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ORIGINAL ARTICLE

Safety, pharmacokinetics, and antiviral activity of the capsid inhibitor AB-506 from Phase 1 studies in healthy subjects and those with hepatitis B

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Abstract

AB-506 is a potent, pan-genotypic small molecule capsid inhibitor that inhibits hepatitis B virus (HBV) pregenomic RNA encapsidation. We assessed the safety, pharmacokinetics, and antiviral activity of AB-506 in two randomized, double-blinded Phase 1 studies in healthy subjects (HS) and subjects with chronic HBV infection (CHB). Single ascending and multiple doses of AB-506 or placebo (30–1000 mg or 400 mg daily for 10 days) were assessed in HS. AB-506 or placebo was assessed at either 160 mg or 400 mg daily for 28 days in subjects with CHB. A second follow-up study examined AB-506 or placebo

Amy C. H. Lee and Bhavna Paratala were employed by Arbutus Biopharma at the time of the study.

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at 400 mg daily for 28 days in 14 Caucasian and 14 East-Asian HS. Twenty-eight days of AB-506 at 160 mg and 400 mg produced mean HBV-DNA declines from baseline of 2.1 $\log_{10}$ IU/ml and 2.8 $\log_{10}$ IU/ml, respectively. Four subjects with CHB (all Asian) had Grade 4 alanine aminotransferase (ALT) elevations (2 at each dose) as HBV DNA was declining; three events led to treatment discontinuation. In the second follow-up study, 2 Asian HS had serious transaminitis events leading to treatment and study termination. No subjects had bilirubin elevations or signs of hepatic decompensation. *Conclusion*: AB-506 demonstrated mean HBV-DNA declines of >2 $\log_{10}$; however, transient but severe ALT flares were observed in 4 Asian subjects with CHB. In the follow-up study in HS, 2 additional Asian HS had Grade 4 flares, suggesting that AB-506 hepatotoxicity contributed to the ALT elevations. The AB-506 development program was terminated because of these findings.

INTRODUCTION

Hepatitis B virus (HBV) remains the leading cause of cirrhosis and cirrhosis-related deaths globally, and the second most common cause of liver cancer, despite the availability of an effective preventative vaccine.[1,2] An estimated 257 million people worldwide are living with chronic hepatitis B infection (CHB).[3] Of these, up to 25% will develop primary liver cancer or cirrhosis.[4]

There are two currently approved therapeutic strategies for those with CHB: (1) therapies of finite duration using the immunomodulator pegylated interferon-α (PEG-IFNα), and (2) long-term suppressive treatment with nucleos(t)ide analogs (NAs).[5,6] PEG-IFNα is often poorly tolerated, and responses can be heterogenous; and while NAs are effective suppressors of viral replication (as measured by HBV DNA), in most subjects they do not lead to functional cure.

HBV capsid assembly is an essential step in the HBV life cycle and is a validated target for the discovery and development of anti-HBV agents.[7] Capsid assembly involves the recruitment of core protein dimers that associate with the priming complex of the viral polymerase and pregenomic RNA (pgRNA) template, followed by synthesis of the HBV genomic relaxed circular DNA (rcDNA) through reverse transcription of the pgRNA, leading to a fully encapsidated and enveloped infectious virion secreted into the bloodstream.[8,9] In addition to its role in forming the viral capsid structure, the core protein has been shown to interact with covalently closed circular DNA (cccDNA) and regulate viral transcription.[10] Small molecules that disrupt the encapsidation of pgRNA, also known as capsid inhibitors (CIs), are in clinical trials and have been associated with notable reductions of HBV DNA and HBV RNA in patients with CHB.[11–16]

AB-506 is an orally bioavailable small-molecule HBV CI. AB-506 blocks HBV-pgRNA encapsidation by accelerating assembly of intact but empty capsids, reduces rcDNA production, and prevents the formation of new cccDNA in HBV cell culture models including HBV-infected primary human hepatocytes, presumably via inhibition of the capsid uncoating step. In a hydrodynamic injection mouse model of HBV infection, oral administration of AB-506 resulted in a profound, dose-dependent antiviral effect with up to 3.0 $\log_{10}$ reductions in serum HBV DNA after 7 days of treatment. AB-506 has activity against all HBV genotypes, and its antiviral potency is maintained against nucleos(t)ide-resistant variants in cell culture systems. Nonclinical toxicology studies supported the initiation of clinical trials in human subjects.

Here we report the safety, pharmacokinetics (PK), and antiviral activity of AB-506 in two Phase 1 studies: a Phase 1a/1b single and multiple dose (MD) study in HS and subjects with CHB, and a follow-up Phase 1 28-day MD safety study in Asian and Caucasian HS.

EXPERIMENTAL PROCEDURES

Study AB-506-001 was a Phase 1 trial in HS and subjects with CHB conducted in three parts (Figure S1A). Study AB-506-003 was a follow–up Phase 1 trial conducted in HS (Figure S1B). These studies received ethics committee/institutional review board approval for each country/site. All subjects gave written informed consent before any screening procedures in accordance with Good Clinical Practice and the Declarations of Helsinki and Istanbul. All authors had access to the study data and reviewed and approved the final manuscript.

Study design

AB-506-001

Part 1 was a randomized, placebo-controlled single-ascending dose assessment of AB-506 safety, tolerability, and PK in HS. Two cohorts of 8 HS each were randomized 6:2 to receive alternating single doses of AB-506 (30, 100, 300, 500, 800, and 1000 mg) or placebo after an overnight fast, with a washout period of at least 14 days between each dose level. AB-506 was administered orally as 15 mg or 100 mg tablets with matching placebo. Subjects remained in the clinic for 3 days after dosing and were then furloughed to complete the remaining visits as outpatients. Dose escalation was performed after review of accumulated safety and PK data from prior panel(s) by the Safety Review Committee (SRC). A food-effect panel to assess the impact of a high-fat meal on AB-506 PK was included at the 500-mg dose level.

Part 2 was a randomized, placebo-controlled MD assessment of the safety, tolerability, and PK of AB-506 400 mg once daily (QD) or placebo for 10 days in 12 HS (randomized 10:2) after an overnight fast. Subjects remained in the clinic for the entire dosing period plus 3 days after dosing and were then furloughed to complete the remaining visits as outpatients. Safety and PK data from Part 2 and any available data from Part 1 were reviewed by the SRC before beginning Part 3.

Part 3 was a randomized MD assessment of the safety, tolerability, PK, and antiviral activity of AB-506 or placebo administered once daily for 28 days in subjects with CHB. Two cohorts of 12 subjects with CHB were randomized 10:2 to receive AB-506 400 mg QD or placebo after an overnight fast (Cohort D), or AB-506 160 mg QD or placebo without regard to food (Cohort E) based on the result of the food-effect panel in Part 1. Cohort D was initiated first, and Cohort E was opened after review of safety, PK, and antiviral data through at least Day 14 from Cohort D.

AB-506-003

This study was a randomized MD assessment of AB-506 400 mg QD or placebo in HS for 28 days. Twenty-eight subjects were enrolled in two cohorts of 14 subjects each: One cohort consisted of Caucasian subjects and the other consisted of East-Asian subjects. Subjects were randomized within each cohort to receive AB-506 or placebo without regard to food and were dosed in groups of 7 staggered by at least 3 days and alternating by cohort. Subjects remained in the clinic for 24 h after the first dose and were then furloughed to complete the remaining visits as outpatients.

Study populations

Healthy subjects (Studies AB-506-001 and AB-506-003)

Eligible subjects were ages 18–45 years, male or female, with body mass index (BMI) between 18 kg/m^2 and 32 kg/m^2 and free from any concomitant medical conditions or clinically relevant laboratory, electrocardiogram (ECG), physical exam, or vital sign abnormalities. Subjects had negative serological tests for human immunodeficiency virus (HIV), hepatitis C virus, and HBV. For study AB-506-003 only, subjects were either White/Caucasian or East Asian (specifically Chinese, Taiwanese, or Korean), and the BMI upper limit was 28 kg/m^2. The remainder of the protocol inclusion and exclusion requirements were similar between the two studies.

Subjects with CHB (Study AB-506-001)

Eligible subjects were ages 18–65, male or female, with BMI between 18 and 38 kg/m^2. HBV DNA at screening was ≥2,000 IU/ml for hepatitis B e antigen (HBeAg)–negative subjects and ≥20,000 IU/ml at screening for HBeAg-positive subjects. All subjects had hepatitis B surface antigen (HBsAg) ≥ 250 IU/ml at screening, and HBV genotype A, B, C, or D. Subjects were either treatment-naïve or treatment-experienced, but off treatment for at least 6 months before screening. Additionally, subjects had liver imaging with no clinically significant abnormalities and either a FibroScan or liver biopsy demonstrating no cirrhosis or advanced fibrosis. Concomitant infection with other hepatitis viruses or HIV was exclusionary, as was a screening alanine aminotransferase (ALT) or aspartate aminotransferase (AST) value > 5 times the upper limit of normal (ULN). ALT ULN thresholds were based on the 2016 American Association for the Study of Liver Diseases Guidelines for Treatment of Chronic Hepatitis B recommendations of 19 U/L for females and 30 U/L for males.[17]

Study procedures

Subjects were evaluated at each study visit by clinical assessment of adverse events (AEs), targeted physical examinations, vital signs, ECG, and clinical laboratory testing (chemistry, liver function tests, complete blood counts, coagulation parameters, and urinalyses). Clinical AEs and laboratory test abnormalities were graded according to the National Institutes of Health Division of Allergy and Infectious Diseases Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Corrected Version 2.1 and

were coded to a MedDRA preferred term and system organ classification.

Pharmacokinetic, virologic, and immunologic (serum cytokines and peripheral blood mononuclear cell [PBMC]) assessments were collected, and sequence analysis of the coding region for HBV core protein was performed according to study protocols (Supporting Methods). AB-506 was quantified in human plasma using a validated liquid chromatography–tandem mass spectrometry assay (QPS). For serial PK sampling days, standard noncompartmental PK parameters were derived using a validated program (Phoenix WinNonlin; Certara). HBsAg, hepatitis B surface antibody (HBsAb), HBeAg and hepatitis B e antibody were analyzed using the Diasorin Liaison XL platform; HBV DNA was analyzed using the Roche Cobas HBV Quantitative nucleic acid test (Cobas 4800 System); hepatitis B core-related antigen (HBcrAg) was analyzed using the Fujirebio Lumipulse assay; and HBV RNA was analyzed using an investigational rapid amplification of complementary DNA end-based real-time polymerase chain reaction (PCR) assay (modified from van Bommel[18]).

No hypotheses were formally tested in these Phase 1 studies, and no formal power analysis was performed. The sample sizes for these studies were based on clinical rather than statistical rationale.

The study sponsor designed the study, analyzed the data, supervised the clinical research organizations that managed the execution of the study, and in collaboration with the authors interpreted the data, wrote and reviewed the manuscript, and submitted the approved manuscript for publication.

RESULTS

Subject disposition and demographics

Dispositions of the study populations for AB-506-001 and AB-506-003 are shown in Figure S2. Subject baseline characteristics are presented in Table 1. Baseline characteristics in study AB-506-003 were similar to AB-506-001 Parts 1 and 2, with the exception that 50% of the subjects were of East-Asian descent per protocol.

Antiviral activity

The mean changes from baseline in HBV DNA and HBV RNA for subjects in Study AB-506-001 Part 3 are found in Table 2 and Figure 1. HBV-DNA mean declines at Day 28/end of treatment (EOT) were dose-dependent, with the 160-mg dose group demonstrating a 2.1 $\log_{10}$ IU/ml decline from baseline and the 400-mg dose group (excluding 2 subjects who discontinued early due to ALT elevations) demonstrating a 2.8 $\log_{10}$ IU/ml decline. The responses were similar between HBeAg-positive and HBeAg-negative subjects. One subject at the 160-mg dose did not respond to AB-506 treatment due to presence of an I105T variant at baseline. HBV DNA rebounded toward pretreatment values in all subjects after cessation of AB-506 treatment, except in 1 subject who started tenofovir alefenamide after early discontinuation of AB-506. In subjects with detectable HBV RNA at baseline (mostly HBeAg-positive subjects), all experienced at least a 2.0-$\log_{10}$ copies/ml decline or reached levels < lower limit of quantitation (LLOQ) by Day 28/EOT.

One subject in the 400-mg cohort had a significant HBsAg decline in the context of a Grade 4 transaminase flare beginning on Study Day 21, which resulted in the discontinuation of AB-506 on Study Day 24 (see "ALT analysis" section). This subject did not have a notable change from baseline in HBsAg at the time of maximal ALT elevation, but HBsAg began to decline during flare resolution and achieved a maximal 2.2 $\log_{10}$ IU/ml HBsAg decline at the Day 302 follow-up visit. HBsAg showed no notable changes from baseline in the other subjects with CHB at either AB-506 dose level. HBsAb remained negative in all subjects except the 1 subject with HBsAg decline of 2.2 $\log_{10}$ IU/ml, in whom the HBsAb titer rose from < LLOQ to 3.88 IU/ml at Day 302 of follow-up (last available timepoint). There were no meaningful changes observed in HBcrAg or HBeAg.

HBV core protein variants

To assess for pre-existing HBV core protein variants that may affect response to AB-506, samples were analyzed for HBV core protein coding sequence for subjects in Part 3 (Table S1). In cell culture, T33N, T33S, I105T, and T109S as single-point mutations resulted in 2.6-fold to 369-fold change in EC_{50} of AB-506. Sequence analysis of HBV sequences in the blood of subjects with CHB at baseline revealed the preexistence of multiple CI-relevant HBV core variants at frequencies that were 10–100 times greater than the HBVdb-reported prevalence (hbvdb.lyon.inserm.fr)[19] and in one case co-existing in the same subject. One subject treated with AB-506 (160 mg/day) had no HBV-DNA response (NR) to treatment despite adequate drug exposure; baseline sequence analysis revealed this subject had a predominant I105T HBV core protein variant that as a single-point mutation is 20-fold less sensitive to AB-506 in cell culture. In addition to the I105T NR, the 1 active subject with T109S (2.8-fold less sensitive to AB-506 *in vitro*) had the weakest response (1.3 $\log_{10}$ IU/ml HBV-DNA decline) other than the NR. Subjects with T33N and T33S were screen failures for other reasons. Pre-existing and emergent core protein variants associated with reduced susceptibility

TABLE 1 Demographics and baseline characteristics

Baseline measure	AB-506-001 HS[a]				AB-506-001 CHB			AB-506-003 HS		
	Cohort A single doses (n = 11)	Cohort B single doses (n = 10)	Cohort C multiple dose (n = 12)	Overall (n = 33)	Cohort D 400 mg QD (n = 10)	Cohort E 160 mg QD (n = 10)	Pooled placebo (n = 4)	Caucasian (n = 10)	East Asian (n = 10)	Pooled placebo (n = 8)
Age, years (mean [SD])	26.2 (6.7)	27.5 (6.5)	24.8 (4.3)	26.1 (5.8)	41.7 (9.5)	41.3 (12.4)	40.8 (9.3)	25.9 (5.5)	30.0 (7.8)	24.1 (4.6)
BMI, kg/m^2 (mean [SD])	25.2 (2.2)	26.4 (3.4)	24.1 (2.4)	25.2 (2.8)	23.4 (3.5)	25.5 (5.6)	25.8 (2.4)	22.3 (1.7)	23.6 (2.7)	21.5 (1.8)
Male gender (n [%])	11 (100)	10 (100)	12 (100)	33 (100)	5 (50)	5 (50)	0	7 (70)	7 (70)	3 (37.5)
Race (n)										
Asian	0	2	1	3	8	5	2	0	10	4
White	7	4	7	18	1	5	2	10	0	4
Pacific Islander	0	2	0	2	1	0	0	0	0	0
Other	4	2	4	10	0	0	0	0	0	0
Baseline ALT (mean [SD])	18.5 (4.1)	27.5 (9.3)	19.1 (8.6)	21.5 (8.5)	40.45 (22.1)	29.58 (17.1)	26.80 (11.1)	14.5 (6.1)	15.3 (5.6)	14.0 (6.7)
HBV genotype										
A					0	0	0			
B					2	0	0			
C					7	5	2			
D					1	5	2			
IL28B genotype[b]					C/C 9	C/C 8	C/C 2			
					C/T 1	C/T 1	C/T 1			
					T/T 0	T/T 1	T/T 1			
HBeAg positive (n)					3	7	2			
Mean HBV DNA, Log_{10} IU/ml (SD)					6.99 (2.11)	5.20 (1.43)	5.40 (2.18)			
Mean HBV RNA, Log_{10} IU/ml (SD)					5.90 (2.12)	4.68 (1.29)[c]	5.37 (1.99)[d]			
Mean HBsAg, Log_{10} IU/ml (SD)					4.23 (0.66)	3.62 (0.56)	3.52 (0.60)			
Prior HBV treatment (n)					2[e]	1[f]	0			

Abbreviation: BMI, body mass index.

[a]Placebos included due to study design features.

[b]rs12979860.

[c]Three subjects target not detected (TND).

[d]Two subjects TND.

[e]One subject treated with 7.5 months lamivudine and 3.5 months TDF (most recent in 2017) and 1 subject treated with 48 weeks of Pegasys in 2010.

[f]One subject treated with 2 weeks of lamivudine in 2009.

TABLE 2 AB-506-001 antiviral activity

Parameter	Cohort D 400 mg QD[a]			Cohort E 160 mg QD			Pooled PBO
HBeAg status	**HBeAg+ (n = 7)**	**HBeAg-(n = 3)**	**All (n = 10)**	**HBeAg+ (n = 3)**	**HBeAg-(n = 7)**	**All (n = 10)**	**All (n = 4)**
HBV DNA							
Mean (SD) change from baseline (Log_{10} IU/ml)	−2.9 (0.58)	−2.5[b] (0.23)	−2.8 (0.57)	−2.2 (0.39)	−2.0 (1.1)	−2.1 (0.91)	−0.045 (0.16)
Subjects < LLOQ at Day 28/EOT	0	1	1	0	0	0	0
HBV RNA							
Subjects < LLOQ at baseline	0	1	1	1	5	6	0
Mean (SD) change from baseline (Log_{10} IU/ml)	−2.4 (0.50)	All[c] < LLOQ	−2.4 (0.50)	−2.5[d] (0.54)	−2.22[e]	−2.37 (0.40)	0.066 (0.19)
Subjects < LLOQ at Day 28/EOT	0	3	3	2	6	8	0
HBsAg							
Mean (SD) change from baseline (Log_{10} IU/ml)	0.116 (0.208)	0.107 (0.001)	0.113 (0.176)	−0.0213 (0.029)	−0.0214 (0.082)	−0.0213 (0.069)	0.006 (0.07)
C_{trough}							
Mean (SD) μg/ml	3.27 (1.41)			1.16 (0.69)			N/A

Abbreviations: EOT, end of treatment; LLOQ, lower limit of quantitation: PBO, placebo.

[a]Two subjects discontinued for ALT elevations were excluded.

[b]One subject < LLOQ.

[c]One subject < LLOQ at baseline.

[d]N = 2 (1 < LLOQ by Day 28).

[e]N = 1 (5 < LLOQ at baseline; 1 < LLOQ by Day 28).

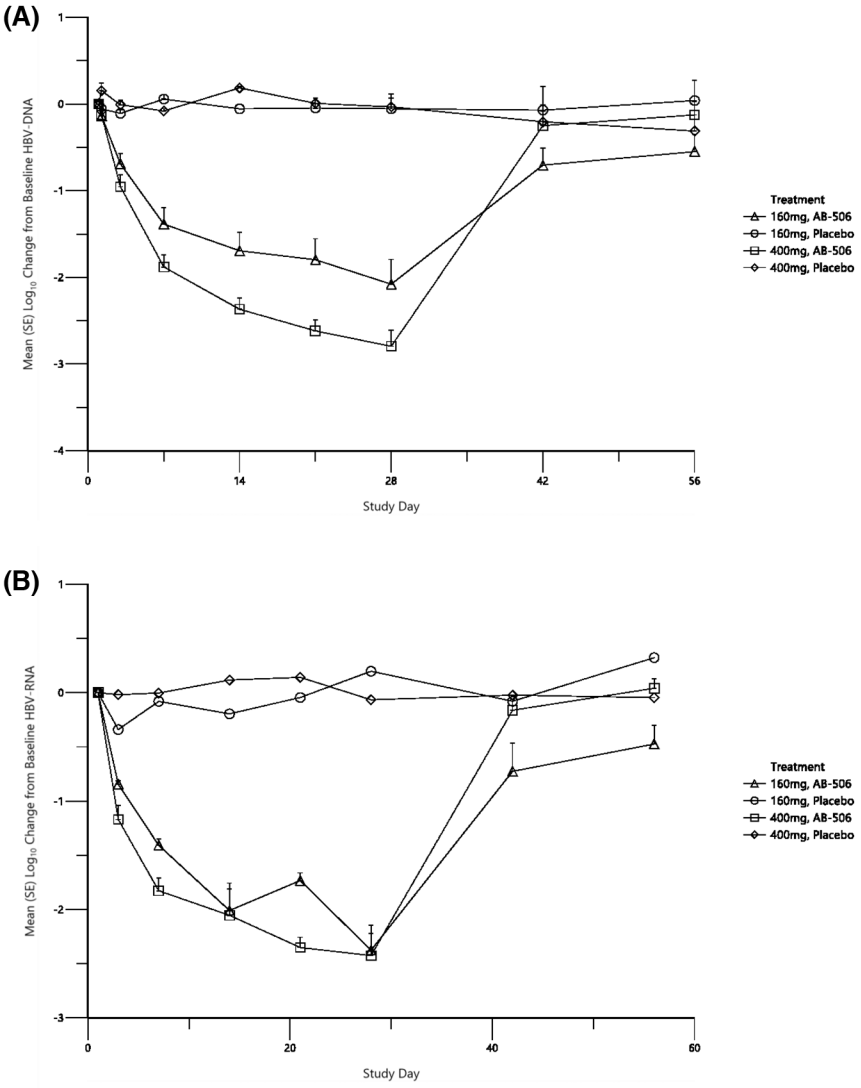


FIGURE 1 On-treatment mean (± SEM) changes from baseline in serum hepatitis B virus (HBV) DNA (A) and serum HBV RNA (B)

have been noted in other CI clinical programs, including T109M[13] and F23Y, T33N, I105T, and Y118F.[20–22]

Pharmacokinetics

PK parameters by dose level and treatment are presented in Tables S2–S5.

Safety

AB-506-001

In HS in AB-506-001 Parts 1 and 2, there were no deaths, serious or Grade 3 or 4 adverse events, or safety-related discontinuations. Among subjects who received active treatment with AB-506 across all single-dose levels, there were 30 treatment-emergent adverse events (TEAEs), of which six (20%) were assessed as related to AB-506. All but one TEAE were Grade 1/mild, with one Grade 2/moderate TEAE of right foot sprain assessed as unrelated. The most frequent TEAE observed was headache (n = 3 events, 10%), and no other TEAE occurred more than once. No dose-related trends in AE frequency or severity were observed (Table S6). No clinically significant abnormalities in laboratory tests, including liver function tests, ECGs or vital signs, were noted. Among subjects in Part 2 who received active treatment with 400 mg AB-506 for 10 days, there were seven TEAEs, none of which were assessed as related to AB-506 and all of which were Grade 1/mild (Table S6).

In subjects with CHB in Part 3, there were no deaths or serious adverse events (SAEs) observed at either dose level. Three subjects discontinued AB-506 due to

TABLE 3 AB-506-001 CHB and AB-506-003 HS safety

AB-506-001 CHB MD				AB-506-003 Healthy Subject MD			
Parameter	**Cohort D 400 mg (n = 10)**	**Cohort E 160 mg (n = 10)**	**Pooled PBO (n = 4)**	**Parameter**	**Cohort A 400 mg (Caucasian) n = 10**	**Cohort B 400 mg (Asian) n = 10**	**Pooled PBO n = 8**
Subjects with ≥1 TEAE, n (%)	7 (70)	8 (80)	3 (75)	Subjects with ≥ 1 TEAE, n (%)	10 (100)	6 (60)	6 (75)
Subjects with ≥1 related TEAE, n (%)	3 (30)	8 (80)	1 (25)	Subjects with ≥1 related TEAE, n (%)	4 (40)	4 (40)	1 (12.5)
SAEs, n (%)	0	0	0	SAEs, n (%)	0	2 (20)	0
Discontinuation due to AE, n (%)	2 (20)	1 (10)	0	Discontinuation due to AE, n (%)	0	3 (30)	0
TEAEs occurring in ≥2 AB-506-treated subjects (any dose)							
Nausea	0	2	0	Abdominal pain	3	2	0
Fatigue	1	1	0	Vomiting	0	2	0
Headache	1	3	0	Dizziness	2	1	1
Rash	0	2	0	Headache	3	1	0
ALT increased	2	3	0	Lethargy	2	1	0
AST increased	2	2	0	Cough	0	2	0
				Arthralgia	1	1	0
				Pyrexia	0	2	1
				Upper respiratory tract infection	1	1	0
				Appetite decreased	0	2	0
Grade 3 or 4 TEAEs							
ALT increased				ALT increased			
Grade 4	2	2	0	Grade 3	1	0	0
				Grade 4	0	0	0
AST increased				Transaminases increased			
Grade 3	0	1	0	Grade 4	0	1	0
Grade 4	2	1	0	Hepatitis acute			
				Grade 4	0	1	0
Grade 3 or 4 laboratory abnormalities							
ALT				ALT			
Grade 3	0	0	0	Grade 3	1[a]	0	0
Grade 4	2	2	0	Grade 4	0	2	0
AST				AST			
Grade 3	0	1	0	Grade 3	0	0	0
Grade 4	2	2	0	Grade 4	0	2	0

TABLE 3 (Continued)

AB-506-001 CHB MD				AB-506-003 Healthy Subject MD			
Parameter	Cohort D 400 mg (n = 10)	Cohort E 160 mg (n = 10)	Pooled PBO (n = 4)	Parameter	Cohort A 400 mg (Caucasian) n = 10	Cohort B 400 mg (Asian) n = 10	Pooled PBO n = 8
Cholesterol				Creatine kinase			
Grade 3	1	0	0	Grade 4	0	0	1
Triglycerides				Phosphate			
Grade 3	1	0	0	Grade 3	0	1	0

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MD, multiple dose; PBO, placebo; SAE, severe adverse event; TEAE, treatment-emergent adverse event (by preferred term).
[a]Occurred during posttreatment follow-up period.

TEAEs across both dose levels. In Cohort D (400 mg), 2 subjects experienced Grade 4 transaminase elevations that met study-stopping rules on Days 24 and 25, both fully resolved (see "ALT analysis" section). In Cohort E (160 mg), 1 subject self-discontinued AB-506 on Day 27 due to a transient Grade 1/mild skin rash. TEAEs were observed in 70%–80% of subjects in each dose level, and most were Grade 1 except for transient Grade 2–4 ALT and/or AST elevations in 5 subjects across both cohorts (n = 2 at 400 mg, n = 3 at 160 mg), and one Grade 2 AE of fatigue (Table 3). No other clinically significant abnormalities in laboratory tests, ECGs, or vital signs were noted.

AB-506-003

There were no deaths in HS in AB-506-003, but two SAEs (transaminase elevations; see "ALT analysis" section) were reported in Asian subjects that led to subject discontinuation and early termination of study drug dosing in all subjects (both SAEs fully resolved). One additional subject discontinued due to a TEAE on Day 10 (Grade 2 rash). A total of 53 TEAEs were reported by 16 subjects who received AB-506 across both cohorts. Fourteen of these TEAEs were assessed as related to AB-506, and all but six events were Grade 1 (Grade 2 rash, pyrexia, abdominal pain; Grade 3 and Grade 4 transaminase elevations; Table 3). Apart from the 2 subjects with SAEs, there were no other clinically significant abnormalities in laboratory tests, ECGs, or vital signs noted.

ALT analysis

The mean baseline ALT levels for HS were within normal limits (Table 1). Among the subjects with CHB in Part 3 of AB-506-001, the mean baseline (±SD) ALT levels (U/L) for the actively treated subjects at the 160-mg and 400-mg dose levels were 29.58 (±17.1, range 11.2–58.9) and 40.45 (±22.1, range 14.2–78.3), respectively. One subject in the 160-mg dose cohort had an asymptomatic ALT elevation at screening (Day −28) of 220 U/L, which was repeated on Day −15 (97.6 U/L) and at dosing Day 1 was 58.9 U/L.

Across both Phase 1 studies, 6 of the 79 total subjects who received at least one dose of AB-506 had treatment-emergent Grade 4 transaminase elevations (7.5%), as shown in Figure 2. All 6 subjects were of Asian descent (6 of 29 total Asian subjects [23%]). Four of the subjects were subjects with CHB in Part 3 of Study AB-506-001, and 2 were HS in the follow-up study AB-506-003. All 6 subjects experienced ALT and/or AST elevations on or after Study Day 18 (Day 18 for HS, between Days 24–27 for subjects with CHB), and none had increases in total bilirubin

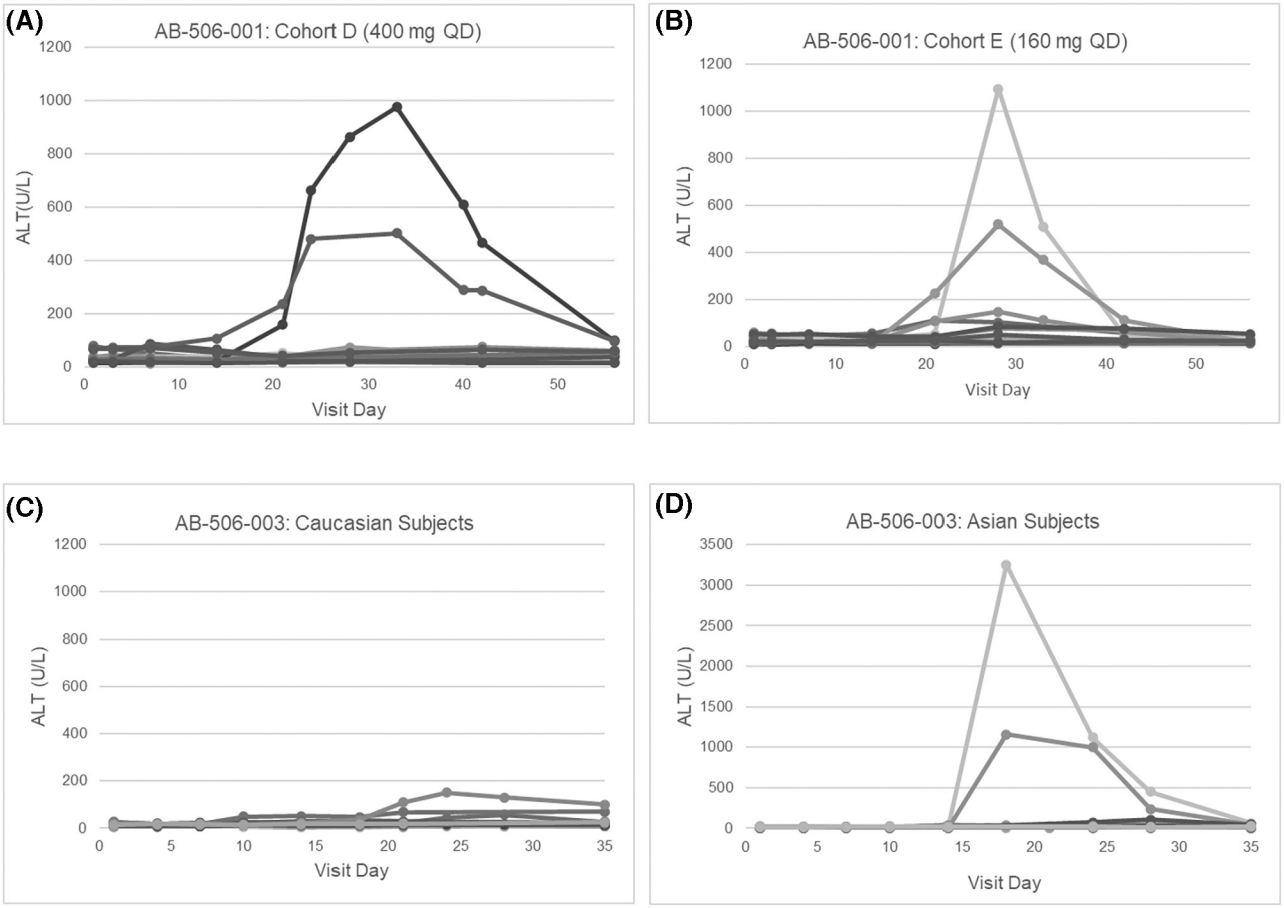


FIGURE 2 Alanine aminotransferase (ALT) levels over time in subjects with chronic hepatitis B (CHB) in AB-506-001 dosing Cohort D (400 mg) (A), subjects with CHB in AB-506-001 dosing Cohort E (160 mg) (B), AB-506-003 Caucasian healthy subjects (HS) (400 mg) (C), and AB-506-003 Asian HS (400 mg) (D). QD, once daily

above the ULN or any evidence of cholestasis or liver synthetic dysfunction. Two of the 4 subjects with CHB were asymptomatic at the time of the flares: 1 subject had Grade 1 rash and the other had Grade 1 headache, flatulence, abdominal discomfort, and Grade 2 fatigue. One of the subjects with CHB was started on TAF during the flare at the Investigator's discretion; the other subjects with CHB were closely observed for resolution of the flares and did not require clinical intervention. Both HS experienced Grade 1–2 symptoms (decreased appetite, fever, headache, cough, vomiting, or abdominal discomfort); 1 was hospitalized for approximately 36 h for observation and administration of fluids, the other was observed in the clinical trial unit overnight. All but 1 of the flare subjects discontinued AB-506 treatment before the end of the planned 28-day treatment period (between Days 18 and 27), and all had full resolution of transaminase elevations following discontinuation of AB-506 (range to resolution 24–56 days). All subjects were evaluated for concomitant viral infections and for autoimmune hepatitis, had repeat ultrasounds performed, and were queried regarding sick contacts, substance abuse, new medications, herbal supplements, or unusual food exposures; all supplemental workups were negative. No pharmacogenomic samples were collected in the AB-506-001 study, but IL28B genotyping (allele-specific PCR for rs12979860) was performed and revealed no notable difference in the subjects who experienced flares (all were C/C, as were 79% of the study participants). A summary of the flare subjects is included in Table S7.

All 4 subjects with CHB with flares returned for follow-up for at least 9 months to monitor for changes in HBV parameters. HBV DNA returned to baseline in 3 of the 4 subjects, whereas in the subject who started TAF on Day 25, HBV DNA was < LLOQ by Day 205. This subject was also the only subject to demonstrate a marked, sustained decline in HBsAg and in HBeAg during the follow-up period (Figure 4D).

A PK subanalysis of subjects with CHB was performed to determine whether there were unusual AB-506 C_{max} and/or area under the curve (AUC) values in subjects with ALT abnormalities that could account for these safety observations, or whether there were ethnic differences in PK parameters between Asian and non-Asian subjects. All evaluations were performed using Day 1 serial PK. In Part 2, the AB-506 Day 10

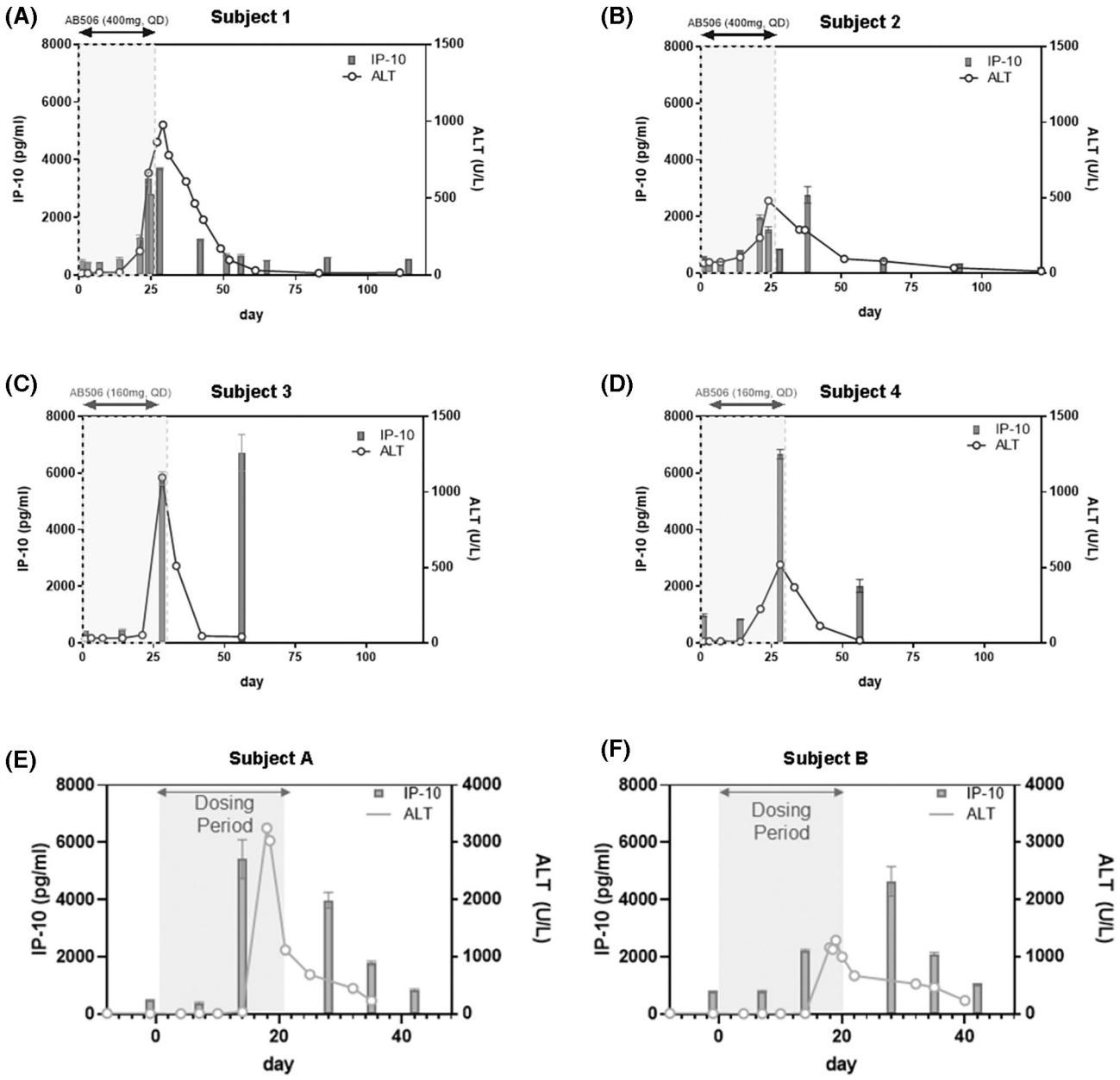
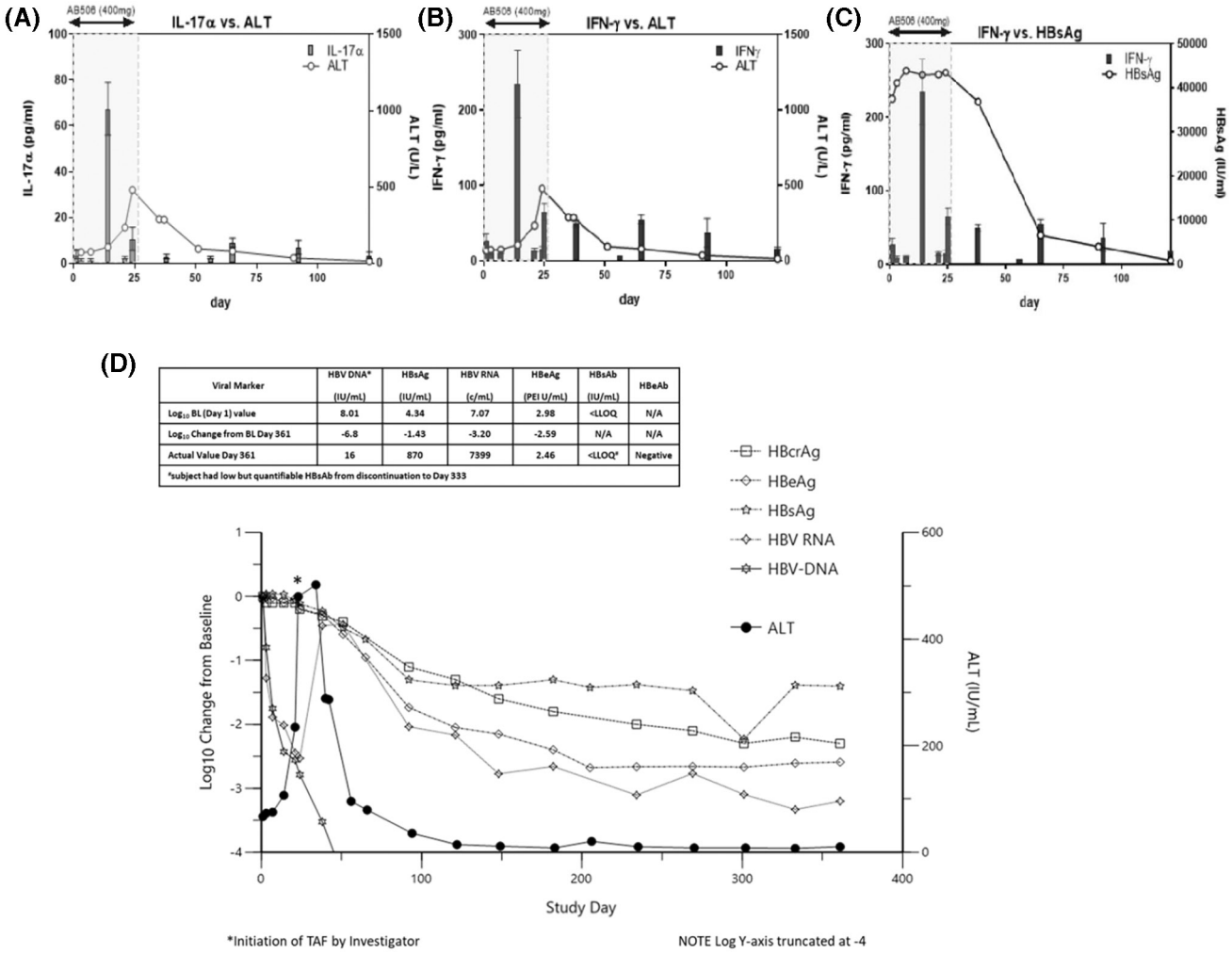


FIGURE 3 Individual subject interferon-gamma inducible protein (IP-10) levels over time in subjects with ALT flares among subjects with CHB in AB-506-001 (A–D) and HS in AB-506-003 (E,F)

C_{max} and AUC_{tau} values were not markedly different from Day 1 (Tables S3 and S4), and in Parts 2 and 3, available C_{trough} values did not increase meaningfully from the first collection on Day 3, further supporting this approach. The assessment of C_{max} or AUC(0–6 h) versus presence or absence of ALT elevations in subjects with CHB (Figure S3A,B) demonstrates considerable overlap of C_{max} or AUC_{tau} between subjects with and without ALT elevations, regardless of race. Several subjects with high C_{max} and AUC(0–6 h) values at the 400-mg dose did not have ALT elevations, further suggesting the lack of dose or exposure dependence. The PK of Asian and non-Asian subjects do not appear to differ, although the populations are equally represented only at 160 mg QD. Within the population with CHB, there were no meaningful trends with weight with respect to PK. A similar exploratory analysis was performed for the AB-506-003 study (Figure S3C,D). As with the patient cohorts in AB-506-001, race, body weight, and PK were all poor predictors of ALT abnormalities and PK did not appear to differ in terms of race.

Immunologic analyses

To evaluate immunologic changes during the transaminase flares, cytokine analysis was performed. All 4



Viral Marker	HBV DNA* (IU/mL)	HBsAg (IU/mL)	HBV RNA (c/mL)	HBeAg (PEI U/mL)	HBsAb (IU/mL)	HBeAb
Log_{10} BL (Day 1) value	8.01	4.34	7.07	2.98	<LLOQ	N/A
Log_{10} Change from BL Day 361	-6.8	-1.43	-3.20	-2.59	N/A	N/A
Actual Value Day 361	16	870	7399	2.46	<LLOQ[a]	Negative

[a]subject had low but quantifiable HBsAb from discontinuation to Day 333

FIGURE 4 Cytokine profiles over time for interleukin 17α (IL-17α) and ALT (A), interferon γ (IFNγ) and ALT (B), and IFNγ and hepatitis B surface antigen (HBsAg) over time (C), and HBV viral marker profile and ALT over time (D) for AB-506-001 CHB flare Subject 2. HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; TAF, tenofovir alefenamide

subjects with CHB and both HS had marked concomitant increases in serum levels of interferon-gamma inducible protein (IP-10) that mirrored the increases in ALT (Figure 3). In 1 of the 4 subjects with CHB, interferon-γ (IFN-γ) and interleukin-17α (IL-17α) levels preceded the rise in ALT, and a marked decline in HBsAg level was observed in this subject after the IFN-γ and IL-17α increase (Figure 4A–C). No significant changes were observed in other measured cytokines.

PBMCs collected for 7 of 10 subjects with CHB dosed with AB-506 at 400 mg were analyzed to assess whether subjects experiencing ALT flares also had associated changes in production of IFN-γ and tumor necrosis factor α by HBV-specific T cells following *in vitro* cell culture in the presence of HBV peptides, or changes in the frequency of HBsAg-specific memory B cells or T-cell and memory B-cell immunophenotypes. No trends were observed, including in the subject who experienced HBsAg decline after the transaminase flare (Figure 4).

DISCUSSION

Study AB-506-001 was a Phase 1a/1b single and MD study in HS and subjects with CHB. AB-506 was well-tolerated in HS after a single dose and through 10 days of 400-mg daily dosing. In subjects with CHB, notable dose-dependent declines in HBV DNA and RNA were observed after 28 days of daily dosing (DNA mean 2.8 log_{10} and 2.1 log_{10} IU/ml declines at the 400 and 160 mg dose levels, respectively), similar to other compounds in this class.[12,13,16] However, hepatic transaminase elevations without accompanying bilirubin elevations were observed in 4 Asian subjects with CHB on or after Day 21 of dosing in the CHB portion of the study, leading to the premature discontinuation of AB-506 despite ongoing declines in HBV DNA of >2.0 log_{10} IU/ml at the time of the flares.

The evaluation of transaminase flares in the context of clinical trials of investigational agents remains a challenge for sponsors and investigators. Clinical trial

subjects with underlying liver disease such as CHB further complicate the assessment, and several recent papers and hepatology society guidance documents have been issued to aid sponsors in the design of studies to limit the risk of and guide interpretation of flares. However, several key questions remain unanswered. First, how are flares defined in patients with CHB as compared to HS without liver disease? Several definitions based on the degree of ALT elevation above-laboratory ULN or consensus-based ULN and/or fold change above prestudy baseline or on-treatment nadir have been proposed.[5,6,23–25] Second, what are the different types of flares and what are the underlying physiological mechanisms that predict potentially beneficial versus adverse outcomes? Transaminase flares may occur spontaneously in untreated patients with CHB, and three types of transaminase flares have been proposed in patients on HBV treatment: Antiviral flares that are likely host-mediated in the context of viral suppression, virus-induced flares following a rise in HBV DNA due to drug resistance or lack of efficacy, and drug-induced flares due to direct toxicity.[23–25] Regrettably, there are no definitive biomarkers available to differentiate with certainty among these different flare types in the context of a clinical trial, and in fact microscopic analyses of liver biopsies from patients with idiosyncratic drug-induced liver injury appear similar to those from patients with viral hepatitis experiencing an immune-mediated flare.[26] Finally, is there guidance regarding ALT threshold values that should signal continuing or discontinuing investigational treatment in the event of a flare? There appears to be no consensus in the context of clinical trials, as differing thresholds of ALT multiples > ULN or fold changes above baseline/prestudy or nadir ALT values achieved during the conduct of the trial have been proposed.[24,25] The nature of the flare is also not considered in these recommendations, which has implications on the benefit–risk assessment: If a subject is experiencing a "beneficial" flare that may lead to seroconversion or other positive change in HBV viral parameters, should they be permitted to continue an experimental treatment?

In untreated patients, ALT flares are associated with rapid rises in HBV DNA and HBsAg and increases in HBeAg/HBcrAg-specific T-cell responses and cytokines, suggesting an important role of the host immune system in these flares.[23] Some of these flares (>50% for those with ALT >5× ULN) may lead to HBeAg seroconversion in HBeAg-positive subjects. The subjects with CHB who had flares in our study did not have antecedent increases from baseline in HBV DNA or HBsAg, but rather had pronounced declines in HBV DNA before the flares, suggesting that they were not related to uncontrolled viral replication.

Flares also occur in subjects with CHB on standard-of-care treatment. During PEG–IFNα 2a (Pegasys) treatment, on-treatment ALT elevations between 5× and 10× ULN and >10× ULN occur in over 25% and 12% of patients, respectively, many of which are accompanied by bilirubin and alkaline phosphatase changes.[27] After PEG-IFNα 2a discontinuation, 7%–18% of patients experience ALT flares of 5×–10× ULN, and when associated with a decline in HBV-DNA after flare appear to be associated with favorable outcomes with regard to HBeAg (~50%) and HBsAg (~30%) loss, suggesting a beneficial immune-mediated response. Similarly, NAs have an 8%–10% incidence of ALT elevations >5× ULN within the first 4–8 weeks of dosing.[28–30] These elevations were not accompanied by signs of hepatic decompensation, and in most cases there was an accompanying decline in HBV DNA of >2 $\log_{10}$ at the time of the flare. These flares are suspected to be due to transient restoration of HBV-specific T-cell responses, and clinical practice with these agents suggests that patients may continue treatment through these flares with ALT normalization. Our 4 subjects with CHB with flares behaved similarly to the subjects described in the NA prescribing information, thus suggesting that they may have experienced host-mediated flares in the context of suppression of viral replication. However, AB-506 treatment was stopped prematurely due to an insufficient clinical safety database accumulated at the time of the events to rule out the possibility of drug-related hepatotoxicity, despite no evidence of transaminase elevations or notable liver pathology in the supporting nonclinical toxicology package.

Interestingly, all 4 subjects with CHB with transaminase flares were of Asian descent, and all events occurred after Study Day 14, which to our knowledge is the longest duration of HS MD data available for any CIs currently in clinical development.[12–14,31–33] Thus, a second Phase 1 study in HS was designed specifically to determine whether AB-506 hepatotoxicity contributed to the transaminase flares by extending AB-506 dosing duration to 28 days to overlap the period where transaminase flares were observed (21–28 days of dosing). Equal numbers of Asian and Caucasian HS were enrolled in two independent cohorts to determine whether racial background was a contributing factor. Two Asian HS experienced dramatic transaminase elevations on or about dosing Day 18, while no Caucasian subject had ALT elevations during dosing. The onset of these elevations was rapid, with both subjects experiencing a change in ALT from normal/Grade 1 to Grade 4 elevation within 4 days. Once AB-506 was discontinued, the transaminase elevations resolved within 3–4 weeks. The PK of AB-506 in these 2 subjects was not markedly different than the PK in the other HS, and there did not appear to be any racial difference in the PK profile of AB-506 in Asians compared with Caucasians.

Other CIs in development have observed on-treatment ALT elevations in subjects with CHB, suggesting either a potential shared safety liability or precipitation of host immune-mediated flares

similar to those occasionally observed with patients on NAs.[12,16,20] A CI (ABI-H2158) in Phase 2 development was recently discontinued due to evidence of hepatotoxicity in 4 subjects with CHB subjects (Assembly Biosciences: press release). There have been no reports of analysis of cytokines or other immune biomarkers from subjects with flares to help explain the pathophysiology of these events. Data from our 6 subjects suggest that elevation in IP-10 levels correlated with increases in transaminase levels, but it remains unclear whether IP-10 is a causative factor or merely a biomarker of hepatocyte injury.

In the subject with CHB who experienced a rapid, multilog decline in HBsAg (Subject 2), the presence of elevated IFNγ and IL-17α but more modest IP-10 elevations before the transaminase flare suggests the presence of host immune reactivation against HBV, perhaps precipitated by the rapid decline in HBV DNA. This cytokine signature was not present in the other 3 subjects with CHB with flares, and not unexpectedly, no other subjects experienced notable HBsAg declines in the context of the transaminase flare. Although no notable HBV-specific immune cell changes were observed, given that HBsAg levels declined rapidly only after Day 28, the timeframe for PBMC assessment may have been too early to capture any cell-mediated immunological responses.

The underlying mechanisms involved in AB-506 liver toxicity have not been identified, and liver biopsies for AB-506 levels or for histological analysis were not included in the protocols. Additional exploratory work is ongoing, including analysis of pharmacogenomic samples (collected from a subset of consenting healthy subjects) to identify potential associations with single-nucleotide polymorphisms in absorption, distribution, metabolism and excretion genes and further evaluation of AB-506 metabolites in preclinical assays. Based on our clinical data, AB-506 and its metabolites were retrospectively assessed *in vitro* for severe drug-induced liver injury (sDILI) potential in a primary human hepatocyte assay based on the reactive oxygen species/adenosine triphosphate (ROS/ATP) depletion ratio from 152 marketed drugs known to cause sDILI.[34] While AB-506 was negative, a primary amine metabolite (present at <10% in preclinical testing and in human plasma; data not shown) was noted to have sDILI potential as suggested by a ROS/ATP ratio equal to or higher than 30% of that for the positive control ketoconazole (data not shown). Transaminase elevations did not correlate with metabolite plasma concentrations in our trial subjects; however, efforts continue to evaluate a potential contribution of this metabolite to AB-506-related hepatotoxicity.

As a result of the hepatotoxicity findings described previously, the AB-506 development program was terminated, and next-generation CIs are being evaluated with different chemotypes to reduce the risk of liver toxicity and improve antiviral activity, including against pre-existing resistant core protein variants.

AUTHOR CONTRIBUTIONS

Data collection: Man-Fung Yuen, Elina Berliba, Wattana Sukeepaisarnjaroen, Sang Hoon Ahn, Tawesak Tanwandee, Young-Suk Lim, Yoon Jun Kim, Kittiyod Poovorawan, Pisit Tangkijvanich, Christian Schwabe, Timothy Eley, Joanne Brown, Karen D. Sims, and Edward J. Gane. *Data analysis*: Man-Fung Yuen, Elina Berliba, Wattana Sukeepaisarnjaroen, Sang Hoon Ahn, Tawesak Tanwandee, Young-Suk Lim, Yoon Jun Kim, Kittiyod Poovorawan, Pisit Tangkijvanich, Timothy Eley, Amy C. H. Lee, Emily P. Thi, Bhavna Paratala, Nagraj Mani, Karen D. Sims, and Edward J. Gane. *Data interpretation*: Man-Fung Yuen, Elina Berliba, Wattana Sukeepaisarnjaroen, Sang Hoon Ahn, Tawesak Tanwandee, Young-Suk Lim, Yoon Jun Kim, Kittiyod Poovorawan, Pisit Tangkijvanich, Christian Schwabe, Timothy Eley, Amy C. H. Lee, Emily P. Thi, Bhavna Paratala, Nagraj Mani, Gaston Picchio, Karen D. Sims, and Edward J. Gane. *Data verification*: Man-Fung Yuen, Timothy Eley, and Karen D. Sims. *Manuscript draft*: Man-Fung Yuen, Timothy Eley, Gaston Picchio, and Karen D. Sims. *Manuscript review*: Man-Fung Yuen, Elina Berliba, Wattana Sukeepaisarnjaroen, Sang Hoon Ahn, Tawesak Tanwandee, Young-Suk Lim, Yoon Jun Kim, Kittiyod Poovorawan, Pisit Tangkijvanich, Christian Schwabe, Timothy Eley, Joanne Brown, Amy C. H. Lee, Emily P. Thi, Bhavna Paratala, Nagraj Mani, Michael J. Sofia, Gaston Picchio, Karen D. Sims, and Edward J. Gane. *Study design*: Christian Schwabe, Timothy Eley, Joanne Brown, Amy C. H. Lee, Emily P. Thi, Bhavna Paratala, Nagraj Mani, Gaston Picchio, Karen D. Sims, and Edward J. Gane. *Study conduct*: Joanne Brown. *Preclinical development of AB-506*: Amy C. H. Lee, Emily P. Thi, Bhavna Paratala, Nagraj Mani, and Michael J. Sofia. All authors reviewed and approved the final manuscript for submission.

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CONFLICT OF INTEREST

M. F. Yuen advises and/or is on the speakers' board for AbbVie, Arbutus Biopharma, Janssen, Bristol-Myers

Squibb, ClearB Therapeutics, Dicerna Pharmaceuticals, Fujirebio Incorporation, Gilead Sciences, GlaxoSmithKline, Merck Sharp and Dohme, Springbank Pharmaceuticals and Sysmex Corporation, and received research funding from Abbott Laboratories, Assembly Biosciences, Bristol Myers Squibb, Gilead Sciences, and Springbank Pharmaceuticals. T. Tanwandee received grant/research support from Arbutus Biopharma, Vir Biotech, Roche, Merck, and Janssen. Y-S Lim advises for Gilead Sciences and Bayer Healthcare. E. J. Gane advises and and/or is on the speakers' board for AbbVie, Gilead Sciences, Janssen, Novartis, Roche, Merck, and Vir Biotechnology. T. Eley, J. Brown, A. C. H. Lee*, E. P. Thi, B. Paratala*, N. Mani, M. J. Sofia, G. Picchio, and K. D. Sims are employees of Arbutus Biopharma (* have since left the company).

DATA AVAILABILITY STATEMENT

Individual participant data will not be shared.

CLINICAL TRIALS REGISTRATION

ANZCTR (https://www.anzctr.org.au): ACTRN12618000987268 (Study AB-506-001), ACTRN12619001255178 (Study AB-506-003).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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