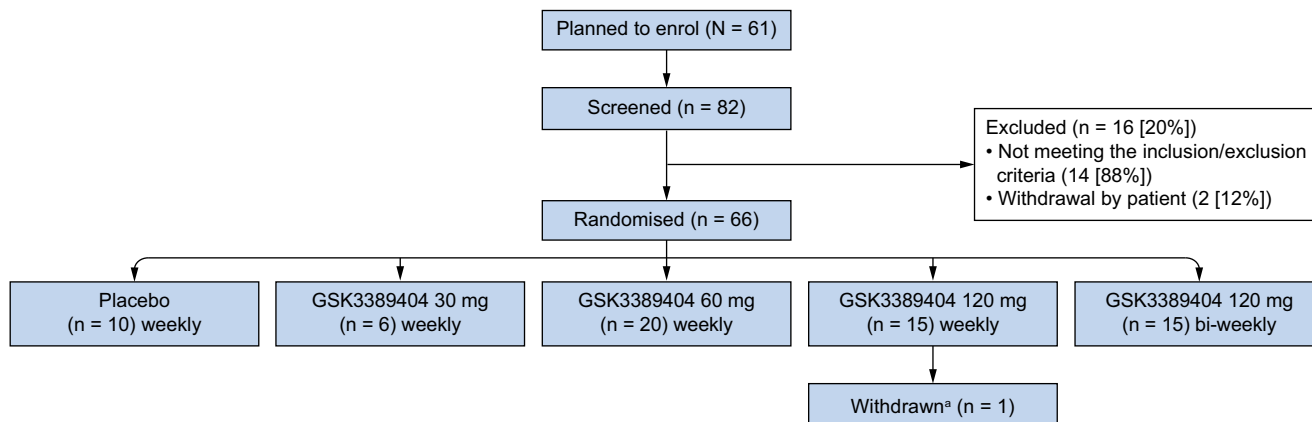


Fig. 2. Patient disposition. Patients enrolled in the Japanese substudy (n = 25) are included in the figure (placebo, n = 4; 30 mg weekly, n = 6; 60 mg weekly, n = 6; 120 mg weekly, n = 3 weekly; 120 mg bi-weekly, n = 3). *Withdrawal due to a non-fatal adverse event of Grade 1 pruritus.

Table 1. Baseline demographics and clinical characteristics (ITT population).

	GSK3389404					Total GSK3389404 (n = 56)	All patients (N = 66)
	Placebo (n = 10)	30 mg weekly (n = 6)	60 mg weekly (n = 20)	120 mg weekly (n = 15)	120 mg bi-weekly (n = 15)		
Age (years), mean (SD)	52.5 (11.2)	52.3 (10.2)	47.4 (10.1)	50.5 (9.1)	47.6 (9.2)	48.8 (9.5)	49.4 (9.8)
Sex, n (%)							
Female	4 (40)	1 (17)	10 (50)	2 (13)	2 (13)	15 (27)	19 (29)
Male	6 (60)	5 (83)	10 (50)	13 (87)	13 (87)	41 (73)	47 (71)
Physical characteristics, mean (SD)							
Body mass index	23.0 (2.1)	22.1 (2.6)	22.8 (2.8)	22.9 (2.1)	23.8 (2.2)	23.0 (2.4)	23.0 (2.4)
Weight (kg)	63.8 (8.5)	65.5 (9.0)	63.6 (12.3)	64.8 (8.9)	67.9 (9.4)	65.3 (10.3)	65.0 (10.0)
Race, n (%)							
East Asian	4 (40)	0	9 (45)	9 (60)	11 (73)	29 (52)	33 (50)
Japanese	4 (40)	6 (100)	7 (35)	5 (33)	3 (20)	21 (38)	25 (38) ^a
Southeast Asian	2 (20)	0	4 (20)	0	1 (7)	5 (9)	7 (11)
Mixed Asian	0	0	0	1 (7)	0	1 (2)	1 (2)
HBeAg status, n (%)							
Positive	3 (30)	1 (17)	8 (40)	2 (13)	4 (27)	15 (27)	18 (27)
Negative	7 (70)	5 (83)	12 (60)	13 (87)	11 (73)	41 (73)	48 (73)
HBsAg (log ₁₀ IU/ml), mean (SD)	3.15 (0.75)	2.73 (0.68)	2.96 (0.54)	2.82 (0.49)	3.00 (0.49)	2.91 (0.52)	2.94 (0.56)
ALT (U/L), mean (SD)	22.9 (12)	24.5 (8)	18.6 (7)	21.1 (10)	19.1 (9)	20.0 (8)	20.4 (9)
Platelet count (10 ⁹ /L), mean (SD)	214 (54)	194 (35)	243 (55)	200 (37)	229 (68)	222 (56)	221 (55)

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; ITT, intent-to-treat.

^aIncludes 22 patients from Japanese substudy and 3 patients from main study.

(<8.5 IU/L) at Day 1 experienced an increase in HBV surface antibody >10 IU/L by Day 85. All other patients had antibodies to surface antigen below the reference range (<8.5 IU/L) at Day 1, Day 85 and Day 169.

HBV RNA and HBcrAg change over time

The majority of patients in the study had HBV RNA below the lower limit of quantitation and consequently no change over time in HBV RNA could be evaluated. Mean (SD) HBcrAg declines at Day 85 in placebo, 30 mg weekly, 60 mg weekly, 120 mg weekly and 120 mg bi-weekly arms were 0.0 (0.23), 0.0 (0.08), 0.2 (0.12), 0.1 (0.17) and 0.2 (0.24) log₁₀ U/ml, respectively.

Safety: Part 1

One patient in the placebo arm reported one AE of nasopharyngitis 30 days after dosing, which resolved prior to the end of the study. One patient in the 120 mg arm reported 7 AEs (increase in C-reactive protein, headache, decrease in lymphocyte count, myalgia, increase in neutrophil count, increase in reticulocyte count and increase in white blood cell count), which occurred 2 or 3 days after dosing and resolved by the end of treatment. All AEs reported during Part 1 of the study were Grade 1 in severity, except for headache, myalgia and nasopharyngitis events, which were Grade 2.

Safety: Part 2

A total of 45 (80%) patients treated with GSK3389404 reported 305 AEs, while 9 (90%) patients in the placebo arm reported 34 AEs (Table 2). Treatment-related AEs occurred in 30 (54%) patients treated with GSK3389404 and in 4 (40%) placebo recipients. One patient in the 120 mg bi-weekly arm withdrew due to Grade 1 pruritus/rash on the neck that was considered related to study treatment. Most AEs were Grade 1 or 2, with no clear relationship to dose; no deaths were reported.

The most frequently reported AEs in patients treated with GSK3389404 were injection site-related events: erythema (18%), injection-site pruritis (13%), injection-site pain (9%) and injection-site reactions (9%) (Table S3); they were generally mild or moderate in severity, self-limiting and resolved without intervention. No trend was observed between injection-site reactions and GSK3389404 dose. Nasopharyngitis (20%), upper respiratory tract infections (13%) and malaise (11%) were frequently reported but were not considered treatment related.

Four patients experienced serious AEs (Table 2). The 3 events in the GSK3389404 arms were renal colic on Day 26 (120 mg bi-weekly arm), Grade 2 Prinzmetal's variant angina on Day 140 (68 days post treatment, 120 mg bi-weekly arm) and putaminal haemorrhage on Day 290 (212 days post treatment, 120 mg weekly arm); all were considered unrelated to study treatment. The patient with the putaminal haemorrhage had a normal platelet count (219 × 10⁹/L at Day 169) and prothrombin international normalised ratio (1.1 on Day 270) prior to the event; however, the patient had consistently high blood pressure at baseline and throughout the study, a known risk factor for such cerebral vascular events. One patient in the placebo arm had a Grade 3 focal hepatic lesion reported on Day 197 and was withdrawn from the optional extended follow-up phase of the study.

Clinical laboratory evaluations of DAIDS Grade ≥3 were reported in 10 (18%) patients treated with GSK3389404 and 3 (30%) patients in the placebo arm (Table S4). Twenty-nine values were categorised as severe (Grade 3) and 2 were categorised as potentially life threatening and were reported as Grade 4 AEs. The 2 Grade 4 laboratory abnormalities observed in the placebo arm were creatine kinase increase and were attributed to physical activity.

AEs of special interest were not formally predefined for this study, but several safety findings of potential interest

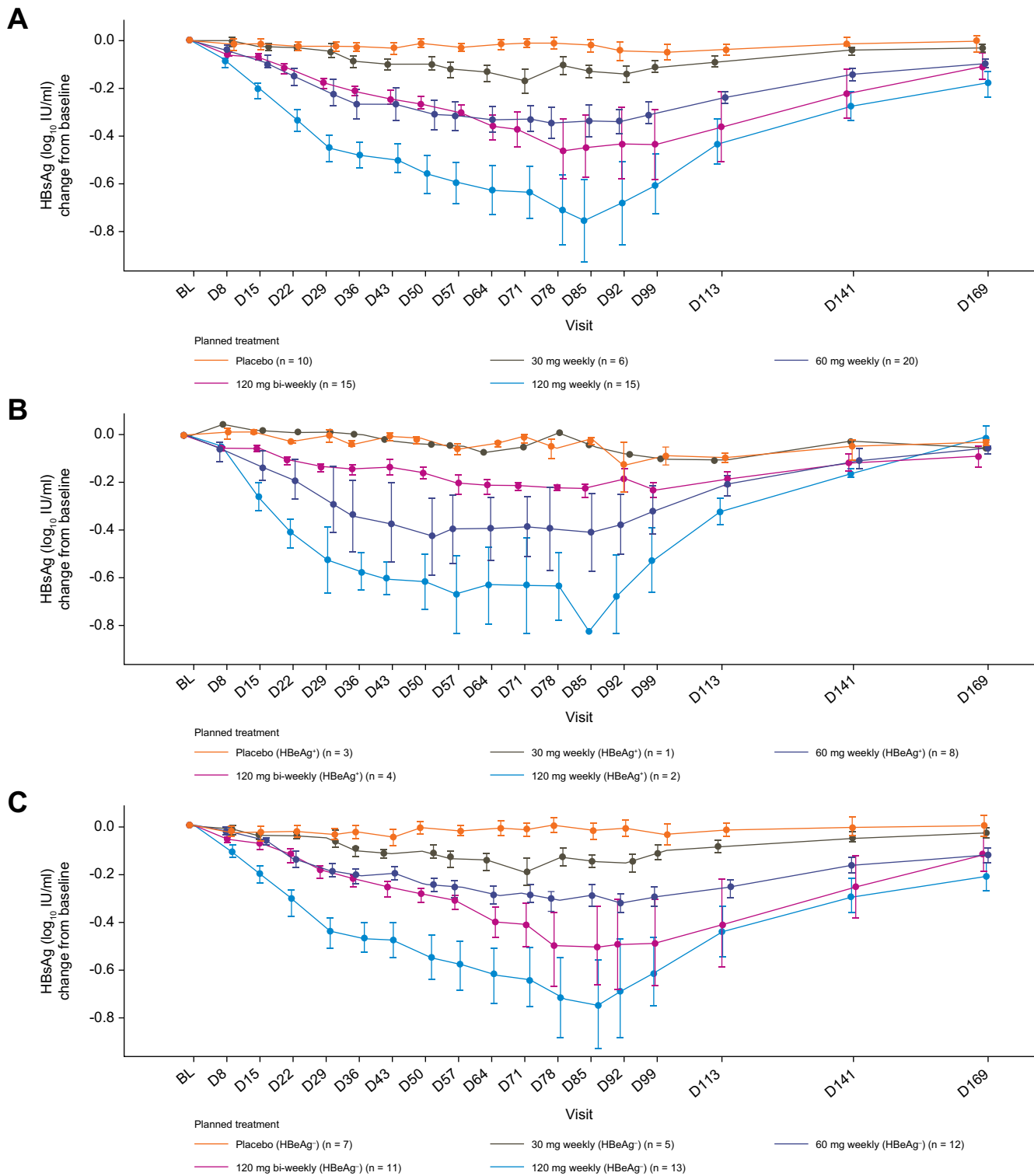


Fig. 3. Mean change from baseline in HBsAg (\log_{10} IU/ml) over time. In (A) the overall population, (B) HBeAg-positive patients and (C) HBeAg-negative patients (ITT population). Error bars represent the standard deviation of the mean. Patients were followed until Day 169. An optional extended post-treatment follow-up period was offered with study visits on Days 270, 360 and 450; HBsAg data to Day 450 are shown in Table S2. BL, baseline; D, day; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; ITT, intent-to-treat.

were noted. These findings are described in detail in the [supplementary safety results](#).

There was no renal safety signal, and no clinically significant ECG findings or vital sign abnormalities were identified.

PK

In the Japanese substudy in Part 2, GSK3389404 plasma concentration peaked at 2–4 hours post dose with a mean plasma half-life of 3–5 hours across dose levels (Table S5). No

Table 2. Overview of AEs (safety population).

AE, n (%)	GSK3389404					Total GSK3389404 (N = 56)
	Placebo (n = 10)	30 mg weekly (n = 6)	60 mg weekly (n = 20)	120 mg weekly (n = 15)	120 mg bi-weekly (n = 15)	
Any AE	9 (90)	4 (67)	18 (90)	12 (80)	11 (73)	45 (80)
Mild (Grade 1)	2 (20)	1 (17)	7 (35)	2 (13)	3 (20)	13 (23)
Moderate (Grade 2)	4 (40)	2 (33)	11 (55)	8 (53)	5 (33)	26 (46)
Severe (Grade 3)	1 (10)	1 (17)	0	2 (13)	3 (20)	6 (11)
Potentially life threatening (Grade 4)	2 (20)	0	0	0	0	0
Treatment-related AEs	4 (40)	3 (50)	11 (55)	8 (53)	8 (53)	30 (54)
Serious AEs	1 (10)	0	0	1 (7)	2 (13)	3 (5)
AEs leading to study withdrawal or treatment discontinuation	0	0	0	0	1 (7) ^a	1 (2)

AE, adverse event.

^aGrade 1 pruritus on the neck.

accumulation of GSK3389404 was observed prior to dosing on Days 1, 29 and 57.

Discussion

This is the first study of GSK3389404 in patients with chronic HBV infection. GSK3389404 demonstrated target engagement, with dose-dependent reductions in plasma HBsAg levels during the 12-week treatment period, followed by subsequent increases in HBsAg levels approximately 2 weeks after treatment completion.

Despite the dose-dependent reductions in HBsAg, only 3/56 patients achieved a $\geq 1.5 \log_{10}$ IU/ml decrease in HBsAg level from baseline during the study. However, as the 3 responders were split across the different dose regimens, the primary objective of identifying an efficacious dose was not achieved. No patient achieved HBsAg seroclearance.

A decrease in HBsAg levels was observed in both HBeAg-positive and -negative patients, with the magnitude of decrease approximately equivalent in the 2 groups, supporting the hypothesis that the GSK3389404 binding site is located away from the integration hot spot.¹³

GSK3389404 was designed to target all HBV RNAs. Interpretation of viral biomarker data (HBeAg, HBcrAg and HBV RNA) is however limited by the low number of patients having detectable levels of these markers at baseline consistent with the predominantly HBeAg-negative, nucleoside suppressed population. Reductions in HBeAg were not observed in HBeAg-positive patients (n = 15 across all GSK3389404 treatment arms). This observation warrants further investigation to understand whether HBeAg-encoding mRNAs are less susceptible to being targeted by GSK3389404, for example, due to different mRNA secondary structure, stability or mRNA location.

To date, 3 ASOs have been tested in patients with chronic HBV infection. Two oligonucleotides, GSK3389404 and RO7062931, are GalNAc-conjugated prodrugs. Although ASOs are distributed to the liver, significant distribution to liver non-parenchymal cells, such as Kupffer and endothelial cells, occurs. Consequently, GalNAc-conjugated oligonucleotides have been developed to improve delivery of the ASO to hepatocytes via the asialoglycoprotein receptor.¹⁴ In other disease areas, GalNAc-conjugated ASOs have demonstrated 10–30-fold improved efficacy over non-GalNAc-conjugated ASOs.¹⁵

Despite the anticipated benefit of the GalNAc conjugation, the findings in this study replicate the rather limited HBsAg reductions observed with RO7062931 after 4 weeks of treatment

(GSK3389404 120 mg weekly mean HBsAg reduction at Day 29 $-0.4512 \log_{10}$ IU/ml, n = 15; RO7062931 3 mg/kg weekly mean HBsAg reduction at nadir $-0.50 \log_{10}$ IU/ml, n = 14).¹⁶ Of note the HBsAg entry criteria was lower in this study compared with the RO7062931 study (50 vs. 1,000 IU/ml). Furthermore, this study has confirmed that by extending treatment to Day 85, a continuous decline in HBsAg is achieved; however, no patient achieved HBsAg seroclearance.

The third ASO, bepirovirsen, has the same sequence as GSK3389404, but is not GalNAc conjugated. Previously, utilising an HBV mouse model, GSK3389404 demonstrated similar efficacy to bepirovirsen at a 5-fold lower dose, consistent with improved hepatocyte delivery.¹¹ A clinical study assessing 4 weeks of bepirovirsen treatment in patients with chronic HBV infection was conducted in parallel to the study reported here, with similar inclusion/exclusion criteria and recruitment from overlapping sites. Surprisingly, bepirovirsen demonstrated more robust dose-related reductions of HBsAg levels than observed with GSK3389404 (bepirovirsen: 300 mg/6 doses/4 weeks, mean HBsAg reduction at Day 29 $-1.56 \log_{10}$ IU/ml n = 12 [naïve] and $-1.99 \log_{10}$ IU/ml n = 5 [NA-treated]).¹⁷ Four of 17 patients at the highest bepirovirsen dose group temporarily achieved HBsAg seroclearance.¹⁷

It is unclear why the GalNAc-conjugated ASOs, in the context of chronic HBV infection, did not deliver the benefit observed with other GalNAc-conjugated ASOs for non-HBV diseases, or the viral antigen reductions observed with GalNAc-conjugated siRNAs targeting HBV.^{18–20} The failure of 2 different GalNAc-conjugated ASOs to deliver substantial reductions in viral antigens does bring into question this approach for the treatment of chronic HBV infection. It has been proposed that the additional benefit observed with the non-conjugated ASO bepirovirsen could simply be due to the higher dose administered (bepirovirsen: 1,800 mg/4 weeks vs. GSK3389404: 480 mg/4 weeks or RO7062931: ~ 800 mg/4 weeks).¹⁶ However, given the anticipated benefit of GalNAc-mediated hepatocyte delivery it is unlikely that this explanation alone accounts for the observed difference in efficacy. It is also unlikely given the GSK3389404 dose-dependent response that saturation of the asialoglycoprotein receptor was the cause of the limited treatment response. This data emphasises the need for improved animal models/cell cultures to enhance our understanding of GalNAc ASOs vs. Non-GalNAc ASOs in terms of efficacy/target engagement.

GSK3389404 demonstrated an acceptable safety profile in patients with chronic HBV infection, with most AEs being

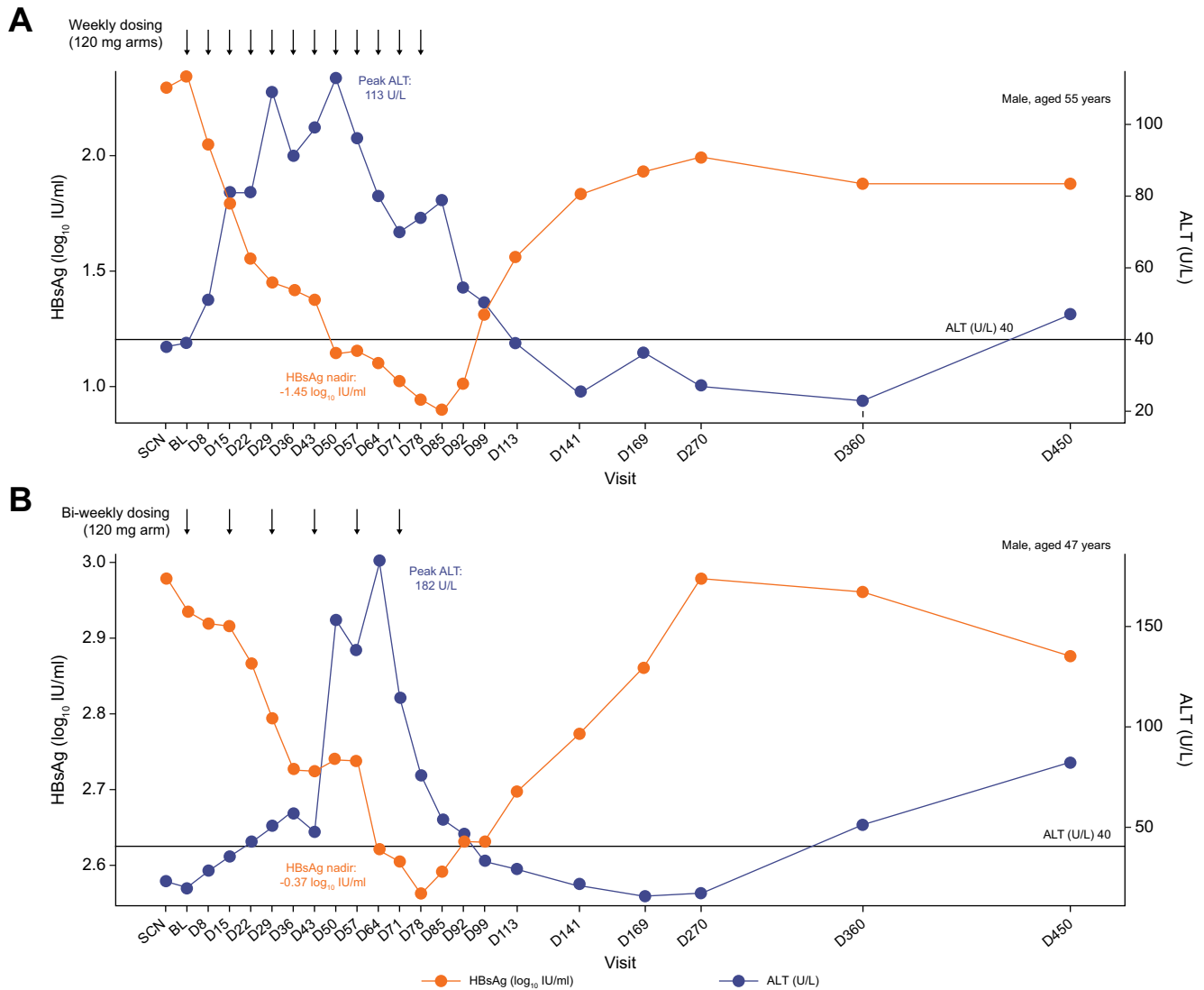


Fig. 4. Individual change from baseline in HBsAg and ALT profile in the 2 patients with ALT flares (>2x ULN). From the (A) GSK3389404 120 mg weekly dose arm and (B) GSK3389404 120 mg bi-weekly dose arm. ALT flares were defined as >2x ULN (ULN was 40 IU/L for males and 33 IU/L for females). ALT, alanine aminotransferase; BL, baseline; D, day; HBsAg, hepatitis B surface antigen; SCN, screening; ULN, upper limit of normal.

mild or moderate in severity, and no unexpected safety signals based on the profile observed in a previous study with healthy volunteers.¹² Injection-site reactions were the most frequent AEs reported; consistent with findings in healthy volunteers, these reactions were generally mild or moderate in severity and self-limiting. Although AEs of special interest were not predefined, several safety findings were of interest, including ALT flare, decreased platelets, aPTT prolongation and complement activation, all of which were observed at a higher incidence with GSK3389404 vs. placebo; however, there were no apparent clinical consequences of these laboratory abnormalities.

Two patients experienced ALT flares (>2x ULN) (Fig. 4). These were probably therapeutic flares given their association with reduction in HBsAg, although the HBsAg reductions did not meet the prespecified definition of responder. The limited study size makes it difficult to exclude the possibility that the ALT increases were caused

directly by the study drug. Therapeutic ALT flares have been shown to correlate with antiviral activity in the blood (HBsAg and HBV DNA reductions).^{21,22} However, none of the 3 HBsAg responders in this study experienced an ALT flare. Outside the setting of disease reactivation or rebound viremia, the aetiology of ALT increase (flares) in patients with chronic HBV infection is uncertain.²³ It has been postulated that ALT flares are evidence of reactivation of the immune system in the liver with accompanying clearance of infected hepatocytes, both during spontaneous HBsAg loss and following therapy.²³ Although we have no good explanations for the absence of ALT flares in these 3 responders, immune-mediated responses may also depend on individuals' immune responsiveness to HBsAg reduction, which involves other individual factors; thus, immune responses may vary in individuals with the same magnitude of HBsAg reduction. The association of ALT levels with intra-hepatic activity in HBV is also uncertain. This variable

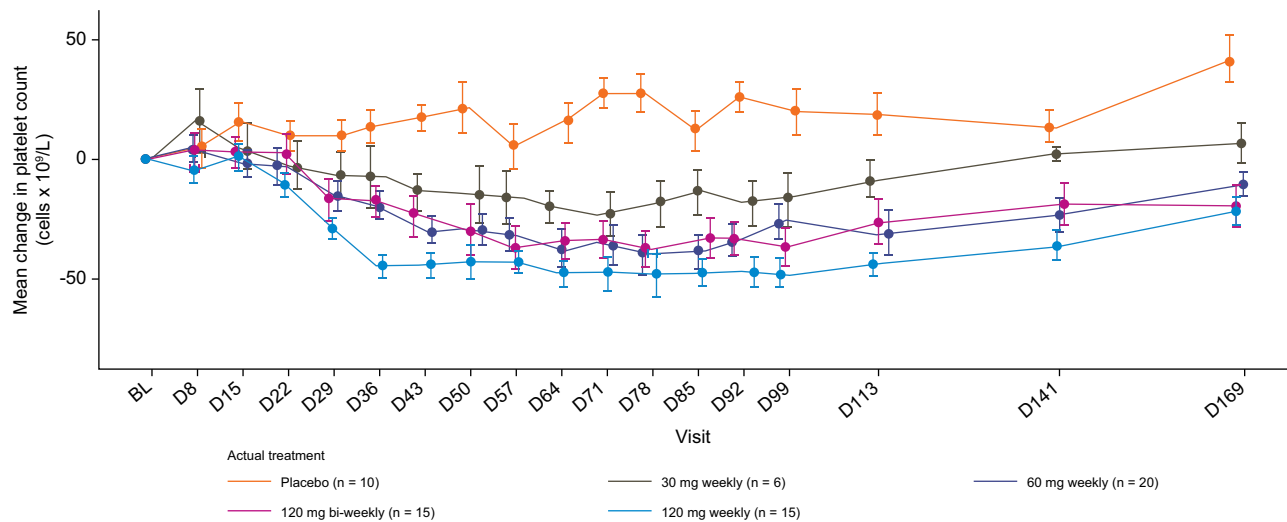


Fig. 5. Mean change from baseline in platelet levels (safety population). Error bars represent standard error of the mean. Patients were followed until Day 169. An optional extended post-treatment follow-up period was offered with study visits on Days 270, 360 and 450; however, laboratory data were only collected from a small number of patients beyond Day 169 (data not shown). BL, baseline; D, day.

immune responsiveness may also underlie the differential anti-HBs findings observed across patients in this study.

Reductions in platelets have been associated with oligonucleotides,²⁴ but they are not established as a class effect of 2'-MOE ASOs.²⁵ In this study, there was an initial dose-dependent decrease in platelet counts that was not considered clinically significant, followed by a recovery of platelet numbers at the end of GSK3389404 treatment (Fig. 5). There were no associated bleeding events or other events of clinical concern. Platelets can be easily monitored in the clinical setting, allowing for dose modification of the ASO in the event of low platelet counts. aPTT prolongation is a well-established clinical class effect associated with higher doses of 2'-MOE ASOs.^{26,27} However, in this study, the reports of aPTT prolongation were Grade 1 and Grade 2, and were not considered clinically significant. Although complement activation was identified as a class effect following studies in monkeys, this has not translated into humans.²⁸ The findings of the current study are consistent with this, as the data from this study were not indicative of complement activation.

GSK3389404 plasma half-life and time to peak plasma concentration were independent of dose, and no plasma accumulation of GSK3389404 was observed following repeat dosing, which is consistent with previous observations in healthy volunteers.¹² Further PK analyses will be published elsewhere.

The limitations of this study include the relatively short duration, despite this being the longest duration of ASO treatment in chronic HBV infection to date, and the narrow dose range, which together may not have been adequate to induce an immune response. Also, the requirement for concomitant NA therapy in Part 2 and the low number of HBeAg-positive patients limit the interpretation of the data to a wider population, such as treatment-naïve patients or those receiving other forms of treatment. Nevertheless, this unexpected finding of greater

efficacy with the unconjugated form compared with the conjugated form has stimulated discussions on the mechanism and PK of this class of agent in the field of hepatitis B. An *in vitro* study in primary human hepatocytes found that GSK3389404 uptake uses a different pathway compared with GSK3228836,¹¹ which may partly underlie the differences in efficacy observed in clinical studies.

In conclusion, GSK3389404 had an acceptable safety profile when administered as repeat doses of 30 mg, 60 mg or 120 mg in patients with chronic HBV infection for 12 weeks. Although dose-dependent reductions in mean HBsAg were observed regardless of HBeAg status, no efficacious dose of GSK3389404 was identified. Given the absence of the benefit that would be expected based on GalNAc conjugation-mediated hepatocyte targeting, development of GSK3389404 has been terminated in favour of development of the GalNAc-unconjugated ASO, GSK3228836.

Abbreviations

AE, adverse event; anti-HB, antibodies against HBsAg; aPTT, activated partial thromboplastin time; ALT, alanine aminotransferase; ASO, antisense oligonucleotide; DAIDS, Division of Acquired Immune Deficiency Syndrome; ECG, electrocardiogram; GalNAc, N-acetyl galactosamine; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LLOQ, lower limit of quantification; MOE, 2'-O-methoxyethyl; NA, nucleos(t)ide analogue; PEG-IFN, pegylated interferon; PK, pharmacokinetics; ULN, upper limit of normal.

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Conflict of interest

MFY has been an advisor for and received consulting fees from AbbVie, Arbutus, Bristol-Myers Squibb (BMS), Dicerna, GSK, Gilead, Janssen, Merck Sharp & Dohme (MSD), Clear B Therapeutics, Springbank and Roche; and received grants from Assembly biosciences, Arrowhead, BMS, Fujirebio, Gilead, MSD, Springbank and Sysmex. JHeo, YS, QX, JJ, YK, JLT, WX, ZD, S-JP, RK, Y-OK and HJY report no conflicts of interest. HK has received teaching fees from MSD, Gilead, AbbVie, Eisai and Dainippon Sumitomo. FS has received teaching fees from AbbVie and Gilead. JLT has received consulting fees from Aligos, Assembly, Gilead Sciences, Johnson & Johnson, Roche; lecturer fees from Gilead, Johnson & Johnson, Roche and grants from BMS. KC has received grants from AbbVie and Dainippon Sumitomo; and has received teaching fees from MSD, BMS and Gilead. MI received grants and teaching fees from BMS. S-GL has been an advisor for and received grants from Abbott Diagnostics, Gilead, Roche and MSD; and has been an advisor for Kaleido Bioscience, Arbutus, Assembly Grifols, Janssen, Fibronostics and GSK. YTanaka received grants from Gilead, Janssen and Chugai; and teaching fees from Gilead and Fujirebio, Inc. J-HY received grants from GSK, Dicerna Pharmaceuticals, Roche, AstraZeneca, Daewoong and Hanmi. MK was an advisor for Gilead and GSK; and received speaking fees from Gilead, AbbVie, MSD, Eisai, Chugai and Bayer. MEL has been an advisor for and received speaking fees for Abbott Diagnostics, Hi-Eisai, Menarini, Mylan and Roche. Y Tao, JC, RE, MD, SB-B, KH, FMC, MP and DT are employees of GSK and hold GSK stocks/options.

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

Authors' contributions

MFY, HK, FS, YS, JJ, WX, S-GL, YTanaka, MI, KC, J-HY, J-LHou, JLT, MEL, DT, RE, KH, SB-B, MD and JC were involved with the conception/design of the study. MFY, HK, FS, YS, JJ, WX, S-GL, YTanaka, MI, KC, J-HY, J-LH, JT, EL, JHeo, QX, YK, MK, S-JP, ZD, RK, Y-OK and HJY were involved with data acquisition. MFY, DT, RE, KH, MD, JC, MP, FMC and Y Tao were involved with data analysis/interpretation.

Data availability statement

Anonymised individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

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Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2022.05.031>.

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