Title Page

Protocol Title:

A Randomized, Open-Label, Multicenter Study Investigating AB-729, Nucleos(t)ide Analogue and Pegylated Interferon Alfa-2a Treatment in Subjects with Chronic Hepatitis B Infection

Short Title: Open-Label Study of AB-729, Nucleos(t)ide Analogue and Pegylated Interferon Alfa-2a in Subjects with Chronic Hepatitis B Infection

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AB-729-201

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AB-729

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at bottom of this page

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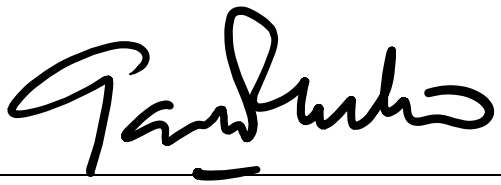

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1. PROTOCOL SUMMARY

1.1. Synopsis

Protocol Title: A Randomized, Open-Label, Multicenter Study Investigating AB-729, Nucleos(t)ide Analogue and Pegylated Interferon Alfa-2a Treatment in Subjects with Chronic Hepatitis B Infection

Short Title: Open-Label Study of AB-729, Nucleos(t)ide Analogue and Pegylated Interferon Alfa-2a in Subjects with Chronic Hepatitis B Infection

Rationale:

AB-729 is a potent, selective, subcutaneously (SC) administered, *N*-Acetylgalactosamine (GalNAc) conjugated small interfering ribonucleic acid (siRNA) inhibitor of hepatitis B virus (HBV) that cleaves and degrades HBV RNA, resulting in downstream silencing of viral proteins, DNA replication and virion production. Development of therapeutic agents that target HBV protein expression, particularly HBV surface antigen (HBsAg), may provide a valuable addition to current approved therapies (nucleos[t]ide analogues [NA] and pegylated interferon alfa [Peg-IFNα]) and possibly increase current HBV cure rates. In an ongoing phase 1 study, AB-729 has been administered as either single or repeat doses to over 40 chronic hepatitis B (CHB) subjects on stable NA therapy. After a single dose of AB-729 60 mg, the mean HBsAg decline at Week 12 was ~1.0 $\log_{10}$ IU/mL (N=6), similar to the decline observed at Week 12 in subjects receiving repeat doses of AB-729 60 mg every 4 weeks (N=7) or every 8 weeks (N=7). However, subjects receiving AB-729 60 mg every 4 weeks or every 8 weeks continued to experience reductions in HBsAg beyond Week 12, with mean maximum $\Delta\log_{10}$ HBsAg of -1.9 $\log_{10}$ IU/mL and -1.8 $\log_{10}$ IU/mL, respectively. Furthermore, 10 of 14 subjects receiving AB-729 60 mg with either dosing interval have achieved HBsAg <100 IU/mL (2 of whom reached <10 IU/mL) to date, with treatment ongoing in both groups.

Recent data using a more sensitive HBV DNA assay suggest that ongoing viral replication occurs even in the context of stable NA use, which may continue to place patients at risk for liver-related morbidity and mortality. Current longitudinal data regarding the likelihood of achieving functional cure (defined as sustained HBV DNA suppression and HBsAg loss with or without hepatitis B surface antibody [HBsAb] seroconversion that is maintained >6 months after discontinuation of antiviral therapy) in subjects who are receiving standard of care (SOC) therapy (NA or Peg-IFNα) suggests that the rate is quite low: <3% for NA-treated subjects and <7% for Peg-IFNα monotherapy after 48 weeks of therapy. Given the ongoing unmet medical need for novel therapeutic combinations to induce functional cure, this study will assess the safety and antiviral efficacy of the addition of AB-729 to ongoing SOC NA therapy in HBV DNA suppressed, hepatitis B virus e-antigen (HBeAg) negative, non-cirrhotic CHB subjects for 24 weeks to reduce HBsAg levels, followed by addition of Peg-IFNα-2a with or without continued AB-729 treatment for either 12 or 24 weeks as consolidation therapy to further decrease HBsAg levels and promote anti-HBV immune reawakening. Subjects will remain on NA therapy during the initial 24-week follow up period, and then may be eligible to discontinue NA treatment if stopping criteria are met. Upon NA discontinuation, the subject will enter a more intensive follow-up period for 48 weeks to monitor for potential rebound of viral markers

and safety events, as well as for sustained viral response and HBsAg loss. Subjects who remain on NA therapy will be followed for an additional 24 weeks (48 weeks total post-end of treatment).

Objectives and Endpoints

Primary Objective

The primary objective of the study is as follows:

Objective	Endpoint
• To evaluate the safety and tolerability of AB-729 plus Peg-IFNα-2a in subjects with NA-suppressed CHB infection	• The frequency and severity of treatment emergent adverse events (TEAEs), discontinuations due to adverse events (AEs), and laboratory abnormalities after dosing with AB-729 plus Peg-IFNα-2a

Secondary Objectives

The secondary objectives of the study are as follows:

Objectives	Endpoints
Secondary	
• To evaluate changes in HBsAg concentration and other virologic parameters during and following repeat doses of AB-729 plus Peg-IFNα-2a	• Change from baseline in HBsAg, HBV DNA, HBV RNA, HBsAb, hepatitis B virus e-antibody (HBeAb) and hepatitis B virus core-related antigen (HBcrAg) concentration at each timepoint • Proportion of subjects with a change in HBsAg from baseline meeting response criteria (≥0.5, 1, 2, or 3 $\log_{10}$ reduction; ≤lower limit of quantitation [LLOQ] or target not detected [TND]) • Proportion of subjects with a change in HBV RNA from baseline meeting response criteria (≥0.5, 1, 2, or 3 $\log_{10}$ reduction; ≤LLOQ or TND) • Proportion of subjects with a change in HBcrAg from baseline meeting response criteria (≥0.5, 1, 2, or 3 $\log_{10}$ reduction; ≤LLOQ or TND)
• To evaluate the proportion of subjects with HBsAb seroconversion	• Proportion of subjects with HBsAb seroconversion at each timepoint

Objectives	Endpoints
Secondary	
• To evaluate the proportion of subjects who meet NA therapy discontinuation criteria	• Proportion of subjects who are eligible to stop NA after Week 24 of follow up
• To evaluate the proportion of subjects who discontinue NA therapy and subsequently restart NA therapy during the follow-up period	• Proportion of subjects who discontinue NA and subsequently restart NA therapy after meeting criteria
• To evaluate the proportion of subjects who experience clinical and/or viral relapse in the follow-up period after discontinuing NA therapy	• Proportion of subjects who discontinue NA and subsequently meet protocol-defined clinical relapse criteria • Proportion of subjects who discontinue NA and subsequently meet protocol-defined viral relapse criteria • Proportion of subjects who have HBV DNA <LLOQ at each timepoint after discontinuation of NA therapy • Proportion of subjects who have HBsAg <100 IU/mL or <10 IU/mL at each timepoint after discontinuation of NA therapy
• To evaluate plasma concentrations of AB-729	• Post-dose plasma concentrations of AB-729 anti-sense (AS), AB-729 AS(N-1)3′, and AB-729 AS(N-2)3′ at selected timepoints

Overall Design:

This is a randomized, open label, multicenter Phase 2 study investigating the safety and antiviral activity of AB-729 in combination with ongoing NA therapy and short courses of Peg-IFNα-2a in subjects with CHB.

The study will enroll 40 stably NA-suppressed, HBeAg negative, non-cirrhotic CHB subjects. After a 24-week lead-in period of AB-729 60 mg SC every 8 weeks (Q8W) added to ongoing SOC NA, subjects will be randomized into one of 4 groups:

- A1: AB-729 + NA + weekly Peg-IFNα-2a for 24 weeks (N = 12)
- A2: NA + weekly Peg-IFNα-2a for 24 weeks (N = 12)
- B1: AB-729 + NA + weekly Peg-IFNα-2a for 12 weeks (N = 8)
- B2: NA + weekly Peg-IFNα-2a for 12 weeks (N = 8)

Treatment assignment will be stratified by HBsAg level at Week 24 (HBsAg ≤100 IU/mL vs >100 IU/mL).

After completion of the assigned Peg-IFNα-2a treatment period, all subjects will remain on NA therapy for the initial 24-week follow up period, and then will discontinue NA treatment if the following criteria are met:

- Alanine aminotransferase (ALT) <2 × upper limit normal (ULN), and
- Undetectable HBV DNA, and
- At least one of the following:
  - HBsAg undetectable (via conventional assay) for at least 24 weeks after the last dose of AB-729
  - HBsAg <100 IU/mL at two consecutive visits at least 24 weeks after the last dose of AB-729
  - HBsAb positive for at least 24 weeks after the last dose of AB-729

If subjects stop NA therapy, they will enter a more intensive follow-up period for 48 weeks to monitor for potential rebound of viral markers and safety events as well as for sustained viral response and HBsAg loss. Subjects who remain on NA therapy will be followed for an additional 24 weeks.

Number of Subjects:

Approximately 80 subjects will be screened to achieve approximately 40 dosed subjects.

Safety Review Committee:

Safety will be continuously monitored by the Investigator, Sponsor's Medical Monitor, and study personnel.

A Data Monitoring Committee (DMC) will periodically review safety data from this study. The DMC will be a group of 3 independent members who will operate in accordance with a pre-specified charter and make recommendations to the Sponsor.

Treatment Groups and Duration:

All subjects will continue their ongoing SOC NA (either entecavir [ETV], tenofovir disoproxil fumarate [TDF] or equivalent, or tenofovir alafenamide [TAF]) and receive AB-729 60 mg SC Q8W (4 doses) for 24 weeks. Subjects will then be randomized into one of 4 groups:

- A1: AB-729 60 mg SC Q8 weeks (2 doses) + NA + Peg-IFNα-2a 180 mcg SC every week (QW) for 24 weeks (N = 12)
- A2: NA + Peg-IFNα-2a 180 mcg SC QW for 24 weeks (N = 12)
- B1: AB-729 60 mg SC Q8 weeks (1 dose) + NA + Peg-IFNα-2a 180 mcg SC QW for 12 weeks (N = 8)
- B2: NA + Peg-IFNα-2a 180 mcg SC QW for 12 weeks (N = 8)

Treatment assignment will be stratified by HBsAg level at Week 24 (HBsAg ≤100 IU/mL vs >100 IU/mL).

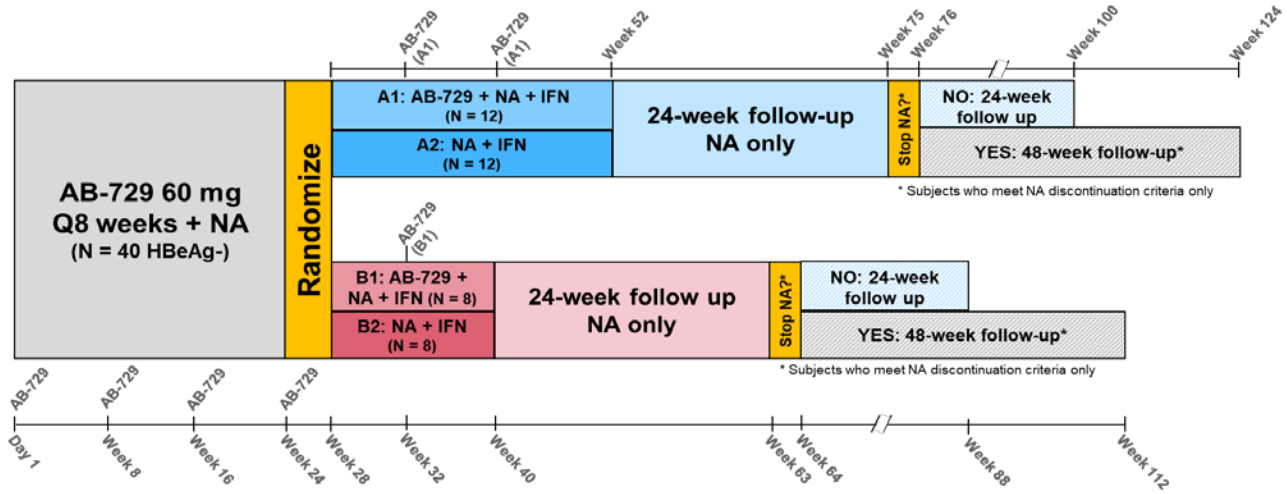
The study will be conducted for approximately 130 weeks as follows:

- The Screening Visit will occur within 45 days prior to the first dose of AB-729.
- Dose 1 of AB-729 will be administered on Day 1 and subjects will be confined to the clinic for a minimum of 6 hours on Day 1 for pharmacokinetic (PK) assessments.
- All subjects will return to the clinic for monthly visits through Week 24 (6 visits) as shown in Table 1.
  - During the Week 24 visit, subjects will be confined to the clinic for a minimum of 6 hours for PK assessments.
- Subjects randomized to Cohorts A1 and A2 will have between 19 (through Week 100) and 32 (through Week 124) visits depending on whether they qualify for NA discontinuation. See Table 2 and Table 3 for details.
- Subjects randomized to Cohorts B1 and B2 will have between 16 (through Week 88) and 29 (through Week 112) visits depending on whether they qualify for NA discontinuation. See Table 4 and Table 5 for details.

1.2. Schema

The study design is represented schematically in Figure 1.

Figure 1. Schematic of Study AB-729-201



Abbreviations: HBeAg- = hepatitis B virus e-antigen negative; IFN = interferon; NA = nucleos(t)ide analogue; Q8 = every 8.

1.3. Schedule of Activities

Table 1 summarizes the schedule of study-related activities for the 24 Week Lead-In Treatment period for all subjects; Table 2 is the Cohort A Consolidation Treatment period and NA-only Post-Treatment follow up (Periods 1 and 2); Table 3 is the Cohort A NA-discontinuation period (for subjects who qualify). Table 4 is the Cohort B Consolidation Treatment period and NA-only Post-Treatment follow-up (Periods 1 and 2), and Table 5 is the Cohort B NA-discontinuation period (for subjects who qualify).

All laboratory assessments including clinical laboratory tests (safety assessments), virology, biomarker and PBMC collections should be drawn pre-dose (AB-729 or Peg-IFNα-2a) when applicable. Additional details of the various laboratory tests are found in Appendix 4 Clinical Laboratory Tests, and Section 8.

Table 1. Schedule of Activities for 24 Week Lead-In Treatment Period (All Subjects)

Assessment	Screening Day -45 to Day -1[a]	Lead-In Treatment Period[b,d]								Notes
		Baseline Day 1	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Early Termination	
Written informed consent	X									
Review inclusion/exclusion criteria	X	X								
Demographics	X									
Height and BMI	X									
Weight	X									
Medical and medication history	X	X								
Concomitant medications	X	X	X	X	X	X	X	X	X	
Eye examination	X									Subjects must provide documentation of an eye examination including a retinal evaluation within 6 months prior to Day 1.
Physical examination	X	X	X	X	X	X	X	X	X	Complete PEs will be conducted at SCR> From Day 1, targeted PEs may be completed at any study visit based on any reported changes to the subject's health.
Vital signs	X	X	X	X	X	X	X	X	X	BP, HR, RR, and temp to be performed pre-dose, when applicable.

Assessment	Screening Day -45 to Day -1[a]	Lead-In Treatment Period[b,d]								Notes
		Baseline Day 1	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Early Termination	
ECG	X	X			X			X	X	Will be performed pre-dose, when applicable.
Clinical laboratory tests	X	X	X	X	X	X	X	X	X	Includes clinical chemistry (including fasting plasma glucose), hematology, coagulation tests, serology, and urine as specified in Appendix 4, and are to be completed after an overnight fast of ≥8 hrs.
HbA1C	X									
TSH/Free T4	X									
AFP	X									
ANA, ASMA, anti-LKM1 serology	X									
Pregnancy test (females only)	X	X	X	X	X	X	X	X	X	Urine pregnancy test is required at SCR for all female subjects, and at subsequent study visits for WOCBP only. If the urine test is positive or not interpretable, a serum/plasma pregnancy test is to be conducted.
FSH (post-menopausal females only)	X									
Fibroscan®	X									Is only required if results from a previous study performed within 6 mos of Day 1 are not available. For subjects who do not have a Fibroscan® result, a prior liver biopsy (performed within 12 mos of Day 1) may be used to determine noncirrhotic status for the purposes of determining eligibility.
Liver ultrasound	X									Is only required if results from a previous study performed within 6 mos of Day 1 are not available.
HIV, HCV, HDV antibody	X									Positive antibody tests may reflex to a confirmatory PCR test.
Qualitative HBcAb IgM	X									
Qualitative HBeAg	X	X			X			X	X	
Quantitative HBsAg[c]	X	X	X	X	X	X	X	X	X	

Assessment	Screening Day -45 to Day -1[a]	Lead-In Treatment Period[b,d]								Notes
		Baseline Day 1	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Early Termination	
HBsAg Ultrasensitive[c]	X	X	X	X	X	X	X	X	X	To be assessed only if quantitative HBsAg result is <LLOQ
Quantitative HBsAb[c]		X			X			X	X	
Quantitative HBV DNA[c]	X	X	X	X	X	X	X	X	X	
Quantitative HBV RNA[c]		X	X	X	X	X	X	X	X	
Quantitative HBcrAg[c]		X	X	X	X	X	X	X	X	
Quantitative HBeAg[c]		X			X			X	X	To be assessed only if positive result obtained with HBeAg qualitative test
Qualitative HBeAb[c]		X			X			X	X	
HBsAg isoforms[c]		X	X	X	X	X	X	X	X	
HBsAg immune complex[c]		X			X			X	X	
Resistance Sample[c]	X	X	X	X	X	X	X	X	X	
HBV Genotype Sample		X								
IL28B GT		X								
Exploratory Immune genes SNP Sample		X								Will be stored and analyzed only if a non-response in HBsAg is observed.
miRNAs[c]		X			X			X	X	Will be stored and analyzed only if a HBsAg effect is observed
RNAi PD marker[c]		X	X	X	X	X	X	X	X	
Immune biomarker/cytokines sample[c]		X		X		X		X	X	Unscheduled samples will be collected in the event of any unexpected safety finding.
PBMCs[c]		X		X		X		X	X	Unscheduled samples will be collected in the event of any unexpected safety finding.
Randomization								X		Subjects will be randomized at the Week 24 visit into Cohort A (A1 or A2) or Cohort B (B1 or B1).
Drug dispensing/ accountability		X	X	X	X	X	X	X	X	Subjects will record all NA doses in a dosing diary, which will be reviewed at each study visit.
AB-729 dosing		X		X		X		X		Doses administered in the clinic only.

Assessment	Screening Day -45 to Day -1[a]	Lead-In Treatment Period [b,d]								Notes
		Baseline Day 1	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Early Termination	
Record AEs		X	X	X	X	X	X	X	X	Will be collected from the time of the start of study treatment through the last Post-Treatment F/U visit.
Record SAEs	X	X	X	X	X	X	X	X	X	Will be collected from the signing of the ICF through the last Post-Treatment F/U visit and followed until resolution of any event.
AB-729 PK sampling		X						X		Will be collected on Day 1 and at Week 24 according to the schedule provided in Appendix 7. On PK collection days, subjects will remain in the clinic a minimum of 6 hours post-dose.

Abbreviations: AEs = adverse events; AFP = alpha-fetoprotein; BMI = body mass index; ANA = antinuclear antibody; anti-LKM1 = liver/kidney microsomal antibody type 1; ASMA = anti-smooth muscle antibody; BP = blood pressure; ECG = electrocardiogram; FSH = follicle stimulating hormone; F/U = follow up; HBcAb = hepatitis B core-related antibody; HBcrAg = hepatitis B virus core-related antigen; HBeAb = hepatitis B virus e-antibody; HBeAg = hepatitis B virus e-antigen; HBsAb = hepatitis B virus surface antibody; HBsAg = hepatitis B virus surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; HDV = hepatitis D virus; HbA1C = hemoglobin A1C; HIV = human immunodeficiency virus; HR = heart rate; hrs = hours; ICF = informed consent form; IgM = immunoglobulin M; IL28B GT = interleukin 28B genotype: LFTs = liver function tests; miRNAs = micro RNAs; mos = months; NA = nucleos(t)ide analogue; PBMCs = peripheral blood mononuclear cells; PCR = polymerase chain reaction; PD = pharmacodynamic; PE = physical exam; PK = pharmacokinetic; RNAi = RNA interference; RR = respiration rate; SAE = serious adverse event; SCR = screening; SNP = single nucleotide polymorphism; temp = temperature; TSH = thyroid stimulating hormone; T4 = thyroxine; WOCBP = women of childbearing potential.

a. If the Screening Visit is >45 days from Day 1 (up to a maximum of 60 days from Day 1), only the safety screening labs (hematology, chemistry including LFTs, and coagulation parameters) need be repeated for the subject to enroll.
b. Visit windows are ± 5 days.
c. Samples are to be drawn pre-dose where applicable.
d. If a subject discontinues on or before Week 28 (prior to peg-IFNα-2a treatment), they should complete an Early Termination visit. If they agree to remain in follow-up they should follow the schedule of activities for Early Termination (Lead-in Period only) Table 13.

Table 2. Schedule of Activities for Cohort A: Consolidation Treatment Period and NA-only Post-Treatment Follow-Up Periods 1 and 2

Cohort A							
Assessment	**Consolidation Treatment Period**		**Post-Treatment Follow-Up (NA only) Period 1**		**Post-Treatment Follow-Up (NA only) Period 2[c]**	**Early Termination[e]**	**Notes**
	Weeks 28, 30, 32, 34, 36, 38[a]	**Weeks 40, 44, 48, 52[b]**	**Weeks 56, 60, 64, 68, 72[b]**	**Weeks 75 and 76[a]**	**Weeks 88 and 100 (EOS)**		
Concomitant medications	X	X	X	X	X	X	
Physical examination	X	X	X	X	X	X	Targeted PEs may be completed at any study visit based on any reported changes to the subject's health.
Vital signs	X	X	X	X	X	X	BP, HR, RR, and temp to be performed pre-dose, when applicable.
ECG	X	X	X	X	X	X	Will be performed pre-dose, when applicable.
Clinical laboratory tests[d]	X*	X*	X*	X	X	X	Includes clinical chemistry, hematology, coagulation tests, serology, and urine as specified in Appendix 4, and are to be completed after an overnight fast of ≥8 hrs. *TSH to be drawn only at selected visits (Weeks 28, 40, 52 and 64).
Pregnancy test (WOCBP only)	X*	X	X	X**	X	X	Urine pregnancy tests will be collected as indicated. If the urine test is positive or not interpretable, a serum/plasma pregnancy test is to be conducted. *Samples to be collected at Weeks 28, 32, 36. **Sample to be collected at Week 76.
Quantitative HBsAg[d]	X	X	X	X	X	X	
HBsAg Ultrasensitive[d]	X	X	X	X	X	X	To be assessed only if quantitative HBsAg result is <LLOQ
Quantitative HBsAb[d]	X	X	X	X	X	X	Samples to be collected at Weeks 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 72, 76, 88, 100.
Quantitative HBV DNA[d]	X	X	X	X	X	X	
Quantitative HBV RNA[d]	X	X	X	X	X	X	
Quantitative HBcrAg[d]	X	X	X	X	X	X	
Qualitative HBeAg[d]	X	X	X	X	X	X	Samples to be collected at Weeks 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 72, 76, 88, 100.

Cohort A							
Assessment	**Consolidation Treatment Period**		**Post-Treatment Follow-Up (NA only) Period 1**		**Post-Treatment Follow-Up (NA only) Period 2[c]**	**Early Termination[e]**	**Notes**
	Weeks 28, 30, 32, 34, 36, 38[a]	**Weeks 40, 44, 48, 52[b]**	**Weeks 56, 60, 64, 68, 72[b]**	**Weeks 75 and 76[a]**	**Weeks 88 and 100 (EOS)**		
Quantitative HBeAg[d]	X	X	X	X	X	X	Samples to be collected at Weeks 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 72, 76, 88, 100. To be assessed only if positive result obtained with HBeAg qualitative test.
Qualitative HBeAb[d]	X	X	X	X	X	X	Samples to be collected at Weeks 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 72, 76, 88, 100.
HBsAg isoforms[d]	X	X	X	X	X	X	
HBsAg immune complex[d]	X	X	X	X	X	X	Samples to be collected at Weeks 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 72, 76, 88, 100.
Resistance Sample[d]	X	X	X	X	X	X	
miRNAs[d]	X	X	X	X	X	X	Will be stored and analyzed only if a HBsAg effect is observed. Samples to be collected at Weeks 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 72,76, 88, 100.
RNAi PD marker[d]	X	X	X	X	X	X	
Immune biomarker/cytokines sample[d]	X*	X	X**	X***	X	X	Unscheduled samples will be collected in the event of any unexpected safety finding. *Collected at Week 28, 32 and 36. **Collected at Week 56, 60, 64, and 72. ***Collected at Week 76 only.
PBMCs[d]	X*	X	X**	X***	X	X	. Unscheduled samples will be collected in the event of any unexpected safety finding. * Collected at Week 28, 32 and 36. ** Collected at Week 56, 60,64, and 72. ***Collected at Week 76 only.
Drug dispensing/ accountability	X	X	X	X	X	X	Subjects will record all doses in a dosing diary, which will be reviewed at each study visit.
AB-729 dosing in the clinic (Cohort A1 only)	X	X					Weeks 32 and 40 only.

Cohort A							
Assessment	**Consolidation Treatment Period**		**Post-Treatment Follow-Up (NA only) Period 1**		**Post-Treatment Follow-Up (NA only) Period 2[c]**	**Early Termination[e]**	**Notes**
	Weeks 28, 30, 32, 34, 36, 38[a]	**Weeks 40, 44, 48, 52[b]**	**Weeks 56, 60, 64, 68, 72[b]**	**Weeks 75 and 76[a]**	**Weeks 88 and 100 (EOS)**		
Peg-IFNα-2a dosing	X	X					First Peg-IFNα-2a dose to be administered in the clinic at Week 28 visit, and Peg-IFNα-2a administration will occur in the clinic on visit days. Remainder of Peg-IFNα-2a doses (odd numbered Weeks and Weeks 42, 46 and 50) to be self-administered with last dose at Week 51. Subjects will record all Peg-IFNα-2a doses in a dosing diary, which will be reviewed at each study visit.
Record AEs	X	X	X	X	X	X	Will be collected from the time of the start of study treatment through the last Post-Treatment F/U visit.
Record SAEs	X	X	X	X	X	X	Will be collected from the signing of the ICF through the last Post-Treatment F/U visit and followed until resolution of any event.
AB-729 PK sampling	X	X					Will be collected at Week 32 and Week 40 for Group A1 only according to the schedule provided in Appendix 7. On PK collection days, subjects will remain in the clinic a minimum of 6 hrs post-dose.

Abbreviations: AEs = adverse events; BP = blood pressure; ECG = electrocardiogram; EOS = end of study; ET = Early Termination; F/U = follow up; HBcrAg = hepatitis B virus core-related antigen; HBeAb = hepatitis B virus e-antibody; HBeAg = hepatitis B virus e-antigen; HBsAb = hepatitis B virus surface antibody; HBsAg = hepatitis B virus surface antigen; HBV = hepatitis B virus; HR = heart rate; hrs = hours; ICF = informed consent form; miRNAs = micro RNAs; NA = nucleos(t)ide analogue; RNAi = RNA interference; PBMCs = peripheral blood mononuclear cells; Peg-IFNα-2a = pegylated interferon α-2a; PD = pharmacodynamic; PE = physical exam; PK = pharmacokinetic; RR = respiration rate; SAE = serious adverse event; temp = temperature; WOCBP = women of childbearing potential.

a. Visit windows are ± 3 days.
b. Visit windows are ± 5 days.
c. Visit windows are ± 7 days.
d. Samples are to be drawn pre-dose where applicable.
e. If a subject discontinues between Week 28 and 52, they should complete an Early Termination visit. If they agree to remain in follow-up they should enter the Post-Treatment FU (NA only) Periods 1 and 2 within 4 weeks of the ET visit. The subject may skip the Week 75 visit as they will not be eligible for NA discontinuation.

Table 3. Schedule of Activities for Cohort A: NA-Discontinuation Period (For Subjects who Qualify)

Assessment	Cohort A NA-Discontinuation Period[a]		Notes
	Weeks 78, 80, 82, 84, 86, 88[b]	Weeks 92, 96, 100, 104, 108, 112, 116, 120, 124 (EOS)[c]	
Concomitant medications	X	X	
Physical examination	X	X	Targeted PEs may be completed at any study visit based on any reported changes to the subject's health.
Vital signs	X	X	BP, HR, RR, and temperature.
ECG	X	X	
Clinical laboratory tests	X	X	Includes clinical chemistry, hematology, coagulation tests, serology, and urine as specified in Appendix 4, and are to be completed after an overnight fast of ≥8 hrs .
Pregnancy test (WOCBP only)	X*	X	Urine pregnancy tests will be collected as indicated. If the urine test is positive or not interpretable, a serum/plasma pregnancy test is to be conducted. *Samples to be collected at Weeks 80, 84, and 88.
Quantitative HBsAg	X	X	
HBsAg Ultrasensitive	X	X	To be assessed only if quantitative HBsAg result is <LLOQ
Quantitative HBsAb	X	X	Samples to be collected at Weeks 80, 84, 88, 92, 100, 112, 124.
Quantitative HBV DNA	X	X	
Quantitative HBV RNA	X	X	
Quantitative HBcrAg	X	X	
Qualitative HBeAg	X	X	
Quantitative HBeAg	X	X	To be assessed only if positive result obtained with HBeAg qualitative test.
Qualitative HBeAb	X	X	Samples to be collected at Weeks 80, 84, 88, 92, 100, 112, 124.
HBsAg isoforms	X	X	
HBsAg immune complex	X	X	Samples to be collected at Weeks 80, 84, 88, 92, 100, 112, 124.
Resistance Sample	X	X	
miRNAs	X	X	Will be stored and analyzed only if a HBsAg effect is observed. Samples to be collected at Weeks 80, 84, 88, 92, 100, 112, 124.

Assessment	Cohort A NA-Discontinuation Period[a]		Notes
	Weeks 78, 80, 82, 84, 86, 88[b]	Weeks 92, 96, 100, 104, 108, 112, 116, 120, 124 (EOS)[c]	
RNAi PD marker	X	X	
Immune biomarker/cytokines sample	X*	X**	Unscheduled samples will be collected in the event of any unexpected safety finding. *Collected at Weeks 80, 84, and 88. **Collected at Weeks 92,100, 112, and 124.
PBMCs	X*	X**	Unscheduled samples will be collected in the event of any unexpected safety finding. *Collected at Weeks 80, 84, and 88. **Collected at Weeks 92,100, 112, and 124.
Record AEs	X	X	Will be collected from the time of the start of study treatment through the last Post-Treatment F/U visit.
Record SAEs	X	X	Will be collected from the signing of the ICF through the last Post-Treatment F/U visit and followed until resolution of any event.

Abbreviations: AEs = adverse events; BP = blood pressure; ECG = electrocardiogram; EOS = end of study; F/U = follow up; HBcrAg = hepatitis B virus core-related antigen; HBeAb = hepatitis B virus e-antibody; HBeAg = hepatitis B virus e-antigen; HBsAb = hepatitis B virus surface antibody; HBsAg = hepatitis B virus surface antigen; HBV = hepatitis B virus; HR = heart rate; hrs = hours; ICF = informed consent form; miRNAs = micro RNAs; NA = nucleos(t)ide analogue; PBMCs = peripheral blood mononuclear cells; PD = pharmacodynamic; PE = physical exam; PK = pharmacokinetic; RNAi = RNA interference; RR = respiration rate; SAE = serious adverse event; temp = temperature.

a. Subjects who Early Terminate during this period should discuss restarting NA therapy and entering the NA Restart Follow-Up period (Table 14) or discuss alternative follow-up plans with the study Investigator.
b. Visit windows are ± 3 days.
c. Visit windows are ± 5 days.

Table 4. Schedule of Activities for Cohort B: Consolidation Treatment Period and NA-only Post-Treatment Follow-Up Periods 1 and 2

Cohort B						
	Consolidation Treatment Period[a]	**Post-Treatment Follow-Up (NA only) Period 1**		**Post-Treatment Follow-Up (NA only) Period 2[c]**	**Early Termination[e]**	
Assessment	**Weeks 28, 30, 32, 34, 36, 38, 40**	**Weeks 44, 48, 52, 56, 60[b]**	**Weeks 63 and 64[a]**	**Weeks 76 and 88 (EOS)**		**Notes**
Concomitant medications	X	X	X	X	X	
Physical examination	X	X	X	X	X	Targeted PEs may be completed at any study visit based on any reported changes to the subject's health.
Vital signs	X	X	X	X	X	BP, HR, RR, and temp to be performed pre-dose, when applicable.
ECG	X	X	X	X	X	Will be performed pre-dose, when applicable.
Clinical laboratory tests[d]	X*	X*	X	X	X	Includes clinical chemistry, hematology, coagulation tests, serology, and urine as specified in Appendix 4, and are to be completed after an overnight fast of ≥8 hrs. *TSH to be drawn only at selected visits (Weeks 28, 40, and 52)
Pregnancy test (WOCBP only)	X*	X	X**	X	X	Urine pregnancy tests will be collected as indicated. If the urine test is positive or not interpretable, a serum/plasma pregnancy test is to be conducted. * Samples to be collected at Weeks 28, 32, 36, and 40. **Sample to be collected at Week 64.
Quantitative HBsAg[d]	X	X	X	X	X	
HBsAg Ultrasensitive[d]	X	X	X	X	X	To be assessed only if quantitative HBsAg result is <LLOQ
Quantitative HBsAb[d]	X	X	X	X	X	Samples to be collected at Weeks 28, 32, 36, 40, 44, 48, 52, 60, 64, 76, and 88.
Quantitative HBV DNA[d]	X	X	X	X	X	

Cohort B						
	Consolidation Treatment Period[a]	**Post-Treatment Follow-Up (NA only) Period 1**		**Post-Treatment Follow-Up (NA only) Period 2[c]**	**Early Termination[e]**	
Assessment	**Weeks 28, 30, 32, 34, 36, 38, 40**	**Weeks 44, 48, 52, 56, 60[b]**	**Weeks 63 and 64[a]**	**Weeks 76 and 88 (EOS)**		**Notes**
Quantitative HBV RNA[d]	X	X	X	X	X	
Quantitative HBcrAg[d]	X	X	X	X	X	
Qualitative HBeAg[d]	X	X	X	X	X	
Quantitative HBeAg[d]	X	X	X	X	X	To be assessed only if positive result obtained with HBeAg qualitative test.
Qualitative HBeAb[d]	X	X	X	X	X	Samples to be collected at Weeks 28, 32, 36, 40, 44, 48, 52, 60, 64, 76, and 88.
HBsAg isoforms[d]	X	X	X	X	X	
HBsAg immune complex[d]	X	X	X	X	X	Samples to be collected at Weeks 28, 32, 36, 40, 44, 48, 52, 60, 64, 76, and 88.
Resistance Sample[d]	X	X	X	X	X	
miRNAs[d]	X	X	X	X	X	Will be stored and analyzed only if a HBsAg effect is observed. Samples to be collected at Weeks 28, 32, 36, 40, 44, 48, 52, 60, 64, 76, and 88.
RNAi PD marker [d]	X	X	X	X	X	
Immune biomarker/cytokines sample[d]	X*	X	X**	X	X	Unscheduled samples will be collected in the event of any unexpected safety finding. *Collected at Weeks 28, 32, 36 and 40. **Collected at Week 64 only.
PBMCs[d]	X*	X	X**	X	X	Unscheduled samples will be collected in the event of any unexpected safety finding. *Collected at Week 28, 32,36, and 40. ** Collected at Week 64 only.
Drug dispensing/ accountability	X	X	X	X	X	Subjects will record all NA doses in a dosing diary, which will be reviewed at each study visit.
AB-729 dosing in the clinic (Cohort B1 only)	X					Week 32 only.

Cohort B						
	Consolidation Treatment Period[a]	**Post-Treatment Follow-Up (NA only) Period 1**		**Post-Treatment Follow-Up (NA only) Period 2[c]**	**Early Termination[e]**	
Assessment	**Weeks 28, 30, 32, 34, 36, 38, 40**	**Weeks 44, 48, 52, 56, 60[b]**	**Weeks 63 and 64[a]**	**Weeks 76 and 88 (EOS)**		**Notes**
Peg-IFNα-2a dosing	X					First Peg-IFNα-2a dose to be administered in the clinic at Week 28 visit, and Peg-IFNα-2a administration will occur in the clinic on visit days. Remainder of Peg-IFNα-2a doses (odd numbered Weeks) to be self-administered with last dose at Week 39. Subjects will record all Peg-IFNα-2a doses in a dosing diary, which will be reviewed at each study visit.
Record AEs	X	X	X	X	X	Will be collected from the time of the start of study treatment through the last Post-Treatment F/U visit.
Record SAEs	X	X	X	X	X	Will be collected from the signing of the ICF through the last Post-Treatment F/U visit and followed until resolution of any event.
AB-729 PK sampling	X					Will be collected at Week 32 for Group B1 only according to the schedule provided in Appendix 7 . At this visit, subjects will remain in the clinic for a minimum of 6 hrs post-dose.

Abbreviations: AEs = adverse events; BP = blood pressure; ECG = electrocardiogram; EOS = end of study; ET = early termination; F/U = follow up; HBcrAg = hepatitis B virus core-related antigen; HBeAb = hepatitis B virus e-antibody; HBeAg = hepatitis B virus e-antigen; HBsAb = hepatitis B virus surface antibody; HBsAg = hepatitis B virus surface antigen; HBV = hepatitis B virus; HR = heart rate; hrs = hours; ICF = informed consent form; miRNAs = micro RNAs; RNAi = RNA interference; NA = nucleos(t)ide analogue; PBMCs = peripheral blood mononuclear cells; PD = pharmacodynamic; PE = physical examination; Peg-IFNα-2a = pegylated interferon α-2a; PK = pharmacokinetic; RR = respiration rate; SAE = serious adverse event; temp = temperature; TSH = thyroid stimulating hormone; WOCBP = women of childbearing potential.

a. Visit windows are ± 3 days.
b. Visit windows are ± 5 days.
c. Visit windows are ± 7 days.
d. Samples are to be drawn pre-dose where applicable.

e. If a subject discontinues between Week 28 and 40, they should complete an Early Termination visit. If they agree to remain in follow-up they should enter the Post-Treatment FU (NA only) Periods 1 and 2 within 4 weeks of the ET visit. The subject may skip the Week 63 visit as they will not be eligible for NA discontinuation.

Table 5. Schedule of Activities for Cohort B: NA-Discontinuation Period (For Subjects who Qualify)

Assessment	Cohort B NA-Discontinuation Period a		Notes
	Weeks 66, 68, 70, 72, 74, 76[b]	Weeks 80, 84, 88, 92, 96, 100, 104, 108, 112 (EOS)[c]	
Concomitant medications	X	X	
Physical examination	X	X	Targeted PEs may be completed at any study visit based on any reported changes to the subject's health.
Vital signs	X	X	BP, HR, RR, and temperature.
ECG	X	X	
Clinical laboratory tests	X	X	Includes clinical chemistry, hematology, coagulation tests, serology, and urine as specified in Appendix 4, and are to be completed after an overnight fast of ≥8 hrs and, during the treatment period, should be drawn prior to dosing, when applicable.
Pregnancy test (WOCBP only)	X*	X	Urine pregnancy tests will be collected as indicated. If the urine test is positive or not interpretable, a serum/plasma pregnancy test is to be conducted. *Samples to be collected at Weeks 68, 72, and 76
Quantitative HBsAg	X	X	
HBsAg Ultrasensitive	X	X	To be assessed only if quantitative HBsAg result is <LLOQ
Quantitative HBsAb	X	X	Samples to be collected at Weeks 68, 72, 76, 80, 88, 100, 112.
Quantitative HBV DNA	X	X	
Quantitative HBV RNA	X	X	
Quantitative HBcrAg	X	X	
Qualitative HBeAg	X	X	
Quantitative HBeAg	X	X	To be assessed only if positive result obtained with HBeAg qualitative test
Qualitative HBeAb	X	X	Samples to be collected at Weeks 68, 72, 76, 80, 88, 100, 112.
HBsAg isoforms	X	X	
HBsAg immune complex	X	X	Samples to be collected at Weeks 68, 72, 76, 80, 88, 100, 112.
Resistance Sample	X	X	
miRNAs	X	X	Will be stored and analyzed only if a HBsAg effect is observed. Samples to be collected at Weeks 68, 72, 76, 80, 88, 100, 112.
RNAi PD marker	X	X	

Assessment	Cohort B NA-Discontinuation Period a		Notes
	Weeks 66, 68, 70, 72, 74, 76[b]	**Weeks 80, 84, 88, 92, 96, 100, 104, 108, 112 (EOS)[c]**	
Immune biomarker/cytokines sample	X*	X**	Unscheduled samples will be collected in the event of any unexpected safety finding. *Collected at Weeks 68, 72, and 76. **Collected at Weeks 80,88, 100 and 112.
PBMCs	X*	X**	Unscheduled samples will be collected in the event of any unexpected safety finding. *Collected at Weeks 68, 72, and 76. **Collected at Weeks 80, 88, 100 and 112.
Record AEs	X	X	Will be collected from the time of the start of study treatment through the last Post-Treatment F/U visit.
Record SAEs	X	X	Will be collected from the signing of the ICF through the last Post-Treatment F/U visit and followed until resolution of any event.

Abbreviations: AEs = adverse events; BP = blood pressure; ECG = electrocardiogram; EOS= end of study; F/U = follow up; HBcrAg = hepatitis B virus core-related antigen; HBeAb = hepatitis B virus e-antibody; HBeAg = hepatitis B virus e-antigen; HBsAb = hepatitis B virus surface antibody; HBsAg = hepatitis B virus surface antigen; HBV = hepatitis B virus; HR = heart rate; hrs = hours; ICF = informed consent form; miRNAs = micro RNAs; RNAi = RNA interference; PBMCs = peripheral blood mononuclear cells; PD = pharmacodynamic; PE = physical exam; PK = pharmacokinetic; RR = respiration rate; SAE = serious adverse event; temp = temperature.

a. Subjects who Early Terminate during this period should discuss restarting NA therapy and entering the NA Restart Follow-Up period (Table 14) or discuss alternative follow-up plans with the study Investigator.
b. Visit windows are ± 3 days.
c. Visit windows are ± 5 days.

2. INTRODUCTION

Arbutus Biopharma Corporation (hereafter referred to as Arbutus) is developing AB-729, a potent, selective, SC administered, GalNAc-conjugated siRNA inhibitor of HBV that cleaves and degrades HBV RNA, resulting in downstream silencing of viral proteins, DNA replication and virion production. Development of therapeutic agents that target HBV protein expression, particularly HBsAg, may provide a valuable addition to current approved therapies (NA and Peg-IFNα) and possibly increase current HBV cure rates. In an ongoing phase 1 study, AB-729 has been administered, as either single or repeat doses, to over 40 chronic hepatitis B (CHB) subjects on stable NA therapy and had been well tolerated. After a single dose of AB-729 60 mg, the mean HBsAg decline at week 12 was ~1.0 $\log_{10}$ IU/mL (N=6), similar to the decline observed at Week 12 in subjects receiving repeat doses of AB-729 60 mg every 4 weeks (N=7) or every 8 weeks (N=7). However, subjects receiving AB-729 60 mg every 4 weeks or every 8 weeks continued to experience reductions in HBsAg beyond Week 12, with mean maximum $\Delta\log_{10}$ HBsAg of -1.9 $\log_{10}$ IU/mL and -1.8 $\log_{10}$ IU/mL, respectively. Furthermore, 10 of 14 subjects receiving AB-729 60 mg with either dosing interval have achieved HBsAg <100 IU/mL (2 of whom reached <10 IU/mL) to date, with treatment ongoing in both groups.

A summary of AB-729 preclinical and clinical data is presented in the Investigator's Brochure.[1]

2.1. Background

Hepatitis B is the world's most common serious liver infection, with an estimated 257 million individuals living with chronic hepatitis B (CHB) infection.[2] Unchecked, CHB can lead to liver cirrhosis and liver cancer. Despite the availability of an effective prophylactic HBV vaccine and interferon- and nucleos(t)ide analogue (NA)-based therapies, there remains an unmet medical need for novel, finite, and more efficacious CHB treatments that result in improved long-term outcomes, including an increased chance for functional cure (defined as sustained HBV DNA suppression and hepatitis B surface antigen [HBsAg] loss with or without hepatitis B virus surface antibody (HBsAb) seroconversion that is maintained >6 months after discontinuation of antiviral therapy). Recognizing that monotherapy approaches are unlikely to be successful in promoting functional cure in subjects with CHB, AB-729 is being developed as a key component of a combination treatment strategy that includes NA therapy and potentially drugs with other mechanisms of action, such as direct acting antivirals and/or immunomodulators such as pegylated interferon alfa (PEG-IFNα).

2.2. Study Rationale

Recent data using a more sensitive HBV DNA assay suggest that ongoing viral replication occurs even in the context of stable NA use, which may continue to place patients at risk for liver-related morbidity and mortality.[3, 4] Current longitudinal data regarding the likelihood of achieving functional cure in subjects who are receiving SOC therapy (NA or Peg-IFNα) suggests that the rate is quite low: <3% for NA-treated subjects and <7% for Peg-IFNα-2a monotherapy after 48 weeks of therapy. An alternative and perhaps complementary approach to long-term suppression of the viral polymerase is to reduce the production of HBsAg and other viral

antigens, which are believed to contribute to host immune exhaustion, resulting in inadequate effective T-cell and B-cell responses to CHB infection.[5] By targeting HBsAg and other viral antigen production in addition to suppressing viral replication, a functional cure may be possible. This has been preliminarily explored via clinical trials using GalNAc-conjugated small inhibitory RNAs (siRNAs) and antisense oligonucleotide approaches to prevent the translation of viral messenger RNAs to viral proteins. While these approaches have demonstrated impressive and sustained declines in HBsAg when given in combination with stable NA therapy, none to date have led to on-treatment loss of HBsAg or HBsAb seroconversion even when another novel, direct acting antiviral such as a capsid inhibitor/capsid assembly modulator is added to the combination.[6-9]. Indeed, even suppression of HBsAg levels to <100 IU/mL or <10 IU/mL in a subset of subjects taking these combinations have been insufficient to promote reawakening of the immune system as manifested by ALT flares, HBsAg loss or HBsAb seroconversion. This suggests that profound inhibition of viral replication and/or antigen production alone may be insufficient to promote functional cure, and that addition of a therapeutic agent capable of boosting the host immune system may be required. Indeed, the only experimental regimen to induce notable levels of functional cure has been the combination of NA + nucleic acid polymers (NAPs) + Peg-IFNα, where rapid HBsAg declines were observed during the first 24 weeks of Peg-IFNα-2a treatment.[10]

Pegasys® (peginterferon alfa-2a, Peg-IFNα-2a) is approved for the treatment of CHB, and while the percentage of subjects who achieve functional cure when used as monotherapy for 48 weeks has been somewhat disappointing, in HBeAg positive patients who achieve HBeAg loss during therapy, HBsAg loss in the post-treatment period can reach 30%.[11] A multicenter trial examining combination therapy in treatment naïve or previously treated but off-therapy CHB subjects with tenofovir and Peg-IFNα-2a as dual therapy vs. each as monotherapy found that the combination arm was superior in regard to HBsAg loss at Week 24 post-treatment.[12] Given the known immunostimulatory and HBsAg lowering effects of Peg-IFNα-2a, consolidation with a short pulse of Peg-IFNα-2a therapy in the context of profound suppression of HBV antigens (HBsAg in particular) by AB-729 and of viral replication by a NA may promote immune reawakening and lead to functional cure.[13]

New data examining the role of discontinuing NA therapy in subjects with sustained suppression of HBV DNA to promote HBsAg loss and HBsAb seroconversion has also become available. Discontinuation of nucleos(t)ide analogue therapy in non-cirrhotic CHB-infected subjects has been endorsed in recent hepatology society guidelines in certain subsets of patients.[14-16] Given that HBsAg loss is infrequent during NA monotherapy, recent studies have examined the likelihood of sustained virologic suppression and/or HBsAg loss after NA discontinuation in non-cirrhotic, HBV DNA suppressed subjects with low HBsAg levels. Several recent reviews and meta-analyses suggest that low HBsAg in the context of NA-mediated suppression of HBV DNA may predict the likelihood of maintaining off-therapy virologic suppression and HBsAg loss after NA discontinuation, and that avoidance of premature re-treatment may promote better outcomes in terms of virologic remission. HBsAg thresholds of 100 IU/mL or less appear to be a good predictor of a favorable response to NA cessation in non-cirrhotic, HBeAg negative subjects.[17-21] In addition, there is interest in other biomarkers and prediction tools, such as HBV RNA levels, HBcrAg levels, and composite predictors such as SCALE-B [(35*HBsAg (log IU/mL) + 20*HBcrAg (log U/mL) + 2*age (years) + ALT (U/L) + 40 for tenofovir use]. A

low SCALE-B score (<260 points) may accurately predict a low risk of clinical flare and a higher chance of HBsAg seroclearance.[22-24]

Given the ongoing unmet medical need for novel therapeutic combinations to induce functional cure, this study will assess the safety and antiviral efficacy of the addition of AB-729 to ongoing SOC NA therapy in HBV DNA suppressed, HBeAg negative, non-cirrhotic CHB subjects for 24 weeks to reduce HBsAg levels, followed by addition of Peg-IFNα-2a with or without continued AB-729 treatment for either 12 or 24 weeks as consolidation therapy to further decrease HBsAg levels and promote anti-HBV immune reawakening. Subjects will then discontinue all treatment after completion of the Peg-IFNα-2a consolidation period and enter an intensive follow-up period for 6 months to monitor for potential rebound of viral markers and safety events. Additional follow up for up to a total of 96 weeks will assess for sustained viral response and HBsAg loss.

2.3. Benefit/Risk Assessment

Nonclinical safety pharmacology and Good Laboratory Practice (GLP) toxicology studies in both rat and cynomolgus monkey are described in the IB [1] and demonstrate adequate dose margins for the proposed dose of AB-729 (60 mg every 8 weeks) to be assessed in this study. In addition, preliminary data from the AB-729-001 study have shown an acceptable safety profile for repeat dosing of AB-729 60 or 90 mg every 4 – 12 weeks in NA-suppressed and treatment naïve or off-treatment CHB subjects for a dosing period of up to 48 weeks. The most common TEAEs have been related to the injection site, mostly single events of mild injection site pain, redness or pruritis, that have resolved. The most common laboratory abnormalities have been mild to moderate ALT elevations that have been asymptomatic and transient and have not been considered adverse events by the Investigators. No changes in total or direct bilirubin or markers of liver synthetic function have been observed in subjects with ALT changes. As noted above, all subjects receiving repeat doses of AB-729 have had declines in HBsAg of 1.0 $\log_{10}$ IU/mL or more, with a majority of subjects reaching levels <100 IU/mL after 24 weeks of dosing. These HBsAg declines are sustained throughout the dosing period, and based on AB-729 single dose data, are anticipated to remain suppressed for weeks to months after the end of treatment. A detailed description of available clinical data is available in the IB.[25]

Peg-IFNα-2a is approved as monotherapy for the treatment of CHB, has a well-understood safety profile, and has been tested in combination with NA in many clinical trials.[11, 26, 27] As noted above, HBsAg thresholds of 100 IU/mL or less appear to be a good predictor of a favorable response to NA cessation in non-cirrhotic, HBeAg negative subjects. Given the known immunostimulatory and HBsAg-lowering effects of Peg-IFNα-2a, consolidation with a short pulse of Peg-IFNα-2a therapy in the context of profound suppression of HBV antigens (HBeAg and HBsAg in particular) may promote immune reawakening and lead to functional cure. Short courses of 12 – 24 weeks of Peg-IFNα-2a may also be better tolerated by subjects than a traditional 48-week course of therapy.

Discontinuation of NA therapy in non-cirrhotic CHB-infected subjects has been endorsed in recent hepatology society guidelines in certain subsets of patients.[14-16] In this study, subjects will be assessed for eligibility to stop NA therapy after the first 24 weeks of follow up. Those that meet the criteria described in Section 4.3 will enter an intensive follow-up period for 24 weeks to monitor for potential rebound of viral markers and safety events. Additional follow up for another 24 weeks (48 weeks total) will assess for sustained viral response and HBsAg loss and/or HBsAb seroconversion. Provided that only subjects who are non-cirrhotic and have minimal fibrosis are permitted to discontinue NAs (as reflected by this study population) and close monitoring is performed after NA discontinuation, the risk of hepatic decompensation is low.[21, 28-30] Re-initiation of NA therapy will be permitted should subjects meet ALT and/or HBV DNA criteria as outlined in Section 8.6. Given the addition of a long-acting HBsAg lowering agent (AB-729) with capacity to also reduce other HBV antigens and Peg-IFNα-2a to stable NA therapy, subjects should be better positioned to experience continued viral suppression and potentially HBsAg loss off-treatment.

AB-729 Drug Interaction Potential

Details regarding the disposition of AB-729 are provided in the IB.[1] AB-729 is metabolized by nucleases and is not expected to be a victim of cytochrome P450 (CYP)-mediated drug interactions. *In vitro* investigations of the drug interaction potential of structurally related GalNAc-conjugated antisense oligonucleotides demonstrated no significant inhibition or induction of the major CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4), nor meaningful inhibition on either uptake transporters (organic cation transporter [OCT]1, organic anion transporter [OAT]P1B1/1B3, OAT1, OAT3, OCT2) or efflux transporters (P-glycoprotein [P-gp], breast cancer resistance protein [BCRP], bile salt export pump [BSEP]).[31] Thus, CYP- and transporter-mediated drug interactions with AB-729 are not anticipated.

Nucleos(t)ide Analogue Co-administration with AB-729 and Peg-IFNα-2a

Subjects will remain on their pre-study NA therapy (TAF, TDF or ETV) for the duration of the study treatment period and the first 24-week follow-up period. To date, the safety and PK profile of each of these NAs are well-characterized. Co-administration of AB-729 with TDF, TAF, or ETV is not expected to have a clinically meaningful impact on exposures to either AB-729 nor these NAs. As a result, there are no expectations that specific NA-associated AEs will be exacerbated or changes in efficacy will be observed during or after the administration of AB-729 to these subjects. In on-going clinical study AB-729-001, AB-729 90 mg Q8W and daily TDF are being assessed in CHB subjects who were HBV DNA+ (not taking NA therapy) at baseline. HBV DNA concentrations in these subjects reached unquantifiable levels by Week 16, consistent with the known HBV DNA profile of TDF monotherapy, and suggests that AB-729 does not impact NA efficacy when co-administered. NAs have been co-administered with Peg-IFNα-2a in many clinical trials and there have been no reports of exacerbation of NA-associated or Peg-IFNα-2a associated AEs during co-administration.[11, 26, 27]

Peg-IFNα-2a Co-administration with AB-729 and NAs

While Peg-IFNα-2a is associated with inhibition of CYPA12 [32], this will not have any meaningful impact on exposures to either AB-729 or NAs when co-administered. Peg-IFNα-2a has been co-administered with NAs in many clinical trials and there have been no reports of exacerbation of NA-associated or Peg-IFNα-2a-associated AEs during co-administration. Different injection sites will be recommended for days when AB-729 dosing and Peg-IFNα-2a dosing coincide to minimize subject discomfort and to allow for observation and differentiation of potential injection-related AEs from either compound.

The adverse event profiles of TAF, TDF and ETV are well characterized, and there does not appear to be overlapping toxicity between these NAs and AB-729 based on available non-clinical and clinical data. Ongoing trials with HBV-specific GalNAc-siRNA compounds co-administered with NAs, including AB-729-001, have not revealed exacerbation of any class-associated safety signals attributable to additive or synergistic AE profiles.[1, 6-9] Combination treatment with GalNAc-siRNA compounds and Peg-IFNα-2a in CHB subjects on NAs is being investigated in several ongoing clinical trials, however data regarding safety are not yet available (NCT04412863, NCT04667104, NCT04439539). The adverse event profile of Peg-IFNα-2a includes flu-like symptoms (fever, headache, fatigue, myalgias, anorexia), alopecia, and cytopenias (neutropenia, thrombocytopenia). Of these, only headache has been observed as a related AE in the AB-729-001 program to date (in 2 subjects), and these AEs have not been commonly described in other HBV-specific GalNAc-siRNA programs. ALT elevations have been commonly noted during and after Peg-IFNα-2a therapy in CHB subjects, thus liver function tests will be monitored intensively during this study to allow appropriate dose modification of Peg-IFNα-2a therapy as indicated in the product label.[32] Mild to moderate asymptomatic ALT elevations have been observed in the repeat dosing cohorts of the AB-729-001 study, but none have been considered AEs and have improved with continued dosing of AB-729. The mechanism of these ALT elevations is unknown, but all have occurred in the setting of HBsAg declines and may reflect “beneficial” flares due to restoration of anti-HBV host immune responses in the context of rapid HBV antigen suppression.[33]

Routine safety laboratory testing (including hematology, chemistry, liver function tests, and urinalyses) and clinical assessments will be obtained frequently during the treatment and Follow-up periods to monitor for expected and unexpected AEs during AB-729, NA and Peg-IFNα-2a co-administration. Follow up clinic visits and laboratory testing schedules are more intense during and after changes in treatment strategy, such as during the addition of Peg-IFNα-2a and after NA discontinuation (in eligible subjects). The Investigator should refer to their country-specific approved product labels for additional information about TDF, TAF, ETV and Peg-IFNα-2a use in CHB.

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
• To evaluate the safety and tolerability of AB-729 plus Peg-IFNα-2a in subjects with NA-suppressed CHB infection	• The frequency and severity of TEAEs, discontinuations due to AEs, and laboratory abnormalities after dosing with AB-729 plus Peg-IFNα-2a
Secondary	
• To evaluate changes in HBsAg concentration and other virologic parameters during and following repeat doses of AB-729 plus Peg-IFNα-2a	• Change from baseline in HBsAg, HBV DNA, HBV RNA, HBsAb, HBeAb and HBcrAg concentration at each timepoint • Proportion of subjects with a change in HBsAg from baseline meeting response criteria (≥0.5, 1, 2, or 3 $\log_{10}$ reduction; ≤LLOQ or TND) • Proportion of subjects with a change in HBV RNA from baseline meeting response criteria (≥0.5, 1, 2, or 3 $\log_{10}$ reduction; ≤LLOQ or TND) • Proportion of subjects with a change in HBcrAg from baseline meeting response criteria (≥0.5, 1, 2, or 3 $\log_{10}$ reduction; ≤LLOQ or TND)
• To evaluate the proportion of subjects with HBsAb seroconversion	• Proportion of subjects with HBsAb seroconversion at each timepoint
• To evaluate the proportion of subjects who meet NA therapy discontinuation criteria	• Proportion of subjects who are eligible to stop NA after Week 24 of follow up
• To evaluate the proportion of subjects who discontinue NA therapy and subsequently restart NA therapy during the follow-up period	• Proportion of subjects who discontinue NA and subsequently restart NA therapy after meeting criteria
• To evaluate the proportion of subjects who experience clinical and/or viral relapse in the follow-up period after discontinuing NA therapy	• Proportion of subjects who discontinue NA and subsequently meet protocol-defined clinical relapse criteria • Proportion of subjects who discontinue NA and subsequently meet protocol-defined viral relapse criteria • Proportion of subjects who have HBV DNA <LLOQ at each timepoint after discontinuation of NA therapy

Objectives	Endpoints
	• Proportion of subjects who have HBsAg <100 IU/mL or <10 IU/mL at each timepoint after discontinuation of NA therapy
• To evaluate plasma concentrations of AB-729	• Post-dose plasma concentrations of AB-729 AS, AB-729 AS(N-1)3′, and AB-729 AS(N-2)3′ at selected timepoints
Tertiary/Exploratory	
• To evaluate potential viral resistance to AB-729, as data permit	• Identification of AB-729 target site variants from sequencing of drug resistant HBV variants, if observed
• To evaluate changes in the levels of HBsAg isoforms (small, middle, and large)	• Change from baseline in small, middle, and large isoforms of HBsAg
• To evaluate changes in the levels of immune biomarkers with AB-729 treatment in lead-in period with AB-729 + Peg-IFNα-2a in consolidation period, as data permit • To evaluate changes in the levels of immune biomarkers during and after treatment with AB-729 + Peg-IFNα-2a and NA + Peg-IFNα-2a, as data permit	• Change from baseline in immune-related protein levels (such as soluble programmed death-1 [sPD-1] and cytokines) and HBsAg immune complex levels • Assessment of the relationship between immune-related protein levels, immune complex levels, and virologic responses
• To profile HBV-specific immune function with AB-729 treatment in lead-in period with AB-729 + Peg-IFNα-2a in consolidation period, as data permit • To profile HBV-specific immune function following treatment with AB-729 + Peg-IFNα-2a and NA + Peg-IFNα-2a, as data permit	• Assessment of the relationship between immunologic activity of peripheral blood mononuclear cells (PBMCs) and virologic response
• To characterize interleukin (IL)28B genotype and explore potential correlation to virologic response, as data permit	• Assessment of the relationship between IL28B genotype and virologic response
• To explore relationships between safety and/or pharmacodynamics (PD) with immune/inflammatory gene polymorphisms, as data permit	• Assessment of immune and inflammatory gene polymorphisms via allele-specific oligonucleotide array

Objectives	Endpoints
• To monitor AB-729 RNA interference (RNAi) activity and explore correlation to virologic response, as data permit	• Detection of HBV RNA cleavage products in blood resulting from AB-729 RNAi activity • Assessment of the relationship between detection of HBV RNA cleavage products and virologic responses
• To evaluate changes in levels of HBV-related microRNAs (miRNAs) during and after treatment with AB-729, as data permit	• Change from baseline in selected potentially HBV-related miRNA levels • Assessment of the relationship between HBV-related miRNAs and virologic responses
• To assess the SCALE-B score in predicting post-treatment clinical relapse and HBsAg loss	• Assessment of SCALE-B score at end-of-treatment (EOT) • Proportion of subjects who experience clinical relapse with SCALE-B scores of <260, 260 – 320, and >320 points • Proportion of subjects who experience HBsAg loss with SCALE-B scores of <260, 260-320, and >320 points

4. STUDY DESIGN

4.1. Overall Design

This is a randomized, open label, multicenter Phase 2 study investigating the safety and antiviral activity of AB-729 in combination with ongoing NA therapy and short courses of Peg-IFNα-2a in subjects with CHB.

The study will enroll 40 stably NA-suppressed, HBeAg negative, non-cirrhotic CHB subjects. After a 24-week lead-in period of AB-729 60 mg SC every 8 weeks (Q8W) added to ongoing SOC NA, subjects will be randomized into one of 4 groups:

- A1: AB-729 + NA + weekly Peg-IFNα-2a for 24 weeks (N = 12)
- A2: NA + weekly Peg-IFNα-2a for 24 weeks (N = 12)
- B1: AB-729 + NA + weekly Peg-IFNα-2a for 12 weeks (N = 8)
- B2: NA + weekly Peg-IFNα-2a for 12 weeks (N = 8)

Treatment assignment will be stratified by HBsAg level at Week 24 (HBsAg ≤100 IU/mL vs >100 IU/mL).

All subjects will remain on NA therapy for the initial 24-week follow up period, and then may be considered for NA discontinuation if the following criteria are met:

- ALT <2 × ULN, and
- Undetectable HBV DNA, and
- At least one of the following:
  - HBsAg undetectable (via conventional assay) for at least 24 weeks after the last dose of AB-729
  - HBsAg <100 IU/mL at two consecutive visits at least 24 weeks after the last dose of AB-729
  - HBsAb positive for at least 24 weeks after the last dose of AB-729

If subjects stop NA therapy, they will enter a more intensive follow-up period for 48 weeks to monitor for potential rebound of viral markers and safety events as well as for sustained viral response and HBsAg loss. Subjects who remain on NA therapy will be followed for an additional 24 weeks (48 weeks total post-end of treatment).

4.1.1. Number of Subjects

Approximately 80 subjects will be screened to achieve approximately 40 dosed subjects.

4.1.2. Treatment Groups and Duration

All subjects will continue their ongoing SOC NA (either ETV, TDF or equivalent, or TAF) and receive AB-729 60 mg SC Q8W (4 doses) for 24 weeks. Subjects will then be randomized into one of 4 groups:

- A1: AB-729 60 mg SC Q8 weeks (2 doses) + NA + Peg-IFNα-2a 180 mcg SC QW for 24 weeks (N = 12)
- A2: NA + Peg-IFNα-2a 180 mcg SC QW for 24 weeks (N = 12)
- B1: AB-729 60 mg SC Q8 weeks (1 dose) + NA + Peg-IFNα-2a 180 mcg SC QW for 12 weeks (N = 8)
- B2: NA + Peg-IFNα-2a 180 mcg SC QW for 12 weeks (N = 8)

Treatment assignment will be stratified by HBsAg level at Week 24 (HBsAg ≤100 IU/mL vs >100 IU/mL).

The study will be conducted for approximately 130 weeks as follows:

- The Screening Visit will occur within 45 days prior to the first dose of AB-729.
- Dose 1 of AB-729 will be administered on Day 1 and subjects will be confined to the clinic for a minimum of 6 hours on Day 1 for PK assessments.
- All subjects will return to the clinic for monthly visits through Week 24 (6 visits) as shown in Table 1.
  - During the Week 24 visit subjects will be confined to the clinic for a minimum of 6 hours for PK assessments
- Subjects randomized to Cohorts A1 and A2 will have between 19 (through Week 100) and 32 (through Week 124) visits depending on whether they qualify for NA discontinuation. See Table 2 and Table 3.
- Subjects randomized to Cohorts B1 and B2 will have between 16 (through Week 88) and 29 (through Week 112) visits depending on whether they qualify for NA discontinuation. See Table 4 and Table 5 for details.

4.1.3. Data Monitoring Committee

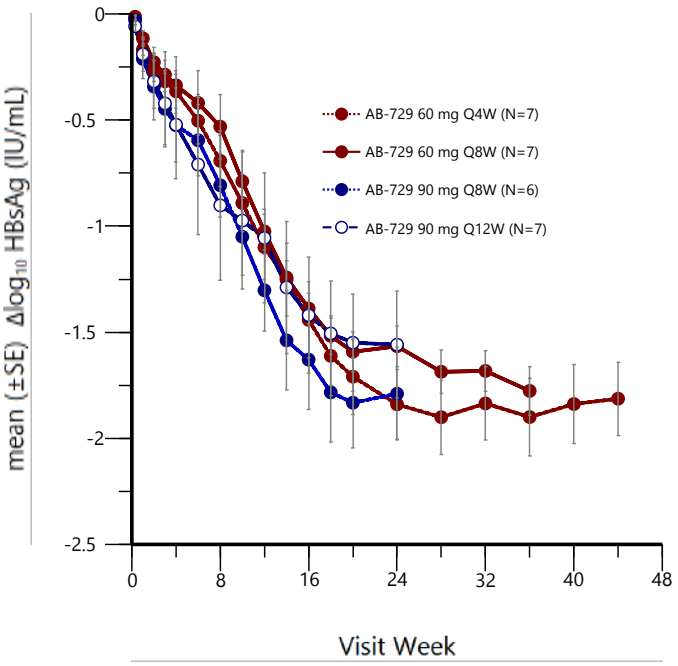
Safety will be continuously monitored by the Investigator, Sponsor's Medical Monitor, and study personnel.

A DMC will periodically review safety data from this study. The DMC will be a group of 3 independent members who will operate in accordance with a pre-specified charter and make recommendations to the Sponsor as needed.

4.2. Scientific Rationale for Study Design

With repeat dosing of AB-729 in CHB subjects at doses of 60 mg or 90 mg every 4 to every 12 weeks for up to 48 weeks, HBsAg declines were observed in all subjects, after which HBsAg response plateaued at approximately Week 20 with no additional meaningful decline in HBsAg with continued dosing of AB-729, regardless of the dose or dosing interval Figure 2.

Figure 2. Mean (±SE) Δlog_{10} HBsAg with Repeat Dosing of AB-729 in Virologically-Suppressed CHB Subjects



Abbreviations: CHB = chronic hepatitis B: HBsAg = hepatitis B virus surface antigen; Q4W = every 4 weeks; Q8W = every 8 weeks; Q12W = every 12 weeks; SE = standard error.
Note: subjects receiving AB-729 60 mg Q4W were switched to AB-729 60 mg Q12W following their Week 20 dose

It is unclear if continued dosing of AB-729 is necessary to maintain the plateau in HBsAg; thus subjects randomized to Cohorts A2 and B2 will receive their final dose of AB-729 at Week 24 (when the course of Peg-IFNα-2a is initiated), while subjects randomized to Cohorts A1 and B1 will continue dosing with AB-729 through Week 40 (Cohort A1) or Week 32 (Cohort B1) (concurrently with the course of Peg-IFNα-2a).

When used as monotherapy for CHB, the recommended treatment duration for Peg-IFNα-2a is 180 mcg weekly for 48 weeks.[32] However, the optimal duration of Peg-IFNα-2a therapy when used as an "add-on" in subjects on HBV DNA suppressive therapy (NAs) and on HBsAg-lowering therapy (AB-729) is not known. HBsAg response at 12 and 24 weeks of interferon monotherapy may predict sustained response after end of therapy for a traditional 48-week regimen [11, 12, 14, 34, 35], but there is limited data regarding outcomes after shorter courses of Peg-IFNα-2a therapy. In a study in CHB subjects examining two combination regimens of TDF plus Peg-IFNα-2a (TDF + Peg-IFNα-2a for 48 weeks and TDF for 48 weeks with Peg-IFNα-2a for the first 16 weeks) versus TDF alone for 120 weeks or Peg-IFNα-2a monotherapy for 48 weeks, the 16 week course of Peg-IFNα-2a + TDF performed as well as 48 weeks of Peg-IFNα-2a monotherapy, and the rates of sustained response (HBV DNA suppression) 24 weeks post-end of treatment was similar across all Peg-IFNα-2a-containing regimens, regardless of duration.[26] A more recent pilot study examined short course Peg-IFNα-2b monotherapy (median 16 weeks) in untreated CHB inactive carriers with low HBsAg (<20 IU/mL) and found a 94% rate of HBsAg loss and 31% rate of anti-HBs seroconversion during the treatment period that was

sustained 9 months post-end of treatment.[27] A shorter course of Peg-IFNα-2a may also be better tolerated than a full 48-week treatment course.

4.3. Justification for Dose

The ongoing Part 3 of Study AB-729-001 is assessing safety and virologic activity of AB-729 60 mg and 90 mg administered at varying dosing intervals: every 4 weeks and every 8 weeks for the 60 mg dose and every 8 weeks and every 12 weeks for the 90 mg dose. Figure 2 above displays the mean ± standard error (SE) HBsAg decline with repeat dosing of AB-729 in virologically-suppressed CHB subjects. At Week 24, there is no meaningful difference ($p \geq 0.2$) in mean (SE) HBsAg decline between the four dosing regimens: -1.84 (0.16), -1.57 (0.09), -1.79 (0.22), -1.56 (0.25) with AB-729 60 mg Q4W (Q12W after Week 20), AB-729 60 mg Q8W, AB-729 90 mg Q8W, and AB-729 90 mg Q12W, respectively. Furthermore, HBsAg decline appears to plateau beyond Week 20, with continued dosing of AB-729 resulting in no meaningful additional decline regardless of dose or dosing interval. At Week 36, AB-729 60 mg Q4W (Q12W after Week 20) and 60 mg Q8W resulted in similar mean (SE) HBsAg declines ($p = 0.6$): -1.90 (0.18) and -1.78 (0.11, N = 6). Thus, the more conservative and convenient AB-729 60 mg Q8W dosing schedule will be assessed in this study.

In this study, the recommended dose of Peg-IFNα-2a for adults with CHB (180 mcg) will be administered subcutaneously for either 24 or 12 weeks depending on assignment to Cohort A or B, respectively (Section 4.2). Dose reduction is permitted for adverse events as described in Section 6.6 and the product label.[32]

4.4. Nucleos(t)ide Discontinuation Criteria

Discontinuation of NA therapy in non-cirrhotic CHB-infected subjects has been endorsed in recent hepatology society guidelines in certain subsets of patients.[14-16] These include HBeAg positive patients who achieve HBeAg loss, anti-HBe seroconversion, and HBV DNA suppression on stable NA therapy and receive consolidation therapy for 6 – 12 months (or longer). Subjects who are HBeAg negative and demonstrate HBsAg loss with or without anti-HBs seroconversion may also be considered for treatment discontinuation. However, given that HBsAg loss is infrequent during NA monotherapy, recent studies have examined the likelihood of sustained virologic suppression and/or HBsAg loss after NA discontinuation in non-cirrhotic, HBV DNA suppressed subjects with low HBsAg levels. These data suggest that on-treatment HBsAg levels at the time of NA discontinuation may predict the likelihood of HBsAg loss after treatment discontinuation. Several recent reviews and meta-analyses suggest that low HBsAg in the context of NA-mediated suppression of HBV DNA may predict the likelihood of maintaining off-therapy virologic suppression and HBsAg loss after NA discontinuation. HBsAg thresholds of 100 IU/mL or less appear to be a good predictor of a favorable response to NA cessation in non-cirrhotic, HBeAg negative subjects.[17-20]

Discontinuation of NA therapy will occur when the following criteria are met by individual subjects upon completion of the first 24 week follow up period:

- ALT <2 × ULN, and
- Undetectable HBV DNA, and
- At least one of the following:
  - HBsAg undetectable (via conventional assay) for at least 24 weeks after the last dose of AB-729
  - HBsAg <100 IU/mL at two consecutive visits at least 24 weeks after the last dose of AB-729
  - HBsAb positive for at least 24 weeks after the last dose of AB-729

Subjects who discontinue NA therapy will enter a more intensive follow up period to monitor for safety, evidence of clinical or virologic relapse, defined as follows:

Clinical relapse: HBV DNA >2000 IU/mL AND ALT >2x baseline and ≥2 × ULN, confirmed by repeat 4 weeks apart

Virologic relapse: HBV DNA > 2000 IU/ml confirmed by repeat 4 weeks apart

Resumption of NA therapy during the second follow-up period should occur if any of the following scenarios occur and after discussion with the Sponsor Medical Monitor:

- Persistent ALT elevations ≥2 × baseline, AND ≥2 – 5 × ULN, AND HBV DNA >2000 IU/mL for 12 weeks
- Persistent ALT elevations ≥2 × baseline, AND ≥5 – 10 × ULN, AND HBV DNA >2000 IU/mL for 4 weeks
- HBV DNA > 20,000 IU/mL regardless of ALT level, confirmed by repeat
- ALT > 10 x ULN confirmed by repeat
- ALT > baseline and > ULN, AND:
  - increased direct or total bilirubin ≥2 × ULN and ≥2 × baseline confirmed by repeat, OR
  - INR increase of ≥0.5 from baseline, confirmed by repeat.

Subjects who restart NA therapy will be assessed every 2 weeks until clinically stable (i.e. ALT and HBV DNA declining on 2 consecutive visits) and will be followed for 24 weeks total via unscheduled visits as needed prior to study discharge (see Appendix 9).

4.5. End of Study Definition

A subject is considered to have completed the study if s/he has completed all phases of the study including the final Post-Treatment Follow-up visit for the study Cohort in which the subject is enrolled.

The end of the study is defined as the date of the last visit of the last subject in the study, globally.

5. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1. Inclusion Criteria

Subjects are eligible to be included in the study only if all the following criteria apply:

1. Subjects must be 18 (or other appropriate age of consent) to 60 years of age, inclusive, at the time of signing the informed consent.
2. Body mass index (BMI) ≥18 kg/m^2 and ≤38 kg/m^2.
3. Male or female subjects as follows:

    a. Male subjects:

    A male subject is eligible to participate if he does not have a female partner who is pregnant or who intends to become pregnant during the study. Male subjects must agree to use contraception as detailed in Appendix 3 starting 4 weeks prior to the first dose of study drug, during the Lead-In Treatment Period, the Consolidation Treatment Period, and throughout the Post-Treatment Follow Up Period. If a subject discontinues the study early, they should continue to follow the contraceptive guidance for 6 months after the last dose of AB-729 study treatment or 28 days after the last dose of Peg-IFNα-2a, whichever is later, and refrain from donating sperm during this period. Male subjects should also be advised of the benefit for their female partners (who are woman of childbearing potential [WOCBP]) to use a highly effective method of contraception as detailed in Appendix 3, as a condom may break or leak.

    b. Female subjects:

    A female subject is eligible to participate if she is not pregnant, not breastfeeding, does not intend to become pregnant during the study, and at least one of the following conditions applies:

        i. Not a WOCBP as defined in Appendix 3.

        OR

        ii. A WOCBP who agrees to follow the contraceptive guidance in Appendix 3 starting 4 weeks prior to the first dose of study drug, during the Lead-In Treatment Period, the Consolidation Treatment Period, and throughout the Post-Treatment Follow Up Period. If a subject discontinues the study early, they should continue to follow the contraceptive guidance for 6 months after the last dose of AB-729 study treatment or 28 days after the last dose of Peg-IFNα-2a, whichever is later.

4. Documented chronic HBV infection via the following:

    a. Positive HBsAg, HBV DNA, or HBeAg at least 6 months prior to the Screening Visit (historical documentation must be provided), AND

    b. Negative serum immunoglobulin M (IgM) anti-hepatitis B core-related antibody (HBcAb) at the Screening Visit.

5. Subjects must be HBeAg-negative at the Screening Visit to participate.
6. HBV DNA must be <LLOQ at Screening.
7. HBsAg between 100 and 5,000 IU/mL (via conventional assay) at Screening.
8. Subjects must have been receiving either TAF, TDF (or equivalent), or ETV consistently for ≥12 months prior to Day 1 and are willing to continue with the same NA treatment through the final study visit (switching from TDF to TAF or vice versa at least 28 days prior to dosing Day 1 is permitted).
9. Liver ultrasound with absence of clinically significant abnormalities is required within 6 months prior to Day 1.
10. All subjects must have assessment of fibrosis demonstrating non-cirrhotic status available at Screening. Non-cirrhotic subjects are defined by a Fibroscan® result of ≤8.5 kPa within 6 months prior to Day 1.
11. Subjects must provide documentation of an eye examination including a retinal evaluation within 6 months prior to Day 1. Any abnormalities must be reviewed with the Sponsor Medical Monitor prior to Day 1.
12. Thyroid stimulating hormone (TSH) and free thyroxine (T4) levels within the normal laboratory reference range, or clinically euthyroid on a stable medication regimen.
13. Capable of giving signed informed consent, able to understand and comply with protocol requirements, instructions, and protocol-related restrictions, and likely to complete the study as planned.

5.2. Exclusion Criteria

A subject is not eligible for inclusion in this study if any of the following criteria apply during Screening:

Medical Status or History

1. Known co-infection with any of the following:
    a. Human immunodeficiency virus (HIV),
    b. Acute hepatitis A virus (HAV),
    c. Hepatitis C virus (HCV),
    d. Hepatitis D virus (HDV), OR
    e. Acute hepatitis E virus (HEV).
2. Any known preexisting medical or psychiatric condition that could interfere with the subject's ability to provide informed consent or participate in study conduct, or that may confound study findings including, but not limited to:
    a. History of any clinically significant medical condition associated with chronic liver disease (e.g., hemochromatosis, autoimmune hepatitis, Wilson's disease, α-1-

antitrypsin deficiency, alcoholic liver disease, non-alcoholic steatohepatitis, or toxin exposures) that may affect the ability to respond to HBV therapy.

b. Immunologically mediated (autoimmune) disease such as autoimmune hepatitis, Crohn's disease, ulcerative colitis, rheumatoid arthritis, idiopathic thrombocytopenic purpura, systemic lupus erythematosus, scleroderma, multiple sclerosis, or sarcoidosis.

c. Significant immunosuppression from, but not limited to, immunodeficiency conditions such as common variable hypogammaglobulinemia or receipt of systemic immunosuppressive medications during the study or ≤2 months prior to the first dose of study treatment, including but not limited to: prednisone (>10 mg/day), azathioprine, methotrexate, and/or cyclosporine.

d. Current or history of any clinically significant cardiac abnormalities/dysfunction such as congestive heart failure, myocardial infarction ≤6 months prior to the Screening Visit, pulmonary hypertension, complex congenital heart disease, significant arrhythmia, and/or active cardiac ischemia. Additional cardiac exclusion criteria include:

   i. A known history of marked prolongation of QT/QTc interval (e.g., repeated demonstration of a QTcF interval >450 msec for males or >470 msec for females).

   ii. History of additional risk factors for Torsades de Pointes (e.g., heart failure, hypokalemia, family history of Long QT Syndrome).

e. Current uncontrolled hypertension or past medical history of hypertensive crisis or hypertensive retinopathy.

f. Uncontrolled thyroid disease (hyper- or hypothyroidism), on or off medication.

g. Uncontrolled diabetes mellitus or subjects with a history of diabetic retinopathy.

h. Ocular disease including but not limited to retinopathy, macular edema, retinal artery or vein thrombosis, optic neuritis or retinal detachment. Documentation of an eye examination with retinal evaluation within 6 months of Day 1 must be provided.

i. History of cirrhosis at any time, or evidence of decompensated liver disease including, but not limited to, a history or presence of clinical ascites, bleeding esophageal varices, hepatorenal syndrome, liver transplantation and/or hepatic encephalopathy.

j. Liver ultrasound or other imaging with findings suggestive of hepatocellular carcinoma (HCC) at any time.

k. Clinically unstable medical condition ≤2 weeks prior to the first dose of study treatment.

l. Unstable or clinically severe psychiatric condition(s), including but not limited to severe depression, suicidal or homicidal ideation and/or attempt.

m. Epilepsy or central nervous system dysfunction

3. Evidence of active or suspected malignancy, or a history of malignancy ≤3 years prior to the Screening Visit (except adequately treated carcinoma in situ and basal cell carcinoma of the skin). Subjects under evaluation for malignancy are not eligible.
4. Any known or suspected hypersensitivity or previous severe reactions to any of the constituents of AB-729 or Peg-IFNα-2a, to biologics such as vaccines, or a history of previous severe hypersensitivity reactions (i.e., anaphylaxis, Stevens-Johnson Syndrome, toxic epidermal necrolysis).
5. Poor venous access that precludes the peripheral blood sampling required for this study.
6. Incapable of self-administration or assisted administration of Peg-IFNα-2a at home.

Findings/Diagnostic Assessments

7. QTcF interval >450 msec for males or >470 msec for females at Screening or Baseline (Day 1) Visit, confirmed by repeat reading.
8. ALT >2 × ULN of the laboratory reference range, confirmed by repeat.
9. Direct or total bilirubin >1.5 × ULN of the laboratory reference range, confirmed by repeat.
10. Serum albumin <3.2 g/dL, confirmed by repeat.
11. INR >ULN of the laboratory reference range, confirmed by repeat.
12. Any of the following hematologic criteria, confirmed by repeat (growth factors may not be used to achieve study entry requirements):
    a. Hemoglobin: <12 g/dL for females and <13 g/dL for males; OR
    b. Neutrophils <1500/mm^3 (African descent: <1200/mm^3); OR
    c. Platelets <150,000/mm^3.
13. Estimated creatinine clearance <60 mL/min, calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. The formula can be found at https://www.kidney.org/professionals/kdoqi/gfr_calculator/. Age, gender, race (non-black or black) and creatinine must be entered. Cystatin is to be left blank.
14. Poorly controlled diabetes mellitus with whole blood hemoglobin A1c (HbA1c) ≥7.5% and/or Screening fasting plasma glucose of ≥126 mg/dL, confirmed by repeat.
15. Laboratory data (such as positive ANA plus positive ASMA and/or anti-LKM1, confirmed by repeat) and/or clinical signs or symptoms suggestive of autoimmune hepatitis.
16. Alpha fetoprotein (AFP) >10 ng/mL.

Medications, Substances, Lifestyle

17. Clinical diagnosis of substance abuse with alcohol, narcotics, or cocaine ≤12 months prior to the Screening Visit, except for those subjects monitored in an opioid substitution maintenance program.

18. Previous treatment with an experimental HBV-directed RNA-interference or antisense oligonucleotide product is exclusionary. History of any other prior experimental HBV treatment must be approved by the Sponsor Medical Monitor.
19. Previous serious adverse events or Grade 4 adverse events due to prior treatment with Peg-IFNα-2a are exclusionary.
20. Participation in any investigational drug, vaccine, or device study within 30 days before study treatment administration, or 90 days for a biologic study, or at any time during participation in the study.

5.3. Lifestyle Considerations

5.3.1. Meals and Dietary Restrictions

All laboratory safety assessments in the clinic should be conducted after subjects have fasted for a minimum of 8 hours. Subject must take their NA therapy with or without a meal as prescribed by the product labeling for their NA therapy.

5.3.2. Alcohol and Tobacco

Alcohol consumption is to be strongly discouraged. During the study, subjects should consume no more than an average of 2 standard drinks of alcohol daily.

The definition of a standard drink (as adapted from the National Institute on Alcohol Abuse and Alcoholism web site [36] is defined as:

- 360 mL (12 fluid oz.) of regular beer,
- 240-270 mL (8-9 fluid oz.) of malt liquor,
- 150 mL (5 fluid oz.) of table wine,
- 90-120 mL (3-4 fluid oz.) of fortified wine,
- 60-90 mL (2-3 fluid oz.) of liqueur, or
- 45 mL (1.5 fluid oz.) of brandy, cognac, or 80-proof distilled spirits.

Subjects are discouraged from smoking, tobacco use, or use of nicotine-containing products for the entire duration of the study.

5.3.3. Activity

Strenuous exercise is strongly discouraged during the study from 96 hours before Day 1 dosing and for 96 hours prior to any clinic visit.

5.4. Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently assigned to treatment. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria,

and any serious adverse event (SAE). Subjects who have been deemed as screen failures may be rescreened for possible participant at a later date, if approved by the Sponsor.

6. STUDY TREATMENT

Study treatment is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study subject according to the study protocol.

6.1. Study Treatment(s) Administered

Table 6. Tabular Summary of Study Treatment(s)

Study Treatment Name:	**AB-729**	**Tenofovir Disoproxil Fumarate (TDF)**	**Tenofovir Alafenamide (TAF)**	**Entecavir (ETV)**	**Pegylated interferon alfa-2a (Peg-IFNα-2a)**
Dosage Formulation:	Sterile solution for injection	Tablet	Tablet	Tablet	Sterile solution for injection
Unit Dose Strength(s)/ Dosage Level(s):	180 mg/mL	300 mg TDF equivalent to 245 mg of tenofovir disoproxil	25 mg	0.5 and 1 mg	180 mcg/ 0.5mL in a pre-filled syringe
Route of Administration:	Subcutaneous injection	Oral	Oral	Oral	Subcutaneous injection
Dosing Instructions:	See Pharmacy Manual	See Pharmacy Manual	See Pharmacy Manual	See Pharmacy Manual	See Pharmacy Manual and Section 6.6
Packaging and Labeling:	AB-729 will be provided in vials and labeled as required per country requirement	To be sourced locally or to be provided by Sponsor and packaged/ labelled in accordance with local regulatory requirements	To be sourced locally or to be provided by Sponsor and packaged/ labelled in accordance with local regulatory requirements	To be sourced locally or to be provided by Sponsor and packaged/ labelled in accordance with local regulatory requirements	To be sourced locally or to be provided by Sponsor and packaged/ labelled in accordance with local regulatory requirements

Study Treatment Name:	**AB-729**	**Tenofovir Disoproxil Fumarate (TDF)**	**Tenofovir Alafenamide (TAF)**	**Entecavir (ETV)**	**Pegylated interferon alfa-2a (Peg-IFNα-2a)**
Storage:	Store under controlled refrigeration (2 – 8°C) and protect from light	Store at 20 – 25°C If provided locally, store according to package insert/label instructions	Store below 30°C If provided locally, store according to package insert/label instructions	Store at 20 – 25°C If provided locally, store according to package insert/label instructions	Store in the refrigerator at 2 – 8°C and protect from light If provided locally, store according to package insert/label instructions

See the Pharmacy Manual for further dosing instructions.

6.2. Preparation/Handling/Storage/Accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.

Only subjects enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatment must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff when the study treatment is kept at the site.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study treatments are provided in the Pharmacy Manual.

6.3. Measures to Minimize Bias: Randomization and Blinding

This is an open label study where after completion of a 24-week lead-in period of AB-729 60 mg SC Q8W added to ongoing SOC NA, subjects will be randomized into one of 4 groups (Cohort A1 or A2: Cohort B1 or B2). Treatment assignment will be stratified by HBsAg level at Week 24 (HBsAg ≤100 IU/mL vs >100 IU/mL).

Each subject will be issued a unique randomization number. The randomization schedule will be generated by the clinical contract research organization (CRO) or by a statistician supporting the study and retained for the study. The randomization sequence along with the assigned study treatment for each number in the randomization sequence will be held confidentially. A manual

randomization process will be followed. A Pharmacist will assign the subjects to randomization numbers to be assigned sequentially from a master randomization schedule.

This study is open-label, so no formal blinding will be performed. However, virology data or other laboratory/analyte results that could bias the conduct of the study will not be reported to investigative sites or other personnel until the subject reaches the end of the first 24 week follow up period to evaluate if NA discontinuation criteria have been met.

6.4. Study Treatment Compliance

All doses of AB-729 will be administered at the study site by study staff members. Each dose administered will be recorded in the patient's Case Report Form (CRF).

Subjects will record all NA doses in a dosing diary, which will be reviewed at each study visit.

Peg-IFNα-2a will be administered weekly for 24 weeks (Cohorts A1 and A2) or 12 weeks (Cohorts B1 and B2). Subjects will record all self-administered Peg-IFNα-2a doses in a dosing diary, which will be reviewed at each study visit.

For Cohort A, the first Peg-IFNα-2a dose will be administered in the clinic at the Week 28 visit, and Peg-IFNα-2a administration will occur in the clinic on visit days (Weeks 30, 32, 34, 36, 38, 40, 44, 48). The remainder of Peg-IFNα-2a doses will be self-administered (Weeks 29, 31, 33, 35, 37, 39, 41, 42, 43, 45, 46, 47, 49, 50, 51). The last dose of Peg-IFNα-2a will be self-administered at Week 51.

For Cohort B, the first Peg-IFNα-2a dose will be administered in the clinic at the Week 28 visit, and Peg-IFNα-2a administration will occur in the clinic on visit days (Weeks 30, 32, 34, 36, 38). The remainder of Peg-IFNα-2a doses will be self-administered (Weeks 29, 31, 33, 35, 37, 39). The last dose of Peg-IFNα-2a will be self-administered at Week 39.

Subjects should take their dose of Peg-IFNα-2a on the same day and approximately same time each week. Subjects should also be advised that if they miss a dose, but remember within 2 days, to take their missed dose as soon as they remember and then to take their next dose on the day they normally do. If they remember when more than 2 days have passed, patients should be advised to consult their Study doctor. Subjects should also be advised to consult their Study doctor if the full dose is not received (e.g., leakage around the injection site).

For further details, see the Pharmacy Manual.

6.5. Concomitant Therapy

All medications or vaccines (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) taken by subjects at the time of enrollment (Screening) prior to first dose of study treatment through the last study visit, will be documented in the CRF as concomitant treatment. Any concomitant treatment given for any reason must be recorded on the CRF, including dosage, start and stop dates, and reason for use.

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the subject is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

Regarding COVID-19 vaccination, the following timing of vaccine administration in relation to AB-729 and Peg-IFNα-2a dosing is suggested:

- When COVID-19 vaccination is anticipated AFTER an AB-729 dose, wait 72 hours prior to administering the COVID-19 vaccine administration when possible
- When AB-729 dosing is to occur AFTER COVID-19 vaccination, wait 7 days after vaccination prior to dosing AB-729 when possible
- Changes to the administration schedule of AB-729 outside of the allowed visit windows due to COVID-19 vaccination should be discussed with the Sponsor, and must be documented as COVID-19-related protocol deviations
- COVID-19 vaccination should be avoided during the period of Peg-IFNα-2a dosing due to the potential for overlapping side effects

The Sponsor's Medical Monitor and/or Clinical Pharmacologist should be contacted if there are any questions regarding concomitant or prior therapy.

The prescribing label, product monograph, or package insert of all ongoing concomitant medications used for the treatment of other medical conditions should be evaluated by the Investigator for continued administration during the subject's participation in the study. The medication list for each potential subject should be provided to the Sponsor's Medical Monitor and/or Clinical Pharmacologist for review as soon as possible after the Screening Visit to minimize the risk of unexpected drug interactions. Any medications added after the Screening Visit prior to the subject's randomization must be documented in the CRF and approved by the Sponsor's Medical Monitor and/or Clinical Pharmacologist.

Subjects must refrain from taking any new prescription or nonprescription drugs (including vitamins and dietary or herbal supplements) within 7 days or 5 half-lives (whichever is longer) before the start of study treatment until completion of the last Post-Treatment Follow-up visit, unless, in the opinion of the Investigator and Sponsor's Medical Monitor and/or Clinical Pharmacologist, the medication will not interfere with the study. Routine vaccinations should be avoided during the screening period.

6.5.1. Prohibited Medications

Investigational agents, with the exception of AB-729, are prohibited. Provisions regarding concomitant medications described in the respective Peg-IFNα-2a and NA package inserts must be followed during the study and throughout the follow-up period.

The Sponsor's Medical Monitor or Clinical Pharmacologist should be contacted if there are any questions regarding concomitant or prior therapy.

6.6. Dose Modification

6.6.1. AB-729

Modification of the AB-729 dose level or interval for a given subject is not allowed at any time.

6.6.2. Peg-IFNα-2a

Peg-IFNα-2a dose modifications may be made as follows after consultation with the Sponsor Medical Monitor according to the approved product label.[32] Following improvement of the neutropenia, thrombocytopenia, or ALT elevation, consider re-escalation of the dosage back to the previous dosage.

Hematologic adverse reactions and dose adjustments for Peg-IFNα-2a are outlined in Table 7.

Table 7. Hematologic Adverse Reactions and Dose Adjustments for Peg-IFNα-2a

Laboratory Value	**Recommended Peg-IFNα-2a Dosage Adjustment**	**Peg-IFNα-2a prefilled syringe (180 mcg/0.5 mL) volume to be injected[a]**
Neutropenia		
ANC ≥500 but <750 cells/mm^3	Reduce to 135 mcg SC weekly	Use 0.375 mL
ANC <500 cells/mm^3	Hold doses until ANC ≥1000 cells/mm^3 then restart at 90 mcg SC weekly and monitor ANC.	Use 0.25 mL
Thrombocytopenia		
Platelets ≥25,000 but <50,000 cells/mm^3	Reduce to 90 mcg SC weekly	Use 0.25 mL
Platelets <25,000 cells/mm^3	Discontinue treatment until resolved	N/A

Abbreviations: ANC = absolute neutrophil count; N/A = not applicable; Peg-IFNα-2a = pegylated interferon alfa 2a; SC = subcutaneous.
a. Syringe marked at 90 mcg, 135 mcg, and 180 mcg graduations.
Source: PEGASYS USPI [32]

ALT elevations and dose adjustments for Peg-IFNα-2a are outlined in Table 8.

Table 8. ALT Elevations and Dose Adjustments for Peg-IFNα-2a

Laboratory Value	Recommended Peg-IFNα-2a Dosage Adjustment	Peg-IFNα-2a prefilled syringe (180 mcg/0.5 mL) volume to be injected[a]
ALT >2 × baseline and 5 × ULN but <10 × ULN	Reduce to 135 mcg SC weekly or hold dose(s) until ALT elevation resolved	Use 0.375 mL
ALT >5 × baseline and 10 × ULN for ≥2 weeks	Discontinue treatment until ALT elevation resolved	N/A

Abbreviations: ALT = alanine aminotransferase; N/A = not applicable; Peg-IFNα-2a = pegylated interferon alfa 2a; SC = subcutaneous; ULN = upper limit normal.
a. Syringe marked at 90 mcg, 135 mcg, and 180 mcg graduations.
Source: PEGASYS USPI [32]

Management of ALT elevations in the context of AB-729 treatment is discussed in Section 8.6 ALT Elevation Management.

Subjects who develop new ocular symptoms should receive a prompt and complete eye examination. Peg-IFNα-2a treatment discontinuation should be considered in patients who develop new or worsening ophthalmologic disorders as recommended in the approved product label and after discussion with the Sponsor Medical Monitor.

Subjects who develop Peg-IFNα-2a-related depression or worsening of existing depression while on treatment should be referred for psychiatric consultation and dose reduction of Peg-IFNα-2a should be considered as recommended in the approved product label.[32]

6.7. Intervention after the End of the Study

No study treatment will be provided to subjects enrolled in this study after the study follow up period has been completed. NAs will continue to be provided during the study follow up period for those who do not qualify for NA discontinuation.

7. STUDY TREATMENT DISCONTINUATION AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Study Treatment for Individual Subjects

General Subject Discontinuation Criteria:

AB-729 study treatment will be discontinued for any subject who meets any of the following criteria:

- The subject requests to stop study treatment.
- Any AE, laboratory abnormality, or intercurrent illness which, in the opinion of the Investigator, indicates that continued participation in the study is not in the best interest of the subject
- Any Grade 3 or higher (Division of AIDS [DAIDS] Table for Grading the Severity of Adult and Pediatric Adverse Events)[37] AE or clinically significant laboratory abnormality (confirmed by repeat testing) considered related to AB-729, excluding ALT elevations (see Section 8.6 ALT Elevation Management)
- Pregnancy
- Termination of the study by the Sponsor
- Loss of ability to freely provide continuing consent because of imprisonment or incarceration for any reason, including for treatment of either a psychiatric or physical illness (e.g., infectious disease)
- Failure to comply with the dosing, evaluations, or other requirements of the study

Subjects may interrupt or discontinue Peg-IFNα-2a treatment due to Peg-IFNα-2a-related adverse events as indicated in Section 6.6 or the product label.[32] Subjects may continue dosing AB-729 and their NA and may remain in the study Consolidation Treatment Period if the Investigator and Sponsor Medical Monitor agree it is in the best interest of the subject.

Hepatic Discontinuation Criteria during the Treatment Period:

- Evidence of confirmed hepatic decompensation (Child Pugh Class B or C)
- Evidence of persistent ALT ≥5 × baseline AND ≥10 × ULN for ≥2 weeks without alternate cause
- ALT ≥2 × baseline AND ≥5 × ULN accompanied by changes in direct bilirubin ≥2 × baseline, confirmed by repeat and without alternate cause
- Laboratory test(s) must be repeated as soon as possible and prior to the next study visit; the Sponsor's Medical Monitor should be informed of results for which discontinuation of the subject is considered. If the laboratory test results are confirmed and the Principal Investigator or Sub-Investigator assess that the above criteria have been met, the subject will discontinue study treatment.

Discontinuation from treatment does not mean discontinuation from the study. Subjects who prematurely discontinue their assigned treatment regimen should immediately undergo the assessments listed for the Early Termination visit and then continue scheduled follow-up assessments.

7.2. Study Discontinuation Criteria

Any of the following events reported will result in a halt of dosing and review of all accumulated safety data by the DMC:

- 2 or more subjects develop SAEs that are at least possibly related to AB-729
- 2 or more subjects develop severe laboratory abnormalities (DAIDS Grade 3 or greater) of the same character that are at least possibly related to AB-729, excluding ALT abnormalities
- 3 or more subjects who meet the hepatic discontinuation criterion of persistent ALT ≥5 × baseline AND ≥10 × ULN for ≥2 weeks without alternate cause
- 3 or more subjects who meet the hepatic discontinuation criterion of ALT ≥2 × baseline AND ≥5 × ULN accompanied by changes in direct bilirubin ≥2 × baseline, confirmed by repeat and without alternate cause

FDA and relevant health authorities will be notified if study discontinuation criteria are met. If the DMC agrees to resume dosing, the minutes from the meeting, rationale, and supportive data will be submitted to the FDA and other relevant health authorities, as well as the Institutional Ethics Committee(s)/Institutional Review Board(s) (IEC[s]/IRB[s]) overseeing the study. IEC/IRB approval will be required before resuming dosing.

7.3. Procedures for Subject Discontinuation or Withdrawal from the Study

A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons.

If the subject withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a subject withdraws from the study, s/he may request destruction of any samples taken and not tested, and the Investigator must document this in the site's study records.

See the Schedule of Activities (Section 1.3) for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

Procedures for Discontinuation of Subjects:

If a subject discontinues prior to completion of the study, the reason for the discontinuation should be obtained. The date of the last dose of study medication and the date of the last assessment and/or contact should be obtained. This information should be documented in the appropriate section of the CRF. A follow-up contact (telephone or visit) should be arranged, as appropriate.

It is vital to obtain follow-up data on any subject withdrawn because of an AE. If a subject is discontinued due to an AE, the event will be followed until resolution or until the event becomes chronic, as assessed by the Investigator.

Discontinuation from study treatment and discontinuation from the overall study, including follow-up, will be collected as two separate events.

7.4. Lost to Follow-up

A subject will be considered lost to follow-up if s/he repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

For subjects lost to follow-up, a reasonable effort will be made to contact the subject in order to ascertain the reason(s) for stopping participation, while fully respecting the subject's rights.

7.5. Replacement of Subjects

Subjects who meet individual subject discontinuation rules for safety events assessed by the Investigator as related to AB-729 will not be replaced; however, subjects who discontinue for other safety-related findings may be replaced after discussion with the DMC. Other non-safety related reasons subjects may be replaced without DMC approval include non-compliance with the protocol or an inability to complete all required study visits and procedures (e.g., withdrawal of consent). Any replacements should be made in a timely manner and only after discussion between the Investigator(s) and the Sponsor's Medical Monitor.

8. STUDY ASSESSMENTS AND PROCEDURES

Refer to the tabular Schedule of Activities (Section 1.3) for the timing of assessments. Adherence to the study design requirements, including those specified in the Schedule of Activities, is essential and required for study conduct.

The Investigator must document any deviation from the protocol procedures and notify the Sponsor or CRO.

Immediate safety concerns should be discussed with the Medical Monitor/Sponsor immediately upon occurrence or awareness, to determine if the subject should continue or discontinue study treatment.

8.1. Screening Assessments

A written informed consent form (ICF), signed and dated by both the subject and the Investigator (or authorized designee), must be obtained prior to screening assessments and before any study specific assessments and procedures are initiated. The ICF will be filed in the subject's medical record, with a copy given to the subject.

All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The Investigator will maintain a screening log to record details of all subjects screened, and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of a subject's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be used for screening or baseline purposes, provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the Schedule of Activities (Section 1.3).

After a subject has provided informed consent, the Investigator and other study personnel will determine if the subject is eligible for participation in the study.

Demographic data will include the following and will only be collected during Screening: age, gender, race, ethnicity, height (cm), and BMI.

A complete medical history will be collected during Screening and will be updated before the first study treatment. The medical history should include, but is not limited to chronic medical conditions, any prior HBV therapies, and concomitant medications.

A liver ultrasound to assess for HCC or other abnormalities will be performed if the subject has not had one performed (with documentation of results available) within 6 months of the Day 1 Visit. A Fibroscan® transient elastography assessment (to be read locally, if performed) will be used to assess cirrhosis status if the subject has not had one performed (with documentation of results available) within 6 months of the Day 1 Visit. If the subject had a liver biopsy within the prior 12 months, that result supersedes the Fibroscan® result. In addition, any prior liver biopsy demonstrating cirrhosis or a Metavir fibrosis score ≥F3 is exclusionary. Screening can begin 45 days prior to dosing to allow for a possible Fibroscan® or liver ultrasound, if needed, as specified in the inclusion criteria.

Subjects must provide documentation of an eye examination including a retinal evaluation within 6 months prior to Day 1. Any abnormalities must be reviewed with the Sponsor Medical Monitor prior to Day 1.

Laboratory testing will be performed per the Schedule of Assessments (Section 1.3) and as per Appendix 4 and the Study's PK/Laboratory Manual(s). Clinical safety laboratory abnormalities performed at the Screening assessment that may be exclusionary may be repeated once for confirmation (see Section 5.2).

8.2. Genetics

A pharmacogenomics blood sample for DNA isolation to examine potential polymorphisms in immune and inflammatory genes in humans and their possible associations with safety and/or PD data will be collected. A single exploratory blood sample for immune genes will be collected on Day 1. In the event of DNA extraction failure, a replacement genetic blood sample may be requested from the subject.

See Appendix 5 for a list of the genetic analyses that may be performed. Details on processes for collection and shipment and destruction of these samples can be found in the Laboratory Manual.

8.3. Immune Biomarkers

Immune biomarker samples for the measurement of soluble immune markers (such as sPD-1), immune complexes, and serum cytokines will be collected as described in the Schedule of Activities (Section 1.3). Cytokines to be examined include, but not limited to:

sCD40L, EGF, Eotaxin/CCL11, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, TGF-α, TNF-α, TNF-β, VEGF.

Samples will be collected for PBMC isolation, as described in the Schedule of Activities (Section 1.3) and laboratory manual. PBMC samples will be evaluated for changes in inflammatory immune response profile or HBV-specific T-cells and other immune cell phenotypes relative to baseline (pre-dose).

Details on processes for collection, shipment, and destruction of these samples can be found in the Study PK/Laboratory Manual.

8.4. Pharmacodynamic and Virologic Assessments

Samples will be collected for assessment of pre-existing or treatment-emergent resistance (via viral sequencing, see Resistance Monitoring Plan [Appendix 10] for details), HBV-related miRNA, RNAi PD marker, and HBV virologic markers (including HBV DNA, HBsAg, HBsAg isoforms, HBV RNA, HBcrAg, HBeAg, HBsAb, HBeAb), as described in the Schedule of Activities (Section 1.3). An ultrasensitive HBsAg sample will be collected for exploratory purposes should subjects reach <LLOQ on the standard HBsAg assay (see Section 1.3). Unused samples may be archived.

A single blood sample for analysis of the interleukin 28B (IL28B) genotype will be collected on Day 1 to determine a potential correlation with virologic responses.

Details on processes for collection, shipment, and destruction of these samples can be found in the Study Laboratory Manual.

8.5. Safety Assessments

Planned time points for all safety assessments are provided in the Schedule of Activities (Section 1.3).

8.5.1. Physical Examinations

Complete or targeted physical examinations will be conducted by the Investigator (or authorized designee) at each timepoint indicated in the Schedule of Activities (Section 1.3). A complete physical examination will include, at a minimum, assessments of the Cardiovascular, Respiratory, Gastrointestinal, Dermatologic and Neurological systems. A complete physical examination is required at Screening. A targeted physical examination can occur after Day 1 and will be based on any changes to the subject's health since the last visit.

8.5.2. Vital Signs

Vitals signs to be measured at all applicable visits will include systolic/diastolic blood pressure, heart rate, respiratory rate, and temperature unless otherwise indicated. Blood pressure and heart rate measurements will be assessed using a completely automated device before dosing while the subject is seated or supine (unless an alternative position is medically required), however, the same position must be used at every visit and should be indicated in the CRF. Manual techniques will be used only if an automated device is not available. Blood pressure and heart rate measurements should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (e.g., television, cell phones).

8.5.3. Electrocardiograms

12-lead electrocardiograms (ECGs) will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, RR, QRS, QT, and QTc (preferably QTcF) intervals at the timepoints indicated in Section 1.3. The Investigator or designee is responsible for reviewing the ECG to assess whether the ECG is within normal limits and to determine the clinical significance of the results. These assessments, including PR, RR, QRS, QT, and QTc/QTcF intervals, will be recorded on the CRF.

For any clinically significant abnormal results, the Investigator must contact the Sponsor's Medical Monitor to discuss continued participation of the subject in the study (e.g., ischemic ECG changes, wave/interval changes, or arrhythmia).

The investigator may repeat the ECG to verify the results of the original ECG.

8.5.4. Pregnancy Testing

For female subjects enrolled, a urine pregnancy test (and follicle stimulating hormone [FSH] test, if necessary to document postmenopausal status) will be performed to confirm eligibility at

Screening, and pregnancy tests will be repeated at the time points shown in the Schedule of Activities (Section 1.3). If a urine pregnancy test is positive or not interpretable, a serum/ plasma pregnancy test will also be performed. Subjects who are pregnant are not eligible for study participation.

8.5.5. Clinical Safety Laboratory Assessments

See Appendix 4 for the list of clinical laboratory tests to be performed. Refer to the Schedule of Activities Section 1.3) for details on the timing and frequency of each clinical laboratory test.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory values are those which are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition.

All laboratory values considered clinically significantly abnormal during participation in the study or during the protocol-defined Follow-up period after the last dose of study treatment, should be repeated until the values return to normal or baseline, or are no longer considered clinically significant by the Investigator or Sponsor’s Medical Monitor.

- If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the Sponsor notified.
- All protocol-required laboratory assessments, as defined in Appendix 4 must be conducted in accordance with the Study PK/Laboratory Manual(s) and the Schedule of Activities (Section 1.3).
- If laboratory values from non-protocol specified laboratory assessments performed at the institution’s local laboratory, require a change in subject management or are considered clinically significant by the Investigator (e.g., SAE or AE), then the results from the local laboratory will be entered into the CRF.

The investigator may repeat the laboratory test or request additional tests to verify the results of the original laboratory tests.

8.5.5.1. Virology Screen

Screening tests for HIV, HCV, and HDV will be performed as indicated in the Schedule of Activities (Section 1.3) and in Appendix 4. Refer to the Study Manual for further information, as applicable. Positive results may have to be reported to Public Health Authorities.

8.5.5.2. Clinical Chemistry, Hematology, and Urine Parameters

Detailed information regarding hematology and clinical chemistry testing is provided in the Schedule of Activities (Section 1.3) and in Appendix 4.

Urinalysis will be performed by a dipstick test and reflex microscopic analysis.

Coagulation studies may include an evaluation of prothrombin time (PT) expressed as INR and activated partial thromboplastin time (aPTT) as indicated in Appendix 4. Refer to the Study PK/Laboratory Manual(s) for further information, as applicable.

8.6. ALT Elevation Management

Subjects with ALT ≥2 × baseline AND ≥5 × ULN should be monitored for changes in hepatic function every 1 – 2 weeks via testing of ALT, aspartate aminotransaminase (AST), total and direct bilirubin, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum albumin and international normalized ratio (INR). Unscheduled visits should be added when necessary. Subjects may continue treatment if there is no evidence of declining liver function or if a causal event (such as intercurrent illness, concomitant medication, etc.) is identified and there is no contraindication to continue treatment.

Subjects with persistent ALT elevations as noted above should also be tested for the following:

- HAV IgM
- HDV IgM/IgG and RNA by PCR
- HCV RNA by PCR
- HEV IgM
- Epstein-Barr virus (EBV) DNA by PCR
- Cytomegalovirus (CMV) DNA by PCR

Subjects with persistent ALT ≥5 × baseline AND ≥10 × ULN for ≥2 weeks OR ALT ≥2 × baseline AND ≥5 × ULN accompanied by changes in direct bilirubin ≥2 × baseline without alternate cause, or symptoms of liver inflammation (such as jaundice, fatigue, nausea, vomiting, loss of appetite) should discontinue AB-729 treatment.

Subjects on Peg-IFNα-2a therapy:

- Subjects with confirmed ALT ≥2 × baseline AND ≥5 × ULN who are on Peg-IFNα-2a therapy may be considered for Peg-IFNα-2a dose reduction to 135 mcg.
- If persistent ALT ≥2 × baseline AND ≥5 × ULN is observed after reduction of Peg-IFNα-2a dosage, Peg-IFNα-2a treatment should be interrupted.
- If at any time direct bilirubin ≥2 × baseline, treatment should be interrupted.
- See Section 6.6 for additional guidance.

Subjects in the Post-Treatment follow up period:

Subjects who have confirmed ALT elevations ≥2 × baseline AND ≥2 × ULN should be monitored for changes in hepatic function every 2 weeks via testing of ALT, AST, total and direct bilirubin, ALP, GGT, serum albumin and INR. Additional testing for HBV DNA, HBsAg, and HBeAg should also be performed. Unscheduled visits should be added when necessary, since subjects may only have scheduled visits on a bi-weekly or monthly during this period.

In addition, the following tests may be performed after confirmation of abnormalities and if warranted based on the results of HBV viral parameters noted above:

- HAV IgM
- HDV IgM/IgG and RNA
- HCV RNA by PCR
- HEV IgM
- EBV DNA by PCR
- CMV DNA by PCR

For subjects who discontinued NA therapy per protocol (see Section 4.4), resumption of NA therapy should occur if any of the following scenarios occur and after discussion with the Sponsor Medical Monitor:

- Persistent ALT elevations ≥2 × baseline, AND ≥2 – 5 × ULN, AND HBV DNA >2000 IU/mL for 12 weeks
- Persistent ALT elevations ≥2 × baseline, AND ≥5 – 10 × ULN, AND HBV DNA >2000 IU/mL for 4 weeks
- HBV DNA > 20,000 IU/mL regardless of ALT level, confirmed by repeat
- ALT > 10 x ULN, confirmed by repeat
- ALT > baseline and > ULN, AND:
  - increased direct or total bilirubin ≥2 × ULN and ≥2 × baseline confirmed by repeat, OR
  - INR increase of ≥0.5 from baseline, confirmed by repeat.

Subjects who restart NA therapy will be assessed every 2 weeks until clinically stable (i.e. ALT and HBV DNA declining on 2 consecutive visits) and will be followed for 24 weeks total via unscheduled visits as needed prior to study discharge (see Appendix 9).

8.7. Adverse Events and Serious Adverse Events

The definitions of an AE and SAE can be found in Appendix 6.

AEs may be reported by the subject. AEs can be spontaneously reported or elicited during examination or evaluation of a subject. To prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE, and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the subject to discontinue the study treatment or study.

8.7.1. Time Period and Frequency for Collecting AE and SAE Information

All SAEs will be collected from the signing of the ICF until the last Post-Treatment Follow-up visit at all the time points specified in the Schedule of Activities (Section 1.3).

All AEs will be collected from the start of study treatment until the last Follow-up visit at all the time points specified in the Schedule of Activities (Section 1.3). Other medical occurrences that

begin after obtaining informed consent but before the start of study treatment will be recorded on the Medical History/Current Medical Conditions section of the CRF, not the AE section.

All SAEs will be recorded on the SAE Report Form and reported to the Sponsor or designee within 24 hours via email or fax, as indicated in Appendix 6. The Investigator will submit any follow-up information with an updated SAE form, to the Sponsor or designee, within 24 hours of it being available.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of study participation. However, if the Investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and s/he considers the event to be reasonably related to the study treatment or study participation, the Investigator must promptly notify the Sponsor.

The method of recording, evaluating, and assessing causality of AEs and SAEs, and the procedures for completing and transmitting SAE reports, are provided in Appendix 6.

8.7.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

8.7.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each subject at subsequent visits/contacts. All AEs and SAEs documented at a previous site visit or contact, and are designated as ongoing, will be reviewed at subsequent visits/contacts.

The Sponsor may request that the Investigator perform, or arrange for, the conduct of supplemental measurements and/or evaluations, to elucidate, as fully as possible, the nature and/or causality of the AE or SAE. The Investigator is obligated to assist.

All SAEs and related AEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 7.4). Further information on follow-up procedures for SAEs is given in Appendix 6. Information regarding pregnancies is found in Section 8.7.5.

8.7.4. Regulatory Reporting Requirements for SAEs

Prompt notification by the Investigator to the Sponsor of an SAE is essential, so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study treatment under clinical investigation, are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The Sponsor or their designee will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and Investigators (see Appendix 6).

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and be forwarded to Investigators as necessary.

An Investigator who receives an Investigator Safety Report describing SAEs or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor, will review it and then file it, along with the IB, and will notify the IRB/IEC, if appropriate, according to local requirements.

8.7.5. Pregnancy

The Sponsor must be notified of any female subject that becomes pregnant while participating in the study, or of any pregnancy that occurs in the female partner of a male subject in the study. Pregnancy in a study subject should result in immediate discontinuation of study treatment.

Although pregnancy is a normal human experience and not technically an AE, all pregnancies must be followed to conclusion to determine outcomes for both mother and baby. This information is important for characterizing the investigational product safety profile. It is the responsibility of the Investigator or designee to report any pregnancy in a subject that occurs during the study. In the event of an early discontinuation, it is the responsibility of the Investigator or designee to report any pregnancy they are aware of in a subject that occurs within 6 months after the last dose of the investigational product.

For women who become pregnant after sexual intercourse with a study subject, a Pregnancy Follow-up Consent Form must be completed to permit follow-up of the woman to ascertain outcomes for both mother and baby. Such informed consent and follow-up are necessary during the study, or in the event the subject discontinues early and the Investigator becomes aware of a pregnancy, when the estimated date of conception falls within 6 months or less of the study subject's last study treatment exposure. Follow-up should be performed by the Investigator or designee in accordance with the schedule outlined on the Pregnancy Report Form.

If the pregnancy ends, the Investigator should notify the Sponsor and complete the Pregnancy Report Form. If necessary, other providers may be approached to ascertain pregnancy outcomes per the completed ICF. If the outcome of the pregnancy for either mother or child meets the criteria for an SAE (e.g., spontaneous abortion, fetal death, stillbirth, ectopic pregnancy, neonatal death, postpartum complications, or congenital anomaly including that in an aborted fetus), the Investigator should follow the procedures for reporting an SAE (see Section 8.7.4 and Appendix 6).

8.8. Treatment of Overdose

Overdose of AB-729 will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

The Sponsor does not recommend any specific treatment for an overdose other than supportive care.

In the event of a suspected overdose of AB-729, the Investigator should:

1. Contact the Sponsor's Medical Monitor immediately.
2. Closely monitor the subject for any AE/SAE and laboratory abnormalities for at least 7 days.
3. Obtain a plasma sample for PK analysis as soon as possible from the date of the last dose of study intervention, if requested by the Sponsor's Medical Monitor (determined on a case-by-case basis).
4. Document the quantity of the excess dose, as well as the duration of the overdose in the CRF.

Suspected overdose of NAs or Peg-IFNα-2a should be reported as per local regulatory guidelines for post-marketing adverse drug events.

8.9. Pharmacokinetic Assessments

Plasma samples will be collected at the time-points described in the Schedule of Activities (Section 1.3) and in Appendix 7 and will be collected to achieve those measures detailed in Section 9.4.4. A window of ±2 minutes for samples collected will be allowed for PK samples.

The actual time of sample collection will be documented. All instances of missed samples and sampling time deviations will be recorded and communicated to the Sponsor, prior to the sample analysis.

Additional details regarding PK sample collection and handling will be provided in the Study Laboratory Manual Kits for PK sample collection, including handling instructions, and all necessary materials for PK sample collection and storage, will be provided to each study site. It is critical that the sample handling instructions be followed exactly.

An intravenous catheter will be used for blood collection to avoid multiple skin punctures, when appropriate. Otherwise, blood samples will be collected by direct venipuncture.

8.10. Use of Residual Samples for Optional Future Research

In addition to the study-specific ICF to be signed by each subject participating in the study, a separate consent form, or a separate specific signature within the main ICF will be required to document a subject's agreement to allow the use of unused or residual biomarker, virologic, and/or PK samples collected as part of the protocol assessments for optional future research.

The specimens collected for optional future research will be used to increase knowledge and understanding of the biology of HBV and related diseases and to study the association of biomarkers with disease pathogenesis, progression and/or treatment outcomes, including efficacy, AEs, and the process of drug absorption and disposition.

These specimens may be used also to develop biomarker or diagnostic assays for HBV and establish the performance characteristics of these assays. The collection and analysis of optional future research specimens will facilitate the rational design of new pharmaceutical agents and the

development of diagnostic tests, which may allow for individualized drug therapy for patients in the future.

Samples will be securely stored by Arbutus or at an Arbutus-approved third party storage management facility for the period specified in the informed consent. Samples will be stored in a coded fashion, and no researcher will have access to the key. The key will be securely held by the Investigator at the clinical site such that there will be no direct ability for a researcher to connect a sample to a specific individual. Additional research samples will be retained for the maximum allowed by applicable law.

Further details of sample collection and processing will be provided to the site in the Study's Laboratory Manual.

8.11. Medical Resource Utilization and Health Economics

No Medical Resource Utilization or Health Economics parameters are evaluated in this study.

9. STATISTICAL CONSIDERATIONS

9.1. Statistical Hypotheses

No hypothesis will be formally tested in this study.

9.2. Sample Size Determination

Approximately 80 subjects will be screened to achieve approximately 40 dosed subjects, who are stably NA-suppressed, HBeAg negative, with non-cirrhotic CHB. After a 24-week lead-in period of AB-729 60 mg SC Q8W added to ongoing SOC NA, subjects will be randomized into one of 4 groups:

- A1: AB-729 + NA + weekly Peg-IFNα-2a for 24 weeks (N = 12)
- A2: NA + weekly Peg-IFNα-2a for 24 weeks (N = 12)
- B1: AB-729 + NA + weekly Peg-IFNα-2a for 12 weeks (N = 8)
- B2: NA + weekly Peg-IFNα-2a for 12 weeks (N = 8)

Treatment assignment will be stratified by HBsAg level at Week 24 (HBsAg ≤100 IU/mL vs >100 IU/mL).

The sample size for this study is based on clinical rather than statistical rationale. The sample size is considered adequate to support the planning and design of future studies.

9.3. Populations for Analyses

For purposes of analysis, the following populations are defined:

Table 9. Populations for Analysis

Population	Description
Safety Lead-in	All subjects who take at least 1 dose of study treatment in the Lead-in period
Randomized (intent-to-treat)	All subjects randomly assigned to study treatment
Safety	All subjects who take at least 1 dose of study treatment. Subjects' data will be analyzed according to the treatment they actually received.
Per Protocol	All subjects in the Safety Population who have no major protocol violations
PK	All subjects in the Safety Population with PK samples adequate for the calculation of PK parameters.

9.4. Statistical Analyses

The statistical analysis plan (SAP) will be developed and finalized before database lock and will describe the subject populations to be included in the analyses (see also Table 9), and procedures to account for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary, secondary, and exploratory endpoints.

In general, descriptive summaries will include n, mean, standard deviation, median, minimum, and maximum, for continuous variables, and n and percent for categorical variables. Summaries will present data by dose group and where appropriate, by assessment time. Geometric means, CV% and associated 95% confidence interval will be presented for PK parameters (except time of maximum observed plasma concentration [T_{max}]).

The data summaries will be provided by treatment period and treatment arm.

9.4.1. Safety Analyses

All safety analyses will be performed on the Safety and Safety Lead-in Population. Safety will be assessed based on AEs, clinical laboratory data, vital signs, ECG parameters, and physical examinations.

Number of doses administered and duration of exposure will be summarized, descriptively, by treatment period and treatment arm. Duration of exposure will be defined as the number of days from the first dose to the last.

9.4.1.1. Adverse Events

AEs will be coded to a Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ classification. Any events reported after the initiation of study treatment and up to 12 weeks after the last dose of AB-729 or 28 days after the last dose of Peg-IFNα-2a, are defined as treatment-emergent. The occurrence of treatment-emergent AEs will be summarized using MedDRA preferred terms, system organ classifications, and severity. Separate summaries of treatment-emergent SAEs and AEs considered related to study treatment, and AEs leading to

study treatment discontinuation, will be generated. All AEs will be listed for individual subjects, showing both verbatim and preferred terms.

9.4.1.2. Clinical Laboratory Results

Laboratory abnormalities will be graded according to the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Corrected Version 2.1 [37]. Any graded abnormality that occurs following the initiation of study treatment and represents at least a 1-grade increase from the baseline assessment, is defined as treatment-emergent. The number and percentage of subjects experiencing treatment-emergent graded toxicities will be summarized. Laboratory toxicity shifts from baseline to worst post-baseline assessments will be summarized.

Listings of the clinical laboratory test results will be provided. Abnormal laboratory values will be flagged in the listings.

9.4.1.3. Other Parameters

Descriptive summaries of vital signs, weight, ECG parameters, and clinical laboratory results will be presented.

Concomitant medications will be coded using the World Health Organization (WHO) drug dictionary and summarized.

9.4.2. Immune Biomarkers

The exploratory endpoints of soluble immune markers, immune complex levels, cytokines, inflammatory and HBV-specific immune markers on PBMCs may be reported separately from the clinical study report. The change from baseline in these markers (if applicable) will be summarized by treatment in tabular and graphic format using descriptive statistics.

The exploratory endpoint to examine potential polymorphisms in immune and inflammatory genes in humans may be reported separately from the clinical study report. Listings of polymorphisms (if applicable) will be summarized by treatment in tabular and graphic format using descriptive statistics.

9.4.3. Pharmacodynamic and Efficacy Analyses

The change from baseline in HBV DNA, HBV RNA, HBsAg, HBsAb, HBcrAg, HBeAg, and HBeAb will be summarized by treatment and visit in tabular and graphic format using descriptive statistics.

The proportion of subjects with HBsAg, HBV RNA and/or HBcrAg reduction of ≥0.5, 1, 2, or 3 $\log_{10}$ from baseline; ≤LLOQ or TND will be presented along with 95% exact binomial interval for each treatment group.

In addition to the descriptive statistics, treatment comparisons may be performed. The continuous variables, such as mean change from baseline, will be evaluated using a regression model with baseline values and stratification factor as covariates. The binary variables, such as the proportion of participants who meet the endpoint criterion, will be evaluated using a Cochran-Mantel-Haenszel test adjusting for the stratification factor. Details will be provided in the SAP.

The exploratory endpoints of HBsAg isoforms, RNAi PD marker levels, and HBV-related miRNAs may be reported separately from the clinical study report. The change from baseline in these markers (if applicable) will be summarized by treatment in tabular and graphic format using descriptive statistics.

9.4.4. Pharmacokinetic Analyses

Bioanalytical summary report(s) will include the analytical results, stability of the frozen samples, a summary of the standard curves and quality control samples, and results of the incurred sample reanalysis, if applicable. Residual plasma may be archived for exploratory metabolite analysis that would be reported separately from the clinical study report.

Pharmacokinetic analysis as defined in the SAP will be conducted using the PK population defined in Section 9.3. In the event of discrepancies between analyses described in the SAP and this clinical study protocol, the SAP will supersede the protocol.

- All samples will be analyzed and all concentrations listed.
- The listing of PK concentrations will be flagged for subjects who did not receive all doses and any other significant protocol deviations.
- Descriptive statistics will be performed for all time points available, with the exclusion of subjects who did not receive all doses or any other significant protocol deviations.
- Pharmacokinetic parameters will be derived where possible for all subjects. Data from subjects with incomplete profiles (missed blood draws, lost samples, samples unable to be quantified) may be used if PK parameters can be estimated using the remaining data points.
- Descriptive statistics will be performed on all parameters available, and any missing parameters will be flagged.
- Plasma PK parameters of AB-729 AS, AB-729 AS(N-1)3′, and AB-729 AS(N-2)3′ for each subject will be estimated over the sampling interval using non-compartmental analysis and summarized by treatment group using descriptive statistics. The plasma PK parameters that will be estimated, if feasible, are listed in the table below. Additional parameters may be analyzed, as appropriate.

Table 10. Pharmacokinetic Parameters for AB-729

Pharmacokinetic Parameter	Definition
C_{max}	Maximum observed plasma concentration
T_{max}	Time of maximum observed plasma concentration
AUC_{0-t}	Area under the concentration time- curve from the time of dosing to the last measurable concentration

Actual blood sampling times will be used for PK analysis. In all derivations of PK parameters, zero will be substituted for concentrations below the quantification limit (BQL) of the assay prior to the first quantifiable sample. Samples which are otherwise BQL will be treated as missing.

9.5. Interim Analyses

There are no formal interim analyses planned for this study, however, a DMC will periodically review safety data from this study.

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11. APPENDICES FOR SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

Appendix 1 Abbreviations and Trademarks

Abbreviation	Definition
Arbutus	Arbutus Biopharma Corporation
AE	adverse event
AFP	alpha fetoprotein
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
AS	anti-sense
AST	aspartate aminotransaminase
BMI	body mass index
BQL	below the quantification limit
CHB	chronic hepatitis B
CRF	case report form, electronic or paper
CRO	contract research organization
CYP	Cytochrome P450
DAIDS	Division of AIDS (acquired immunodeficiency syndrome)
DILI	drug induced liver injury
ECG	electrocardiogram
ETV	entecavir
FSH	follicle stimulating hormone
GalNAc	*N*-Acetylgalactosamine
GCP	Good Clinical Practice
HAV	hepatitis A virus
HBcAb	hepatitis B core antibody
HBcrAg	hepatitis B virus core related antigen
HBeAb	hepatitis B virus e-antibody
HBeAg	hepatitis B virus e-antigen
HBsAb	hepatitis B virus surface antibody
HBsAg	hepatitis B virus surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDV	hepatitis D virus
HEV	hepatitis E virus
HIV	human immunodeficiency virus
HRT	hormonal replacement therapy
IB	Investigator’s Brochure
ICF	informed consent form
ICH	International Council on Harmonisation
IEC	Institutional Ethics Committee
IFNα	interferon alpha

Abbreviation	Definition
IgM	immunoglobulin M
IL	interleukin
INR	international normalized ratio
IRB	institutional review board
IUD	intrauterine device
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
miRNA	microRNAs
NA	nucleos(t)ide analogue
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamic
Peg-IFNα	pegylated interferon alfa
PK	pharmacokinetic(s)
PT	prothrombin time
QTcF	QT interval corrected using Fridericia's formula
QW	every week
RNA	ribonucleic acid
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	subcutaneous
siRNA	small interfering ribonucleic acid
sPD-1	soluble programmed death-1
TAF	tenofovir alafenamide
TDF	tenofovir disoproxil fumarate
TEAE	treatment emergent adverse event
TND	target not detected
ULN	upper limit of normal
WOCBP	women of childbearing potential

Appendix 2 Regulatory, Ethical, and Study Oversight Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
  - Applicable International Council on Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., subject recruitment materials) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.
- The Investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.
  - Providing oversight of the conduct of the study at the site and adherence to applicable requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies, and all other applicable local regulations.

Financial Disclosure

Investigators and Sub-Investigators must provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the study and for 1 year after completion of the study.

Informed Consent Process

- The Investigator or his/her representative will explain the nature of the study to the subject and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary. Subjects will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) or other privacy law requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the subject.

A subject who is rescreened is not required to sign another ICF if the rescreening occurs within 60 days from the previous ICF signature date.

A separate ICF will be provided that addresses the use of remaining mandatory samples for optional exploratory research. The Investigator or authorized designee will explain to each subject the objectives of the exploratory research. Subjects will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. If a study subject refuses or withdraws consent to have their samples used for optional exploratory research, the samples will be destroyed accordingly as per local requirements. A signature on the separate, optional ICF will be required to document a subject's agreement to allow any remaining specimens to be used for exploratory research.

Data Protection

- Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.
- The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject.

- The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Data Monitoring Committee Structure

The DMC will be a group of 3 independent members who will operate in accordance with a pre-specified charter and make recommendations to the Sponsor.

Data Quality Assurance

- All subject data relating to the study will be recorded on printed or electronic CRF(s) unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct, and to confirm this by physically or electronically signing the CRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for 5 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Source Documents

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Study and Site Closure

The Sponsor or their designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local Health Authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the Investigator
- Discontinuation of further study treatment development.

Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Appendix 3 Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

    Note: Documentation can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.
3. Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high FSH level in the postmenopausal range (>40 mIU/mL) may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
    - Females on HRT and whose menopausal status is in doubt will be required to use one of the non- hormonal contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

Male subjects

Male subjects with female partners of childbearing potential are eligible to participate if they agree to ONE of the following during the protocol-defined time frame in Section 5.1:

- Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent.

- Agree to use a male condom **plus** partner-use of a contraceptive method with a failure rate of <1% per year as described in Table 11 when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.

Male subjects with a pregnant partner are not eligible to enroll in this study.

Female subjects

Female subjects of childbearing potential are eligible to participate only if they agree to use a highly effective method of contraception consistently and correctly as described in Section 5.1. Female study subjects and female partners of male study subjects may use any of the highly effective methods shown in Table 11.

Table 11. Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.* Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation**:** • Oral • Intravaginal • Transdermal Progestogen only hormonal contraception associated with inhibition of ovulation**:** • Oral • Injectable Sexual abstinence *Sexual abstinence is considered a highly effective method **only** if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.*
Highly Effective Contraceptive Methods That Are User-Independent[b] • Implantable progestogen only hormonal contraception associated with inhibition of ovulation • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • Bilateral tubal occlusion • Vasectomized partner: *A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.*

a. Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for subjects participating in clinical studies.
b. User-independent methods do not require consistent interaction (between the user and the method) to assure their success rate.

Less effective contraceptive methods that are NOT permitted as the primary contraceptive method for use in this study include:

- Progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
- Male or female condom, with or without spermicide (male and female condoms used together do not constitute a highly effective method)
- Cervical cap, diaphragm or sponge with spermicide

Any of these methods above may be used as an additional method of contraception in addition to one of the highly effective methods in Table 11 if required by local regulations.

Ineffective contraceptive methods that are NOT permitted for use in this study include:

- Periodic abstinence (calendar, symptothermal, post-ovulation methods)
- Withdrawal (coitus interruptus)
- Spermicides only
- Lactational amenorrhea method

Pregnancy Testing:

- WOCBP should only be included after a confirmed menstrual period and a negative urine pregnancy test.
- Additional pregnancy testing should be performed during the study period and after the last dose of study treatment as in Section 1.3, Schedule of Activities, and as required locally.
- Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected

Collection of Pregnancy Information:

Male subjects with partners who become pregnant

The Investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study. This applies only to male subjects who receive active AB-729, if treatment assignment is known at the time the pregnancy is reported.

After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor as soon as possible after learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female subjects who become pregnant

The Investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a subject's pregnancy. The subject will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the subject and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the

estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such. Any post-study pregnancy-related SAE considered reasonably related to the study treatment by the Investigator will be reported to the Sponsor as described in Section 8.7.4. While the Investigator is not obligated to actively seek this information in former study subjects, he or she may learn of an SAE through spontaneous reporting.

Any female subject who becomes pregnant while participating in the study will discontinue study treatment and be withdrawn from the study.

Appendix 4 Clinical Laboratory Tests

Laboratory tests are to be completed after a fast of at least 8 hours (including refraining from ingestion of alcohol or caffeine-containing products) and, during the treatment period, should be drawn prior to dosing.

Local laboratory results are only required if the central laboratory results are not available in time for either study treatment administration and/or response evaluation. If a local sample is required, it is important that the sample for central analysis is obtained at the same time. Additionally, if the local laboratory results are used to make either a study treatment decision or response evaluation, the results must be entered into the CRF.

Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Virology data or other laboratory/analyte results that could bias the conduct of the study will not be reported to investigative sites until the subject reaches the end of the first 24 week follow up period to evaluate if NA discontinuation criteria have been met.

Investigators must document their review of each laboratory safety report.

Table 12. Protocol-Required Laboratory Assessments

Laboratory Assessments	Parameters	
Hematology	Platelet Count RBC Count Hemoglobin Hematocrit	<u>WBC count with differential:</u> Neutrophil count Lymphocyte count Monocyte count Eosinophil count Basophil count
Clinical Chemistry	Sodium Potassium Glucose Chloride CO_2 BUN Creatinine Calcium Phosphorus Magnesium Uric acid Lactate dehydrogenase Lipase Albumin Total protein CPK	<u>Liver Function Tests</u>: AST ALT Alkaline phosphatase Bilirubin (total and direct) GGT
Coagulation	PT / INR aPTT	
Endocrine	TSH	
Urine Parameters	<u>Urinalysis</u>: Specific gravity pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick Microscopic examination (reflex if dipstick is abnormal)	
Virology Tests	HBV Genotype <u>Quantitative tests:</u> HBsAb HBsAg HBsAg isoforms HBcrAg HBeAg HBV DNA HBV RNA Ultrasensitive HBsAg	Resistance sample <u>Qualitative tests:</u> HBcAb IgM (screening only) HBeAg (screening only) HBeAb

Laboratory Assessments	Parameters
Biomarkers	HBV-related miRNAs Soluble immune biomarkers/immune complexes PBMCs
Genotype (24 Week Lead-In Period only)	IL28B Genotype
Pharmacogenomics (24 Week Lead-In Period only)	Exploratory immune genes SNP Sample
Other Screening Tests	FSH (as needed in postmenopausal females only) hCG pregnancy test (as needed for women of childbearing potential only)[a] Serology– HIV, HCV, and HDV antibody AFP, HbA_1C, and TSH/Free T4 ANA, ASMA, anti-LKM1 serology

Abbreviations: AFP = alpha fetoprotein; ALT = alanine aminotransferase; ANA = antinuclear antibody; anti-LKM1 = liver/kidney microsomal antibody type 1; aPTT = activated partial thromboplastin time; ASMA = anti-smooth muscle antibody; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CO_2 = carbon dioxide; CPK = creatine phosphokinase; DNA = deoxyribonucleic acid; FSH = follicle stimulating hormone; GGT = gamma glutamyl transpeptidase; HbA_1C = whole blood hemoglobin $A1_c$; HBcAb = hepatitis B core antibody; HBV = hepatitis B virus; HBeAb = HBV e-antibody; HBeAg = HBV e antigen; HBsAb = HBV surface antibody; HBsAg = HBV surface antigen; HBcrAg = HBV core antigen; hCG = human chorionic gonadotropin; HCV = hepatitis C virus; HDV = hepatitis D virus; HIV = human immunodeficiency virus; IL28B = interleukin 28B; INR = international normalized ratio; miRNA = micro ribonucleic acid; PBMCs = peripheral blood mononuclear cells; PT = prothrombin time; RBC = red blood cells; RNA = ribonucleic acid; TSH = thyroid stimulating hormone; T4 = thyroxine; WBC = white blood cells.

a. Local hCG pregnancy urine testing will be standard for the protocol unless serum/plasma testing is required by local regulation or IRB/IEC. See Schedule of Activities (Section 1.3) for required pregnancy testing throughout the study.

Appendix 5 Immune and Inflammatory Genes for Pharmacogenomic Testing

Immune and inflammatory genes for analysis will be tested via the Affymetrix® GeneChip® Human Immune and Inflammation 9K SNP Kit:

http://tools.thermofisher.com/content/sfs/brochures/humanimmune_9k_snp_datasheet.pdf

Specific genes and polymorphisms are listed in the following file:
http://www.affymetrix.com/Auth/support/technical/panelfiles/Immu-Inflammation9K_rev1.zip

AFFYMETRIX®

Data Sheet

Affymetrix® GeneChip® Human Immune and Inflammation 9K SNP Kit

The Affymetrix GeneChip® Human Immune and Inflammation 9K SNP Kit contains over 9,000 SNPs in approximately 1,000 genes that have been implicated in, or are thought to be involved in, immune and inflammatory responses in humans. The 1,000 covered genes were selected in collaboration with leading researchers in the field. HapMap data from multiple populations were used to select SNPs to tag common (>5%) polymorphisms in these genes. In addition, the panel includes ~800 validated non-synonymous SNPs. The panel allows researchers engaged in studies of the immune system and inflammatory response to perform cost-effective genotyping. The Immune and Inflammation 9K SNP Kit is designed to work with the Affymetrix GeneChip® Scanner 3000 (with the Targeted Genotyping upgrade).

Features and Benefits

- A gene-focused approach to immune and inflammation studies has the potential to reduce genotyping and multiple testing costs.
- The use of tagging SNPs results in high genomic coverage for over 1,000 genes involved in immune and inflamation response.
- Tagging SNPs were selected using samples from multiple populations, resulting in a panel that is suitable for genotyping both Caucasian and non-Caucasian populations.
- The inclusion of potentially functional non-synonymous SNPs increases the possibility of directly detecting the causative variant.
- Significant associations can be linked directly to biological pathways and gene function.

Key Specifications

- Accuracy ≥99.25 percent
- Data Completeness ≥98 percent
- Repeatability ≥99.25 percent
- Quantity of genomic DNA required without amplification is 4.0 µg
- Throughput of 48 samples per day (~0.5 million genotypes/day)
- SNPs selected from HapMap #17. SNP and gene annotations from Ensembl build 34 (dbSNP build 124)

Panel Design

Using Molecular Inversion Probe (MIP) technology, Affymetrix has developed an application-specific SNP genotyping panel for studying the immune system and inflammatory response. The panel is designed to cover over 1,000 candidate genes related to immunity and inflammation that were selected in collaboration with leading researchers in the field. The panel is suitable for studies in multiple ethnic populations. These studies can exploit the MIP technology's ability to develop large multiplex panels in a single assay to conduct affordable, large-scale genetic studies in two important areas: identifying the genes responsible for autoimmune diseases such as lupus, multiple sclerosis and rheumatoid arthritis; and collecting information on individual susceptibility to infectious disease or patient response to immunization, for example, measuring the genetic basis of patient response to the flu vaccine.

The panel is designed to cover ~1,000 genes (coverage of certain selected GO categories is shown in Figure 1). Tagging SNPs were selected to result in an r^2 coverage of ≥0.8 for all HapMap SNPs with MAF greater than 5 percent. These tagging SNPs were selected based on genotypes in the HapMap CEPH and Yoruban samples and covered SNPs from 5 kb upstream and 5 kb downstream of the gene. Additionally, 773 non-synonymous (amino acid-changing) SNPs were selected in these genes from the 20K panel (see the Affymetrix GeneChip® Human 20K cSNP Kit). The

average number of all types of SNPs per gene is 10. Following design and manufacture of MIP probes, conversion to working assays was very high (>92 percent), resulting in a panel of approximately 9,200 working assays.

Panel Performance

In actual studies using this panel, 88 DNA samples from 76 unique individuals (CEPH Utah HapMap samples plus one anonymous sample) were genotyped for a total of 824,729 genotypes. Accuracy, measured using Mendelian inheritance across 25 trios, was 99.90 percent. In addition, reviewing the SNPs that overlapped with reference genotypes generated in the HapMap project, concordance was measured with these genotypes as 99.71 percent. Repeatability, measured across six different individuals who were each genotyped two to eight times, was 99.95 percent. Data completeness, the average call rate across passed samples for passed SNPs, was measured on genotypes across the panel at 99.57 percent.

Figure 1: Representation of certain Gene Ontology (GO) categories for the ~1,000 genes represented on the Affymetrix GeneChip® Human Immune and Inflammation 9K SNP Kit. Note that many genes appear in multiple GO categories.

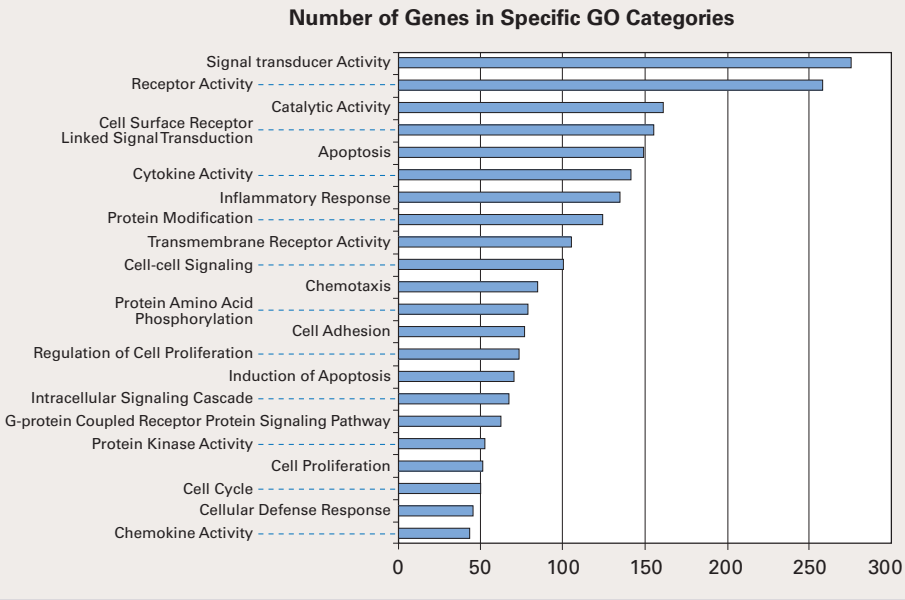
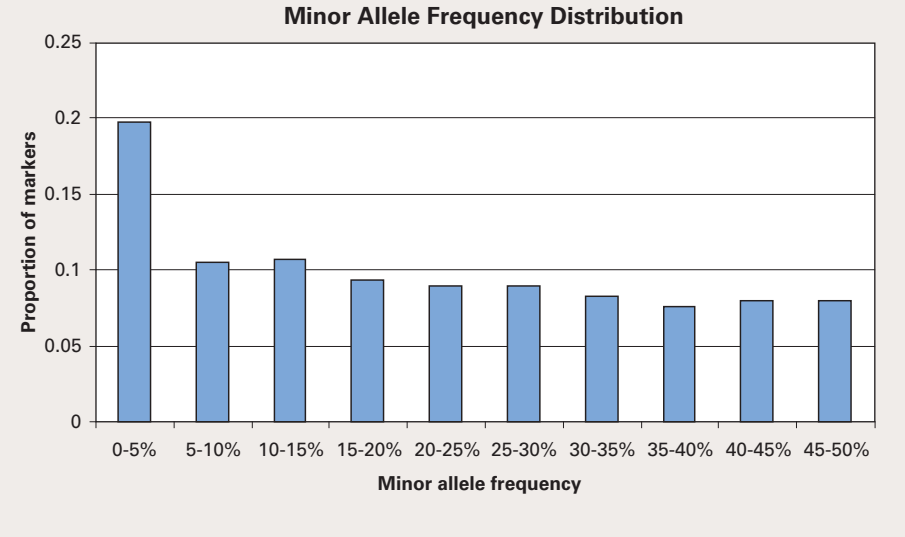


Figure 2: CEPH HapMap sample. Minor allele frequencies of the 9,200 SNPs that are present in the Affymetrix GeneChip® Human Immune and Inflammation 9K SNP Kit. SNPs with low minor allele frequencies in CEPH were either picked as tagging SNPs in the Yoruban HapMap samples (and hence were above 5% in that population) or are non-synonymous SNPs (the panel includes only nsSNPs validated as polymorphic in at least one HapMap population).



Notes:

Ordering Information

Affymetrix GeneChip® Human Immune and Inflammation 9K SNP Kit

900868	*Contains enough reagents to process a total of 24 samples (including one control)*

Affymetrix GeneChip® Universal 10K Tag Array

900604	*(6 pack) Arrays have approximately 10K features on each array that can detect 10K SNPs using the Affymetrix GeneChip® DNA Analysis System incorporating MIP technology*
900580	*(96 pack)*

To Order

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Part No. 702189 Rev. 3

Number	Target Id	TrueTag_10K_A	Tag Id	Assay Allele	Switched?	Assay Id	External Id	Map	Chromosome	Position (bp)	Gene
1	-	270	A/C	0	389095	rs1043730	Human NCBI Build 35	6	112008146	C6orf4	
3	-	921	A/C	0	389121	rs11141915	Human NCBI Build 35	9	87465348	DAPK1	
4	-	1297	A/C	0	389136	rs1165679	Human NCBI Build 35	12	102825705	TRA1	
5	-	1610	A/C	0	389153	rs1256061	Human NCBI Build 35	14	63773346	ESR2	
6	-	1939	A/C	0	389169	rs1322393	Human NCBI Build 35	6	137376639	IL20RA	
7	-	2567	A/C	0	389199	rs1713918	Human NCBI Build 35	7	2717428	CARD11	
10	-	2823	A/C	0	389213	rs1861759	Human NCBI Build 35	16	49303084	CARD15	
12	-	3185	A/C	0	389235	rs2052016	Human NCBI Build 35	7	156910465	PTPRN2	
13	-	3264	A/C	0	389240	rs2071555	Human NCBI Build 35	6	33013064	HLA-DMB	
18	-	4642	A/C	0	389298	rs2280148	Human NCBI Build 35	17	73865975	SOCS3	
19	-	5224	A/C	0	389327	rs2302821	Human NCBI Build 35	9	129581435	PTGES-ENSG00000198665	
20	-	5747	A/C	0	389356	rs25887	Human NCBI Build 35	5	131443960	CSF2	
21	-	6148	A/C	0	389378	rs3132453	Human NCBI Build 35	6	31712023	BAT3-BAT2	
22	-	6414	A/C	0	389398	rs3739070	Human NCBI Build 35	2	239088268	none	
23	-	6438	A/C	0	389400	rs3743249	Human NCBI Build 35	15	97322946	IGF1R-ENSG00000184474	
24	-	6727	A/C	1	389433	rs3802428	Human NCBI Build 35	9	34557200	CNTFR	
26	-	6999	A/C	0	389459	rs406231	Human NCBI Build 35	19	59442877	LILRB5-ENSG00000186152	
30	-	7502	A/C	0	389528	rs5743704	Human NCBI Build 35	4	154983556	TLR2	
31	-	7665	A/C	1	389564	rs6752445	Human NCBI Build 35	2	105930763	NCK2	
32	-	7705	A/C	0	389571	rs6961856	Human NCBI Build 35	7	157366332	PTPRN2	
33	-	7720	A/C	0	389575	rs706780	Human NCBI Build 35	10	6127032	IL2RA	
34	-	7721	A/C	0	389576	rs7079591	Human NCBI Build 35	10	133628642	BNIP3	
35	-	7859	A/C	0	389598	rs7543182	Human NCBI Build 35	1	71051994	PTGER3	
36	-	7891	A/C	0	389603	rs759171	Human NCBI Build 35	7	54860421	EGFR	
38	-	8208	A/C	0	389653	rs9282734	Human NCBI Build 35	7	45730209	IGFBP3	
39	-	8322	A/C	0	389675	rs9911257	Human NCBI Build 35	17	59365575	SCN4A-CD79B	
40	-	8432	A/C	1	389692	rs1042308	Human NCBI Build 35	6	33144831	HLA-DPA1	
42	-	8743	A/C	0	389760	rs13312727	Human NCBI Build 35	16	65745944	TRADD-ENSG00000180561	
43	-	8760	A/C	0	389765	rs14004	Human NCBI Build 35	6	32515687	HLA-DRA-HLA-DRA	
44	-	8805	A/C	0	389778	rs1610555	Human NCBI Build 35	18	65694127	CD226	
45	-	8813	A/C	0	389780	rs1638025	Human NCBI Build 35	7	156946842	PTPRN2	
47	-	8882	A/C	0	389796	rs1801033	Human NCBI Build 35	5	41235716	C6	
49	-	9119	A/C	1	389842	rs2227580	Human NCBI Build 35	10	75341362	C10orf55-PLAU	
51	-	9158	A/C	1	389851	rs2241715	Human NCBI Build 35	12	121217700	ENSG00000176383-ENSG00000158113-DIABLO-ENSG00000197247	
52	-	9194	A/C	1	389856	rs2247584	Human NCBI Build 35	1	156072753	FCER1A-ENSG00000196266	
53	-	9320	A/C	0	389885	rs2304738	Human NCBI Build 35	18	75322195	NFATC1	
54	-	9350	A/C	0	389890	rs2335851	Human NCBI Build 35	7	157425076	PTPRN2	
55	-	9397	A/C	0	389894	rs2523809	Human NCBI Build 35	6	29957598	HLA-G	
56	-	9478	A/C	0	389911	rs2889982	Human NCBI Build 35	9	87528085	DAPK1	
57	-	9556	A/C	0	389926	rs3138038	Human NCBI Build 35	17	29671944	CCL8	
58	-	9598	A/C	0	389931	rs3212254	Human NCBI Build 35	14	23875303	RIPK3-ADCY4	
59	-	9655	A/C	1	389943	rs366510	Human NCBI Build 35	19	6648829	C3	
60	-	9719	A/C	0	389951	rs3741410	Human NCBI Build 35	11	48125240	PTPRJ	
61	-	9735	A/C	1	389953	rs3745376	Human NCBI Build 35	19	7739690	ENSG00000104938	
62	-	10017	A/C	0	389991	rs3806501	Human NCBI Build 35	2	136710801	ENSG00000121966	
63	-	10115	A/C	0	390011	rs3917148	Human NCBI Build 35	14	75516274	TGFB3	

64	-	10302	A/C	0	390035	rs436719	Human NCBI Build 35	1	193519401	CFHL1
65	-	10304	A/C	1	390036	rs4376721	Human NCBI Build 35	1	117434222	ENSG00000134258
66	-	10317	A/C	0	390038	rs446125	Human NCBI Build 35	20	42700679	ADA
67	-	10347	A/C	0	390043	rs4541741	Human NCBI Build 35	6	41772010	TFEB
68	-	10522	A/C	1	390069	rs4987768	Human NCBI Build 35	18	59055497	BCL2
70	-	10649	A/C	0	390092	rs6136	Human NCBI Build 35	1	166295609	SELP
71	-	10659	A/C	0	390094	rs6180	Human NCBI Build 35	5	42754996	GHR
72	-	10744	A/C	0	390105	rs6494457	Human NCBI Build 35	15	62050585	ENSG00000035664
76	-	10974	A/C	1	390140	rs7170924	Human NCBI Build 35	15	79370194	IL16
79	-	11088	A/C	1	390164	rs744690	Human NCBI Build 35	12	50727751	NR4A1
82	-	11352	A/C	0	390204	rs874627	Human NCBI Build 35	12	6804561	LEPREL2-CD4
83	-	11358	A/C	1	390207	rs8773	Human NCBI Build 35	9	90740262	SYK
84	-	11605	A/C	0	390243	rs9958610	Human NCBI Build 35	18	46395496	MAPK4
85	-	11621	A/C	1	390245	rs1002839	Human NCBI Build 35	7	38102637	TRGV9
86	-	11672	A/C	0	390252	rs1009950	Human NCBI Build 35	7	157035924	PTPRN2-ENSG00000197192
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90	-	11879	A/C	0	390300	rs11147439	Human NCBI Build 35	13	30223643	ALOX5AP
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100	-	90	A/C	0	390451	rs1990384	Human NCBI Build 35	12	6213182	CD9
101	-	3367	A/C	0	390465	rs2071409	Human NCBI Build 35	17	53703229	LPO-MPO
102	-	4391	A/C	0	390473	rs2116941	Human NCBI Build 35	19	10195443	EDG5
103	-	4370	A/C	0	390477	rs2228059	Human NCBI Build 35	10	6042374	IL15RA
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108	-	2738	A/C	0	390552	rs2298872	Human NCBI Build 35	1	195456899	PTPRC
110	-	5356	A/C	1	390676	rs3800313	Human NCBI Build 35	6	27318743	PRSS16
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112	-	3144	A/C	1	390705	rs3918245	Human NCBI Build 35	20	44070317	MMP9
113	-	5441	A/C	0	390726	rs4359426	Human NCBI Build 35	16	55950234	CCL22
115	-	1256	A/C	0	390813	rs657770	Human NCBI Build 35	17	44936149	NGFR
116	-	1670	A/C	1	390831	rs6922865	Human NCBI Build 35	6	36202166	MAPK13
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118	-	5780	A/C	0	390899	rs80579	Human NCBI Build 35	22	34864761	APOL3
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587	-	9515	A/C	0	396804	rs3734099	Human NCBI Build 35	5	169055381	DOCK2
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592	-	9555	A/C	1	396838	rs3819409	Human NCBI Build 35	6	135567089	MYB
593	-	9567	A/C	0	396848	rs3918227	Human NCBI Build 35	7	150138594	NOS3-NOS3AS
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659	-	10308	A/C	0	397480	rs7244913	Human NCBI Build 35	18	8241524 PTPRM		
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2327	-	4413	A/G	1	393479	rs1124374	Human NCBI Build 35	3	37825081	ITGA9
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2339	-	5593	A/G	0	393517	rs12477946	Human NCBI Build 35	2	86955453	CD8B1
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2344	-	4466	A/G	1	393526	rs12693932	Human NCBI Build 35	2	201918901	CASP10-CASP8
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4808	-	11575	A/T	0	398544	rs646910	Human NCBI Build 35	11	102214732	MMP3
4809	-	11583	A/T	0	398550	rs6621956	Human NCBI Build 35	X	104333784	IL1RAPL2
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4845	-	11988	A/T	1	398874	rs174405	Human NCBI Build 35	6	112036915	C6orf4
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4940	-	8400	C/G	1	389687	rs10261447	Human NCBI Build 35	7	157449881	PTPRN2
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4942	-	8484	C/G	0	389702	rs1057401	Human NCBI Build 35	12	107188475	CMKLR1
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4944	-	8505	C/G	0	389707	rs10754555	Human NCBI Build 35	1	243910684	CIAS1
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5283	-	3929	C/G	1	393202	rs6474030	Human NCBI Build 35	8	56980702	LYN

5284	-	3222	C/G	1	393215	rs6550507	Human NCBI Build 35	3	37764386	ITGA9
5285	-	437	C/G	0	393223	rs6661764	Human NCBI Build 35	1	204196780	none
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5288	-	4116	C/G	1	393233	rs6741949	Human NCBI Build 35	2	162735730	DPP4
5289	-	5136	C/G	0	393238	rs6796131	Human NCBI Build 35	3	191794834	IL1RAP
5290	-	180	C/G	0	393260	rs7169463	Human NCBI Build 35	15	64532983	MAP2K1
5291	-	4552	C/G	0	393262	rs7183706	Human NCBI Build 35	15	97092712	IGF1R
5292	-	2388	C/G	0	393307	rs7705216	Human NCBI Build 35	5	35229426	PRLR
5293	-	2662	C/G	0	393319	rs7855263	Human NCBI Build 35	9	89315012	SEMA4D
5294	-	2237	C/G	1	393323	rs7896632	Human NCBI Build 35	10	71507697	H2AFY2-AMID
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5300	-	4502	C/G	0	393351	rs866484	Human NCBI Build 35	1	155799550	IFI16
5301	-	235	C/G	0	393352	rs867375	Human NCBI Build 35	9	113404964	RGS3
5302	-	1422	C/G	1	393372	rs9331888	Human NCBI Build 35	8	27524779	CLU
5303	-	636	C/G	1	393375	rs9426418	Human NCBI Build 35	1	29474452	PTPRU
5304	-	5264	C/G	0	393388	rs966065	Human NCBI Build 35	1	165278832	XCL1
5305	-	1909	C/G	0	393426	rs10482810	Human NCBI Build 35	1	214995927	ENSG00000092969
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5310	-	3107	C/G	1	393468	rs1105238	Human NCBI Build 35	1	158413319	ENSG00000132185
5311	-	4071	C/G	0	393474	rs11152370	Human NCBI Build 35	18	58986489	BCL2
5312	-	821	C/G	0	393482	rs1142881	Human NCBI Build 35	19	19523	KIR2DS2-KIR2DS1-KIR2DL2
5313	-	1090	C/G	0	393492	rs1169670	Human NCBI Build 35	3	10198607	IRAK2
5314	-	6220	C/G	0	393503	rs12063780	Human NCBI Build 35	1	203663689	C4BPB-C4BPA
5315	-	5532	C/G	0	393507	rs12099964	Human NCBI Build 35	12	92750261	CRADD
5316	-	5252	C/G	0	393510	rs12255504	Human NCBI Build 35	10	91082169	IFIT3-LIPA
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5319	-	1859	C/G	0	393536	rs130654	Human NCBI Build 35	22	37967154	PDGFB
5320	-	4611	C/G	0	393537	rs13084656	Human NCBI Build 35	3	46255198	CCR3
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5322	-	570	C/G	0	393556	rs1389834	Human NCBI Build 35	5	35916144	IL7R
5323	-	2732	C/G	1	393562	rs1427804	Human NCBI Build 35	3	42845562	CCBP2
5324	-	608	C/G	0	393566	rs1460834	Human NCBI Build 35	9	90649615	SYK
5325	-	4767	C/G	0	393571	rs1473418	Human NCBI Build 35	18	59137529	BCL2
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5328	-	4387	C/G	0	393617	rs1867539	Human NCBI Build 35	5	41254463	C6
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5336	-	2399	C/G	1	393716	rs2292863	Human NCBI Build 35	17	42724129	ITGB3
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5339	-	1777	C/G	0	393731	rs2366216	Human NCBI Build 35	3	61540127	PTPRG
5340	-	8	C/G	0	393732	rs2416806	Human NCBI Build 35	9	120769846	TRAF1
5341	-	507	C/G	0	393733	rs2422666	Human NCBI Build 35	20	1851108 PTPNS1	
5342	-	5281	C/G	0	393758	rs2719240	Human NCBI Build 35	8	57032602	LYN
5343	-	576	C/G	0	393768	rs2806878	Human NCBI Build 35	1	117286563	IGSF2
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5345	-	395	C/G	0	393773	rs2838023	Human NCBI Build 35	21	41645369	FAM3B
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5348	-	896	C/G	0	393824	rs344548	Human NCBI Build 35	19	6636817 C3	
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5352	-	2636	C/G	0	393853	rs3782905	Human NCBI Build 35	12	46552434	VDR
5353	-	394	C/G	0	393860	rs3792231	Human NCBI Build 35	2	196856534	STK17B
5354	-	4500	C/G	0	393865	rs3794345	Human NCBI Build 35	13	23127046	TNFRSF19
5355	-	5215	C/G	0	393870	rs3805433	Human NCBI Build 35	5	150415673	TNIP1
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5357	-	3906	C/G	0	393915	rs4572808	Human NCBI Build 35	3	32292718	CKLFSF8
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5378	-	870	C/G	0	394104	rs7571586	Human NCBI Build 35	2	201965444	CASP8
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5386	-	1523	C/G	0	394202	rs9867325	Human NCBI Build 35	3	138101607	NCK1-ENSG00000181882
5387	-	4818	C/G	0	394206	rs9965844	Human NCBI Build 35	18	59029944	BCL2
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5396	-	980	C/G	0	394273	rs10815925	Human NCBI Build 35	9	8702601	PTPRD
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5403	-	6366	C/G	1	394314	rs11809371	Human NCBI Build 35	1	32424207	LCK-HDAC1
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5417	-	6655	C/G	0	394444	rs1894408	Human NCBI Build 35	6	32894811	TAP2-HLA-DOB
5418	-	6663	C/G	0	394450	rs1956870	Human NCBI Build 35	14	59833852	PPM1A
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5451	-	7124	C/G	0	394860	rs6733162	Human NCBI Build 35	2	162739005	DPP4
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5578	-	8798	C/G	0	396232	rs4298142	Human NCBI Build 35	4	111083352	none
5579	-	8802	C/G	0	396234	rs431144	Human NCBI Build 35	19	11359174	EPOR
5580	-	8806	C/G	0	396236	rs4333080	Human NCBI Build 35	3	61688697	PTPRG
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5648	-	9793	C/G	0	397041	rs7758826	Human NCBI Build 35	6	128734935	PTPRK
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5660	-	10026	C/G	0	397242	rs1860129	Human NCBI Build 35	4	111243947	EGF

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5715	-	11178	C/G	1	398210	rs4465173	Human NCBI Build 35	1	211097093	PTPN14-ENSG00000181931

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7233	-	571	C/T	1	392788	rs1927976	Human NCBI Build 35	9	87488187	DAPK1
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7236	-	5972	C/T	0	392799	rs1998651	Human NCBI Build 35	1	157354381	CD84
7237	-	4374	C/T	0	392800	rs2005154	Human NCBI Build 35	9	36846828	PAX5
7238	-	5648	C/T	0	392802	rs2018414	Human NCBI Build 35	2	239995925	HDAC4
7239	-	2424	C/T	0	392807	rs2042370	Human NCBI Build 35	1	15587048	CASP9
7240	-	151	C/T	0	392808	rs2046136	Human NCBI Build 35	18	58964961	BCL2
7241	-	5758	C/T	0	392810	rs2051308	Human NCBI Build 35	18	46448639	MAPK4
7242	-	1386	C/T	0	392812	rs20541 Human NCBI Build 35	5	132023863	IL13	
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7245	-	2790	C/T	0	392818	rs2069933	Human NCBI Build 35	2	127901918	PROC
7246	-	5010	C/T	0	392819	rs2070016	Human NCBI Build 35	4	155867919	FGA
7247	-	2232	C/T	0	392821	rs2070123	Human NCBI Build 35	1	78827926	IFI44
7248	-	1099	C/T	0	392824	rs2071048	Human NCBI Build 35	2	138555329	HNMT
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7250	-	811	C/T	0	392838	rs220065	Human NCBI Build 35	10	31821010	TCF8
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7252	-	4905	C/T	0	392840	rs221300	Human NCBI Build 35	7	156866999	PTPRN2
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7254	-	1737	C/T	1	392848	rs2237051	Human NCBI Build 35	4	111258802	EGF
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7267	-	1621	C/T	0	392881	rs2291375	Human NCBI Build 35	3	106746819	ALCAM
7268	-	709	C/T	1	392886	rs2298749	Human NCBI Build 35	4	111039109	none

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7273	-	3378	C/T	1	392907	rs2467009	Human NCBI Build 35	12	69503359	PTPRR
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7276	-	3768	C/T	1	392920	rs261074	Human NCBI Build 35	5	169241658	DOCK2
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7404	-	1058	C/T	0	393298	rs7637925	Human NCBI Build 35	3	61998507	PTPRG
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7406	-	2206	C/T	0	393316	rs7759295	Human NCBI Build 35	6	41243828	ENSG00000095970
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8154	-	7738	C/T	1	395367	rs2356350	Human NCBI Build 35	2	191828044	STAT4
8155	-	7740	C/T	0	395368	rs2365936	Human NCBI Build 35	3	61623676	PTPRG
8156	-	7743	C/T	0	395370	rs2414906	Human NCBI Build 35	15	64513305	MAP2K1
8157	-	7744	C/T	0	395371	rs2421735	Human NCBI Build 35	1	71111895	PTGER3
8158	-	7746	C/T	0	395373	rs2430608	Human NCBI Build 35	12	12120154	BCL2L14
8159	-	7750	C/T	1	395376	rs2465810	Human NCBI Build 35	12	69301547	PTPRB
8160	-	7751	C/T	0	395377	rs250103	Human NCBI Build 35	5	141966795	FGF1
8161	-	7758	C/T	0	395381	rs253156	Human NCBI Build 35	12	9897687	ENSG00000110852
8162	-	7760	C/T	0	395382	rs2535694	Human NCBI Build 35	22	16533961	BCL2L13
8163	-	7761	C/T	0	395383	rs2551919	Human NCBI Build 35	2	208255889	CREB1
8164	-	7764	C/T	1	395385	rs26120	Human NCBI Build 35	5	149212406	PPARGC1B
8165	-	7769	C/T	0	395390	rs262880	Human NCBI Build 35	5	169086328	DOCK2
8167	-	7772	C/T	0	395393	rs2661686	Human NCBI Build 35	17	73728259	BIRC5-ENSG00000182802
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8170	-	7789	C/T	0	395407	rs2737139	Human NCBI Build 35	17	3613611	ITGAE
8171	-	7794	C/T	0	395410	rs2792247	Human NCBI Build 35	1	161352444	PBX1-ENSG00000197613
8172	-	7796	C/T	0	395411	rs2792249	Human NCBI Build 35	1	161335780	PBX1-ENSG00000197613
8173	-	7798	C/T	0	395413	rs2816306	Human NCBI Build 35	1	189279702	RGS1
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8182	-	7826	C/T	0	395438	rs3130055	Human NCBI Build 35	6	31605378	ATP6V1G2-ENSG00000189327
8183	-	7835	C/T	0	395446	rs3218312	Human NCBI Build 35	22	35854832	IL2RB
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8190	-	7854	C/T	0	395465	rs3737606	Human NCBI Build 35	1	29451572	PTPRU
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8194	-	7878	C/T	0	395485	rs3770704	Human NCBI Build 35	2	208264624	CREB1

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8204	-	7907	C/T	1	395509	rs380649	Human NCBI Build 35	5	41299606	C6
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8207	-	7925	C/T	1	395523	rs4074080	Human NCBI Build 35	2	45927286	PRKCE
8208	-	7936	C/T	0	395530	rs4247362	Human NCBI Build 35	17	7885267 ALOX15B	
8209	-	7937	C/T	0	395531	rs4251527	Human NCBI Build 35	12	42463130	ENSG00000198001
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8218	-	7962	C/T	0	395552	rs4738412	Human NCBI Build 35	8	75078223	LY96
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8220	-	7974	C/T	0	395561	rs4798593	Human NCBI Build 35	18	7761132 PTPRM	
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8267	-	8122	C/T	0	395696	rs7605560	Human NCBI Build 35	2	179146009	PRKRA
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8283	-	8172	C/T	0	395736	rs861319	Human NCBI Build 35	1	155728990	PYHIN1
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8570	-	9288	C/T	0	396617	rs12821887	Human NCBI Build 35	12	10445946	KLRC4
8571	-	9290	C/T	0	396618	rs1292627	Human NCBI Build 35	6	128815641	PTPRK
8572	-	9293	C/T	0	396621	rs1327575	Human NCBI Build 35	10	101942506	CHUK
8573	-	9295	C/T	0	396623	rs13332786	Human NCBI Build 35	16	45968704	ENSG00000129636
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8576	-	9302	C/T	0	396629	rs1411479	Human NCBI Build 35	1	177738587	MR1
8577	-	9307	C/T	0	396632	rs1423001	Human NCBI Build 35	5	147430048	SPINK5
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8584	-	9326	C/T	0	396648	rs1562303	Human NCBI Build 35	2	113498623	IL1F6-IL1F8
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8588	-	9333	C/T	0	396654	rs1681626	Human NCBI Build 35	11	47972972	PTPRJ
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8593	-	9340	C/T	0	396660	rs1865761	Human NCBI Build 35	18	65690372	CD226
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8598	-	9355	C/T	0	396672	rs1962589	Human NCBI Build 35	15	97154292	IGF1R
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8602	-	9370	C/T	0	396686	rs2034598	Human NCBI Build 35	16	45559127	DNAJA2-ENSG00000129635
8603	-	9374	C/T	1	396690	rs2048607	Human NCBI Build 35	12	69497704	PTPRR
8604	-	9376	C/T	0	396692	rs2051324	Human NCBI Build 35	18	65701339	CD226
8605	-	9381	C/T	0	396697	rs2066804	Human NCBI Build 35	2	191667265	STAT1
8606	-	9383	C/T	0	396699	rs2069718	Human NCBI Build 35	12	66836429	IFNG
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8623	-	9456	C/T	0	396759	rs261076	Human NCBI Build 35	5	169236950	DOCK2
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8633	-	9486	C/T	0	396781	rs3123592	Human NCBI Build 35	1	2119719 PRKCZ	
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8712	-	9752	C/T	1	397004	rs7316037	Human NCBI Build 35	12	63133078	XPOT-TBK1

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8716	-	9758	C/T	0	397010	rs747726	Human NCBI Build 35	12	12561494	DUSP16
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8722	-	9795	C/T	0	397043	rs7808907	Human NCBI Build 35	7	128178035	IRF5
8723	-	9798	C/T	0	397044	rs7870456	Human NCBI Build 35	9	8444921 PTPRD	
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8749	-	9886	C/T	0	397119	rs10774603	Human NCBI Build 35	12	109618667	PPP1CC
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8752	-	9890	C/T	0	397123	rs10826945	Human NCBI Build 35	10	31702492	TCF8
8753	-	9903	C/T	0	397136	rs11120276	Human NCBI Build 35	1	203707597	C4BPA
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8766	-	9943	C/T	0	397169	rs12120762	Human NCBI Build 35	1	195431450	PTPRC
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8769	-	9962	C/T	1	397185	rs12520276	Human NCBI Build 35	5	42600319	GHR
8770	-	9964	C/T	0	397187	rs12546391	Human NCBI Build 35	6	31783276	C6orf21-BAT5-LY6G6E
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9016	-	10763	C/T	1	397865	rs6441929	Human NCBI Build 35	3	45899795	LZTFL1-CCR9	
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9854	-	6970	G/T	0	394722	rs3917407	Human NCBI Build 35	1	166433774	SELE
9856	-	6977	G/T	0	394729	rs410469	Human NCBI Build 35	2	32388643	CARD12
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9867	-	7134	G/T	0	394868	rs6787964	Human NCBI Build 35	3	61639986	PTPRG
9868	-	7144	G/T	1	394876	rs6918223	Human NCBI Build 35	6	32830510	HLA-DQB2
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9870	-	7272	G/T	0	394981	rs871335	Human NCBI Build 35	15	97297469	IGF1R
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10089	-	10466	G/T	0	397612	rs11466649	Human NCBI Build 35	4	38599291	TLR10
10090	-	10491	G/T	0	397633	rs12252639	Human NCBI Build 35	10	35457788	CREM
10091	-	10506	G/T	0	397647	rs13031237	Human NCBI Build 35	2	61047780	REL
10092	-	10537	G/T	0	397674	rs174642	Human NCBI Build 35	16	45861136	ENSG00000129636
10093	-	10555	G/T	1	397690	rs2027993	Human NCBI Build 35	17	23731073	SARM1
10094	-	10570	G/T	0	397702	rs2197209	Human NCBI Build 35	3	107050855	CBLB
10095	-	10625	G/T	0	397746	rs2885370	Human NCBI Build 35	3	191759697	IL1RAP
10096	-	10627	G/T	0	397748	rs3019561	Human NCBI Build 35	11	60500429	CD6
10097	-	10645	G/T	0	397762	rs355960	Human NCBI Build 35	16	65244211	CKLFSF4
10098	-	10650	G/T	0	397766	rs3730556	Human NCBI Build 35	12	67502788	MDM2
10099	-	10665	G/T	0	397778	rs3775296	Human NCBI Build 35	4	187372916	TLR3
10101	-	10680	G/T	0	397793	rs3917289	Human NCBI Build 35	2	102240429	IL1R1
10102	-	10709	G/T	1	397818	rs4698370	Human NCBI Build 35	4	15015213	C1QTNF7
10103	-	10717	G/T	0	397826	rs4799055	Human NCBI Build 35	18	75282991	NFATC1
10104	-	10722	G/T	0	397831	rs487273	Human NCBI Build 35	18	12843458	PTPN2
10105	-	10739	G/T	0	397843	rs5741880	Human NCBI Build 35	X	12647073	TLR7
10106	-	10742	G/T	0	397846	rs5747347	Human NCBI Build 35	22	16620087	BID
10107	-	10743	G/T	0	397847	rs582052	Human NCBI Build 35	10	6509161 PRKCQ	
10108	-	10747	G/T	0	397850	rs5988198	Human NCBI Build 35	X	114074563	IL13RA2

10109	-	10749	G/T	0	397852	rs5999704	Human NCBI Build 35	22	20503345	MAPK1
10110	-	10782	G/T	0	397883	rs6694672	Human NCBI Build 35	1	193677446	CFHL5
10111	-	10787	G/T	1	397888	rs6722418	Human NCBI Build 35	2	45787490	PRKCE
10112	-	10805	G/T	0	397902	rs702275	Human NCBI Build 35	9	5436388	ENSG00000120217
10113	-	10824	G/T	0	397919	rs7522020	Human NCBI Build 35	1	195369510	PTPRC
10115	-	10834	G/T	0	397928	rs7649363	Human NCBI Build 35	3	37579730	ITGA9
10116	-	10836	G/T	0	397930	rs7660964	Human NCBI Build 35	4	87874788	PTPN13
10117	-	10838	G/T	0	397932	rs7706055	Human NCBI Build 35	5	142012664	FGF1
10118	-	10861	G/T	0	397951	rs858545	Human NCBI Build 35	1	164193049	CD3Z
10119	-	10872	G/T	0	397959	rs9321119	Human NCBI Build 35	6	128819664	PTPRK
10120	-	10888	G/T	0	397973	rs9882766	Human NCBI Build 35	3	126441217	ZNF148
10121	-	10891	G/T	1	397976	rs10019269	Human NCBI Build 35	4	87563332	MAPK10
10122	-	10897	G/T	0	397980	rs1009977	Human NCBI Build 35	14	54672755	LGALS3
10123	-	10904	G/T	0	397986	rs10155140	Human NCBI Build 35	4	75087569	PF4V1
10124	-	10910	G/T	0	397992	rs1036583	Human NCBI Build 35	3	106564738	ALCAM
10125	-	10924	G/T	0	398003	rs10494134	Human NCBI Build 35	1	111571954	ENSG00000134216
10126	-	10938	G/T	0	398016	rs1078859	Human NCBI Build 35	1	232238066	ENSG00000143669
10127	-	10966	G/T	0	398038	rs11667351	Human NCBI Build 35	19	54147966	BAX
10128	-	10968	G/T	0	398040	rs11916852	Human NCBI Build 35	3	191863298	IL1RAP
10129	-	10971	G/T	1	398042	rs12082477	Human NCBI Build 35	1	51013074	FAF1
10130	-	10990	G/T	0	398054	rs1268656	Human NCBI Build 35	14	63717399	SYNE2-ESR2
10131	-	10991	G/T	0	398055	rs12721582	Human NCBI Build 35	17	37736992	STAT3
10133	-	11004	G/T	1	398065	rs13384232	Human NCBI Build 35	2	73999365	STAMBP
10134	-	11068	G/T	0	398117	rs2054724	Human NCBI Build 35	12	93145333	PLXNC1
10135	-	11091	G/T	0	398134	rs2259094	Human NCBI Build 35	10	93612303	TNKS2
10136	-	11104	G/T	1	398146	rs2325704	Human NCBI Build 35	1	155722237	PYHIN1
10137	-	11119	G/T	0	398157	rs273479	Human NCBI Build 35	14	23848800	LTB4R-CIDEB-LTB4R2
10138	-	11181	G/T	1	398213	rs4602134	Human NCBI Build 35	18	7967007	PTPRM
10139	-	11211	G/T	0	398239	rs5744048	Human NCBI Build 35	X	12689755	TLR8
10140	-	11223	G/T	0	398248	rs6414769	Human NCBI Build 35	4	15478187	CD38
10141	-	11236	G/T	0	398259	rs653973	Human NCBI Build 35	18	7787536	PTPRM
10142	-	11288	G/T	0	398302	rs7688526	Human NCBI Build 35	4	15400712	ENSG00000109743
10143	-	11289	G/T	0	398303	rs7701605	Human NCBI Build 35	5	42686227	GHR
10144	-	11290	G/T	0	398304	rs7701642	Human NCBI Build 35	5	43430535	CCL28
10146	-	11325	G/T	0	398333	rs9387045	Human NCBI Build 35	6	112268696	FYN
10147	-	11331	G/T	0	398339	rs975074	Human NCBI Build 35	1	169745424	TNFSF18
10148	-	11339	G/T	0	398345	rs9943268	Human NCBI Build 35	1	203680500	C4BPA
10149	-	11349	G/T	0	398354	rs10214916	Human NCBI Build 35	6	167513495	CCR6-ENSG00000187485
10150	-	11379	G/T	0	398377	rs10981818	Human NCBI Build 35	9	113388263	RGS3
10151	-	11404	G/T	0	398396	rs11962214	Human NCBI Build 35	6	128582041	PTPRK
10152	-	11412	G/T	1	398403	rs12089749	Human NCBI Build 35	1	51018350	FAF1
10153	-	11427	G/T	1	398416	rs1377470	Human NCBI Build 35	11	76728362	PAK1
10154	-	11446	G/T	0	398433	rs2009434	Human NCBI Build 35	7	38055406	TRGV9
10155	-	11469	G/T	0	398455	rs2289779	Human NCBI Build 35	5	38562985	LIFR
10156	-	11477	G/T	0	398462	rs2382723	Human NCBI Build 35	X	69156774	IGBP1
10157	-	11505	G/T	0	398485	rs3181166	Human NCBI Build 35	7	142503735	CASP2-ENSG00000178826
10158	-	11507	G/T	1	398487	rs3211820	Human NCBI Build 35	7	79923116	CD36
10159	-	11513	G/T	0	398492	rs3739527	Human NCBI Build 35	9	27516480	IFNK-ENSG00000120162
10160	-	11529	G/T	0	398505	rs4017732	Human NCBI Build 35	1	157593628	LY9

10161	-	11576	G/T	1	398545	rs6490814	Human NCBI Build 35	13	23147957	TNFRSF19
10162	-	11592	G/T	0	398559	rs6914136	Human NCBI Build 35	6	136643588	ENSG00000029363
10163	-	11595	G/T	0	398562	rs6984608	Human NCBI Build 35	8	56990235	LYN
10164	-	11613	G/T	1	398578	rs7612628	Human NCBI Build 35	3	62126488	PTPRG
10165	-	11647	G/T	0	398608	rs9291926	Human NCBI Build 35	5	67635412	PIK3R1
10166	-	11663	G/T	0	398621	rs9944249	Human NCBI Build 35	15	39634468	TYRO3
10167	-	11685	G/T	1	398638	rs10811505	Human NCBI Build 35	9	21198723	IFNA10-ENSG00000137026
10168	-	11729	G/T	0	398672	rs1909757	Human NCBI Build 35	9	8395674 PTPRD	
10169	-	11734	G/T	1	398676	rs1967446	Human NCBI Build 35	6	136638981	ENSG00000029363
10170	-	11737	G/T	1	398678	rs1994276	Human NCBI Build 35	8	120020700	TNFRSF11B
10171	-	11752	G/T	0	398692	rs2278342	Human NCBI Build 35	12	69235100	PTPRB
10172	-	11758	G/T	0	398697	rs2357479	Human NCBI Build 35	14	63862517	ESR2
10173	-	11770	G/T	0	398706	rs264863	Human NCBI Build 35	5	168992855	DOCK2
10174	-	11776	G/T	1	398710	rs2719237	Human NCBI Build 35	8	57089416	LYN
10176	-	11828	G/T	1	398750	rs4940741	Human NCBI Build 35	18	54512757	MALT1-ENSG00000182288
10177	-	11834	G/T	0	398756	rs594454	Human NCBI Build 35	1	71142489	PTGER3
10178	-	11869	G/T	1	398781	rs7095398	Human NCBI Build 35	10	91075587	IFIT3-LIPA
10179	-	11889	G/T	0	398795	rs7656411	Human NCBI Build 35	4	154985260	RNF175-TLR2
10181	-	11915	G/T	0	398815	rs9288815	Human NCBI Build 35	3	106908600	CBLB
10182	-	11928	G/T	0	398827	rs983751	Human NCBI Build 35	10	90736673	ENSG00000026103
10183	-	11938	G/T	1	398835	rs10455097	Human NCBI Build 35	6	74550153	CD109
10184	-	11951	G/T	0	398847	rs11056560	Human NCBI Build 35	12	15609804	PTPRO
10185	-	11993	G/T	0	398879	rs1860235	Human NCBI Build 35	4	15635220	ENSG00000137441
10186	-	12008	G/T	0	398890	rs2017242	Human NCBI Build 35	4	48045522	TEC
10187	-	12029	G/T	0	398907	rs2332096	Human NCBI Build 35	3	123303833	CD86
10189	-	12043	G/T	0	398919	rs3109808	Human NCBI Build 35	1	204336872	MCP
10190	-	12064	G/T	0	398937	rs4251431	Human NCBI Build 35	12	42440957	
ENSG00000198001-ENSG00000129317										
10191	-	12070	G/T	0	398942	rs4561893	Human NCBI Build 35	4	87932051	PTPN13
10192	-	12075	G/T	0	398947	rs4657378	Human NCBI Build 35	1	161531469	PBX1
10193	-	12082	G/T	0	398952	rs4760821	Human NCBI Build 35	12	69489807	PTPRR
10194	-	12085	G/T	0	398954	rs4852983	Human NCBI Build 35	2	73970549	STAMBP
10195	-	12090	G/T	0	398958	rs5743317	Human NCBI Build 35	4	187378908	TLR3
10196	-	12108	G/T	0	398973	rs6877536	Human NCBI Build 35	5	39181010	FYB
10197	-	12113	G/T	1	398977	rs7013397	Human NCBI Build 35	8	16008976	MSR1
10198	-	12184	G/T	0	399038	rs10922153	Human NCBI Build 35	1	193710272	CFHL5
10199	-	12195	G/T	0	399046	rs1263572	Human NCBI Build 35	7	156929342	PTPRN2
10200	-	12241	G/T	0	399084	rs2918415	Human NCBI Build 35	5	142725156	NR3C1
10201	-	12267	G/T	0	399108	rs6151510	Human NCBI Build 35	15	57759664	BNIP2
10202	-	12268	G/T	1	399109	rs636842	Human NCBI Build 35	15	32055908	AVEN
10203	-	12288	G/T	0	399122	rs7857730	Human NCBI Build 35	9	5074049 JAK2	
10204	-	12299	G/T	0	399131	rs896771	Human NCBI Build 35	7	157595602	PTPRN2
10205	-	12322	G/T	0	399150	rs1923143	Human NCBI Build 35	1	155081027	CD1C
10206	-	12335	G/T	0	399161	rs2381301	Human NCBI Build 35	9	35212453	UNC13B-ENSG00000196186
10207	-	12341	G/T	1	399163	rs2709402	Human NCBI Build 35	2	208246483	CREB1
10208	-	12347	G/T	1	399168	rs4253890	Human NCBI Build 35	1	210998217	PTPN14
10209	-	12354	G/T	0	399174	rs4957384	Human NCBI Build 35	5	39242184	FYB
10210	-	12397	G/T	0	399210	rs1768358	Human NCBI Build 35	6	128620465	PTPRK
10211	-	12399	G/T	1	399212	rs2026431	Human NCBI Build 35	10	6550460 PRKCQ	

10213	-	12407	G/T	0	399219	rs2364482	Human NCBI Build 35	12	6372392	LTBR
10214	-	12426	G/T	0	399233	rs7809114	Human NCBI Build 35	7	157663985	PTPRN2
10215	-	12451	G/T	0	399255	rs4748127	Human NCBI Build 35	10	6616887	PRKCQ
10216	-	12467	G/T	1	399267	rs10282832	Human NCBI Build 35	8	75103860	LY96
10217	-	12477	G/T	0	399274	rs1256045	Human NCBI Build 35	14	63799513	ESR2
10218	-	12507	G/T	0	399300	rs2288730	Human NCBI Build 35	12	97568982	APAF1

Appendix 6 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of Adverse Event

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study subject, temporally associated with the use of study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events <u>Meeting</u> the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New condition detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • Clinically significant change in objective findings (e.g., laboratory, ECG, physical examination) should be considered an AE ONLY if it meets the following criteria: o Associated with accompanying symptoms; and/or o Requires medical/surgical interventions; and/or o Leads to a change in study treatment dosing or discontinuation from the study, significant additional concomitant drug treatment or other therapy; and/or o Leads to any of the outcomes included in the definition of an SAE • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of a Serious Adverse Event

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
Results in death
Is life-threatening meaning an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Requires hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
Results in persistent disability/incapacity which means a substantial disruption of a person's ability to conduct normal life functions; this definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

Is a congenital anomaly/birth defect

Is an important medical event

such as medical events that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgement, may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse. Meeting laboratory criteria for drug induced liver injury (DILI) is also considered an important medical event. Suspected transmission of an infectious agent (pathogenic or nonpathogenic) via the study treatment is an SAE.

Although pregnancy, overdose, cancer, and suspected DILI are not always serious by regulatory definition, these events must be handled as SAEs if they meet the definitions as specified in the protocol (see Section 8.7.5 Pregnancy, Section 8.8 Treatment of Overdose, and Section 7.1 Discontinuation of Study Treatment for Individual Subjects [DILI]). A study endpoint (such as all-cause mortality) for which an occurrence is considered *related* to study treatment should be reported as an SAE (e.g., death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported as an SAE) (see below for reporting details).

Hospitalizations NOT Meeting the SAE Definition in Arbutus clinical studies

- A visit to the emergency room or other hospital department <24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- Elective surgery, planned prior to signing the ICF
- Admissions as per protocol for a planned medical/surgical procedure or routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason)

Recording and Follow-up of Adverse Events and/or Serious Adverse Events

AE and SAE Recording

- All SAEs will be collected and be recorded in the CRF from the signing of the ICF until the last Post-Treatment Follow-up visit. The description of the AE will include the onset date, duration, date of resolution, severity (see below), seriousness, and Investigator's assessment of relationship to the study treatment
- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in the CRF, and on the SAE Report Form when an SAE is being reported.
- The initial report of an SAE should include the following information, at a minimum:
- Study name or number
- Subject number
- Event description (including date of the event onset, outcome and reason for it being considered serious)
- Name of the investigational product (including drug dose and administration dates)
- Preliminary assignment of causality
- Person reporting event (include site name or number)
- Dated signature of the Investigator or sub-/co-Investigator

It is **not** acceptable for the Investigator to send photocopies of the subject's medical records to the Pharmacovigilance (PV) department (the CRO) in lieu of completion of the SAE Report Form.

- There may be instances when copies of medical records for certain cases are requested by the CRO PV department. In this case, all subject identifiers, except for the subject number, will be redacted on the copies of the medical records before submission to the CRO PV department.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Severity (Intensity)

- The Investigator will assess intensity for each AE and SAE reported during the study using the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS Table) .
- Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity.
- AEs characterized as intermittent require documentation of the start and stop of each incidence.
- When changes in the intensity of an AE occur more frequently than once a day, the maximum intensity for the experience that day should be noted. If the intensity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates).

Assessment of Causality

- The Investigator is obligated to use clinical judgment to assess the relationship between study treatment and each occurrence of each AE/SAE.
- "Not Related" should be assessed when a causal relationship between study treatment administration and AE is *not considered a reasonable possibility*. The observed AE can be reasonably explained by an alternative cause such as underlying disease, concurrent medication or co-morbidity; or a causal association is considered unreasonable.
- "Related" should be assessed when there is a reasonable possibility that the study drug caused the AE, rather than using only because a relationship cannot be ruled out. The temporal relationship, biological plausibility and absence of confounding with the underlying disease, co-morbidities or other medications make a causal relationship a reasonable possibility.
- The Investigator will also consult the IB and/or Product Information, for marketed products, in making his/her assessment.
- For each AE/SAE, the Investigator **must** document in the medical notes that s/he has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report to the CRO PV department. However, **it is very important that the Investigator always assess causality for every event before the initial transmission of the SAE data to the CRO PV department.** The Investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report or amend the SAE Report Form with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs
• The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the CRO PV department to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a subject dies during participation in the study or during a recognized follow-up period, the Investigator will provide the CRO PV department with a copy of any post-mortem findings including histopathology. • New or updated information will be recorded in the originally completed CRF. • The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

Reporting of Serious Adverse Events

SAE Reporting to the CRO PV Department
• The primary mechanism for reporting an SAE to the CRO PV department will be e-mailing the SAE Report form to the CRO in addition to updating details in the CRF within 24 hours of knowledge of the event. • If the electronic system is unavailable for more than 24 hours, then the site will use the paper SAE data collection tool. • The site will enter the SAE data into the electronic system as soon as it becomes available, within 24 hours. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form. Contacts for SAE reporting can be found in the Safety Management Plan.

Appendix 7 Pharmacokinetic Sampling for AB-729 in Study AB-729-201

Visit	Period	Cohort	Timepoint Relative to Dose (hours)	AB-729 Plasma PK Sample
Day 1	Lead-In Treatment Period	all subjects	pre-dose[a]	X
			0.5 hours	X
			1 hours	X
			2 hours	X
			4 hours	X
			6 hours	X
Week 24	Lead-In Treatment Period	all subjects	pre-dose[a]	X
			0.5 hours	X
			1 hours	X
			2 hours	X
			4 hours	X
			6 hours	X
Week 32	Consolidation Treatment Period	Cohort A1 Cohort B1	pre-dose[a]	X
			0.5 hours	X
			1 hours	X
			2 hours	X
			4 hours	X
			6 hours	X
Week 40	Consolidation Treatment Period	Cohort A1	pre-dose[a]	X
			0.5 hours	X
			1 hours	X
			2 hours	X
			4 hours	X
			6 hours	X

a. Pre-dose sample will be collected within 60 minutes prior to dosing of AB-729.

Appendix 8 Schedule of Activities for Early Termination (Lead-in Period only)

Table 13. Schedule of Activities for Early Termination (Lead-in Period only)

Assessment	Early Termination Follow Up		
	Weeks 4[a], 8, 12, 16, 20, 24[b]	Weeks 36 and 48 (EOS)[c]	Notes
Concomitant medications	X	X	
Physical examination	X	X	Targeted PEs may be completed at any study visit based on any reported changes to the subject's health.
Vital signs	X	X	BP, HR, RR, and temp to be performed pre-dose, when applicable.
ECG	X*	X	*Weeks 12 and 24 only.
Clinical laboratory tests	X	X	Includes clinical chemistry, hematology, coagulation tests, serology, and urine as specified in Appendix 4, and are to be completed after an overnight fast of 8 hrs.
Pregnancy test (WOCBP only)	X	X	If the urine test is positive or not interpretable, a serum/plasma pregnancy test is to be conducted.
Quantitative HBsAg	X	X	
HBsAg Ultrasensitive	X	X	To be assessed only if quantitative HBsAg result is <LLOQ
Quantitative HBsAb	X*	X	*Sample to be collected at Week 12 only.
Quantitative HBV DNA	X*	X	*Samples to be collected at Weeks 8, 16, and 24 only.
Quantitative HBV RNA	X	X	
Quantitative HBcrAg	X*	X	*Samples to be collected at Weeks 8, 16, and 24 only.
Qualitative HBeAg	X*	X	*Samples to be collected at Weeks 8, 16, and 24 only.
Quantitative HBeAg	X*	X	*Samples to be collected at Weeks 8, 16, and 24 only. To be assessed only if positive result obtained with HBeAg qualitative test.
Qualitative HBeAb	X*	X	*Sample to be collected at Week 12 only.
HBsAg isoforms	X*	X	*Samples to be collected at Weeks 8, 16, and 24 only.
Resistance Sample	X	X	Samples will be stored and analyzed only if defined criteria for non-response or rebound met (Appendix 10).

Assessment	Early Termination Follow Up		
	Weeks 4[a], 8, 12, 16, 20, 24[b]	**Weeks 36 and 48 (EOS)[c]**	**Notes**
Immune biomarker/cytokines sample	X*	X	Unscheduled samples will be collected in the event of any unexpected safety finding. *Collected at Weeks 4, 8, 12 and 20 only.
PBMCs	X*	X	. Unscheduled samples will be collected in the event of any unexpected safety finding. *Collected at Weeks 4, 8, 12 and 20 only.
Drug dispensing/ accountability	X	X	Subjects will record all doses in a dosing diary, which will be reviewed at each study visit.
Record AEs	X	X	Will be collected from the time of the start of study treatment through the last Post-Treatment F/U visit.
Record SAEs	X	X	Will be collected from the signing of the ICF through the last Post-Treatment F/U visit and followed until resolution of any event.

Abbreviations: AEs = adverse events; BP = blood pressure; ECG = electrocardiogram; EOS = end of study; F/U = follow up; HBcrAg = hepatitis B virus core-related antigen; HBeAb = hepatitis B virus e-antibody; HBeAg = hepatitis B virus e-antigen; HBsAb = hepatitis B virus surface antibody; HBsAg = hepatitis B virus surface antigen; HBV = hepatitis B virus; HR = heart rate; hrs = hours; ICF = informed consent form; NA = nucleos(t)ide analogue; PBMCs = peripheral blood mononuclear cells; PD = pharmacodynamic; PE = physical exam; PK = pharmacokinetic; RR = respiration rate; SAE = serious adverse event; temp = temperature; WOCBP = women of childbearing potential.

a. First follow up visit will occur 4 weeks after the date the subject terminated from the study during the Lead-In Period.
b. Visit windows are ± 5 days.
c. Visit windows are ± 7 days.

Appendix 9 NA Retreatment Criteria and Follow up Schedule of Activities

For subjects who discontinued NA therapy per protocol (see Section 4.4), resumption of NA therapy should occur if any of the following scenarios occur and after discussion with the Sponsor Medical Monitor:

- Persistent ALT elevations ≥2 × baseline, AND ≥2 – 5 × ULN, AND HBV DNA >2000 IU/mL for 12 weeks
- Persistent ALT elevations ≥2 × baseline, AND ≥5 – 10 × ULN, AND HBV DNA >2000 IU/mL for 4 weeks
- HBV DNA > 20,000 IU/mL regardless of ALT level, confirmed by repeat
- ALT >10 x ULN, confirmed by repeat
- ALT >baseline and >ULN, AND:
  - increased direct or total bilirubin ≥2 × ULN and ≥2 × baseline confirmed by repeat, OR
  - INR increase of ≥0.5 from baseline, confirmed by repeat.

Subjects who restart NA therapy will be assessed at minimum every 2 weeks until clinically stable (i.e., ALT and HBV DNA declining on 2 consecutive visits) and will be followed for 24 weeks total via unscheduled visits as needed prior to study discharge (Table 14). The Sponsor Medical Monitor should be consulted regarding when the subject is felt to be clinically stable, at which time visits may be reduced to monthly. More frequent visits may also be conducted if the subject's clinical status worsens.

Table 14. Schedule of Activities for Subjects who Restart NA Therapy

Assessment	Visit 1[a] (Week 2)	Visit 2[a] (Week 4)	Remaining Visits[b] (every 2 weeks until clinically stable, then every 4 weeks until Week 24/EOS)	Notes
Concomitant medications	X	X	X	
Physical examination	X	X	X	Targeted PEs may be completed at any study visit based on any reported changes to the subject's health.
Vital signs	X	X	X	BP, HR, RR, and temperature.
Clinical Laboratory tests	X	X	X	Includes clinical chemistry, hematology, coagulation tests, serology, and urinalysis as specified in Appendix 4, and are to be completed after an overnight fast of ≥8 hrs
Pregnancy test (WOCBP only)	X		X*	If the urine test is positive or not interpretable, a serum/plasma pregnancy test is to be conducted. *Every 8 weeks until study discharge

Assessment	Visit 1[a] (Week 2)	Visit 2[a] (Week 4)	Remaining Visits[b] (every 2 weeks until clinically stable, then every 4 weeks until Week 24/EOS)	Notes
Quantitative HBV DNA	X	X	X	Local testing can be performed in addition to central lab testing to facilitate more rapid results.
Quantitative HBsAg	X	X	X	If HBsAg becomes <LLOQ an unscheduled HBsAb sample may be obtained
Quantitative HBV RNA	X	X	X	
Quantitative HBcrAg	X	X	X	
Record AEs	X	X	X	
Record sAEs	X	X	X	

Abbreviations: AEs = adverse events; BP = blood pressure; ECG = electrocardiogram; EOS = end of study; F/U = follow up; HBcrAg = hepatitis B virus core-related antigen; HBsAb = hepatitis B virus surface antibody; HBsAg = hepatitis B virus surface antigen; HBV = hepatitis B virus; HR = heart rate; hrs = hours; NA = nucleos(t)ide analogue; PE = physical exam; PK = pharmacokinetic; RR = respiration rate; SAE = serious adverse event; temp = temperature; WOCBP = women of childbearing potential.

a. Visit windows are ± 3 days.

b. Visit windows are ± 3 days of visits are every 2 weeks, ± 5 days if visits are every 4 weeks.

Appendix 10 Resistance Monitoring Plan

The probability of selection of pre-existing resistant variants during AB-729 treatment is anticipated to be low. The AB-729 target site is highly conserved, at ≥99.4% amongst 9,098 HBV genotype A-H sequences available in the hepatitis B virus database (HBVdb). Preclinical *in vitro* activity assessments of the most commonly occurring nucleotide mismatches within the AB-729 HBV target site suggest that AB-729 is able to retain activity against these HBV variants. Given that the target site of AB-729 differs from the known HBV target sites for NA and capsid inhibitor agents and does not overlap with the HBsAg open reading frame (thus negating any impact on approved vaccine epitopes), there is a reasonable expectation that resistant variants to any of these drug modalities will remain susceptible and most likely respond to treatment with the other drugs in a combination treatment setting. Consistent with this expectation, resistant variants against NAs or capsid inhibitor agents were susceptible to inhibition by AB-729 in a cell culture model of HBV. In the clinical setting, the risk of viral resistance emerging to AB-729 is decreased significantly in a combination treatment context where NAs effectively arrest viral replication.

To monitor for selection of resistant variants to AB-729, samples from all subjects undergoing AB-729 treatment will be collected at baseline and different timepoints during treatment and follow-up, and where feasible, next-generation sequencing (NGS) analyses conducted to assess the AB-729 HBV target site sequence in subjects at baseline or who experience a non-response to AB-729 treatment (defined as <0.3 $\log_{10}$ reduction in HBsAg after 24 weeks of dosing) or who show a rebound in either HBsAg (defined as ≥0.5 $\log_{10}$ IU/mL change above nadir) or HBV DNA (defined as ≥1 $\log_{10}$ IU/mL change above nadir or quantifiable HBV DNA after being undetectable) during repeat dosing of AB-729. Where feasible, given limitations on the sensitivity of the assay selected, HBV genotypes of baseline and on-therapy non-response or rebound samples will be determined by sequencing and phylogenetic analysis. Changes in the nucleotide sequence of the AB-729 HBV target site present in on-treatment or follow-up samples will be compared to the AB-729 target site nucleotide sequence in the baseline sample for each subject identified as showing a non-response or who experience a rebound during AB-729 treatment.

HBV viral variants with mismatches in the AB-729 HBV target site identified from subjects with on-therapy non-response or rebound will be cloned into a representative HBV genome background and susceptibility to AB-729 activity will be tested in cell culture systems, such as an HBV plasmid transient transfection model.

A listing of the virological assessments to be conducted for AB-729-201, along with detailed assay descriptions, is provided below.

An overview of the next generation sequencing approach for resistance assessment is provided below.

Virological Assessments in AB-729-201

A listing of the virological assessments to be conducted in AB-729-201 is provided in Table 15.

Detailed assay descriptions and available performance characteristics are provided in the indicated locations for assays that are not currently FDA-approved.

Table 15. Virological Assessments

HBV Marker	Assay	Product Number	Assay Description and Performance Characteristics
HBsAg (Quantitative)	Roche cobas Elecsys HBsAg II quant II	P/N: 07027443119	Roche cobas HBsAg II quant II Information Sheet
HBsAg (Quantitative, Ultra-sensitive)	Abbott Architect HBsAg Next Research Use Only Assay	Not applicable	Sickinger 2020_Abbott Architect HBsAg Next Assay
HBV DNA	Roche cobas HBV 6800	P/N: 07000979190	FDA Approved
HBcrAg	Fujirebio Lumipulse G	231715	Lumipulse G HBcrAg Information Sheet
HBeAg (Qualitative)	Roche cobas Elecsys HBeAg Immunoassay	P/N: 11820583122	FDA Approved
HBeAg (Quantitative)	Roche cobas Elecsys HBeAg Immunoassay	Not applicable	Roche Quantitative HBeAg in Serum Validation Summary
Anti-HBs (Quantitative)	Advia Centaur aHBs2	4670661	FDA Approved
HBeAg Ab (Qualitative)	Roche Elecsys anti-HBe	P/N: 07026838190	FDA Approved
Anti-HBc IgM	Advia Centaur HBc IgM	00504619	FDA Approved
HBsAg Isoforms	Abbott Research Use Only Assay	Not applicable	Rodgers et al., Poster 0675
HBV RNA	Abbott Research Use Only Assay	Not applicable	Anderson M et al., Poster P13
HBsAg Immune Complex	Abbott Research Use Only Assay	Not applicable	To be provided
HBV Genotype	To be identified	To be identified	To be identified

Resistance Assessment: Next Generation Sequencing Approach

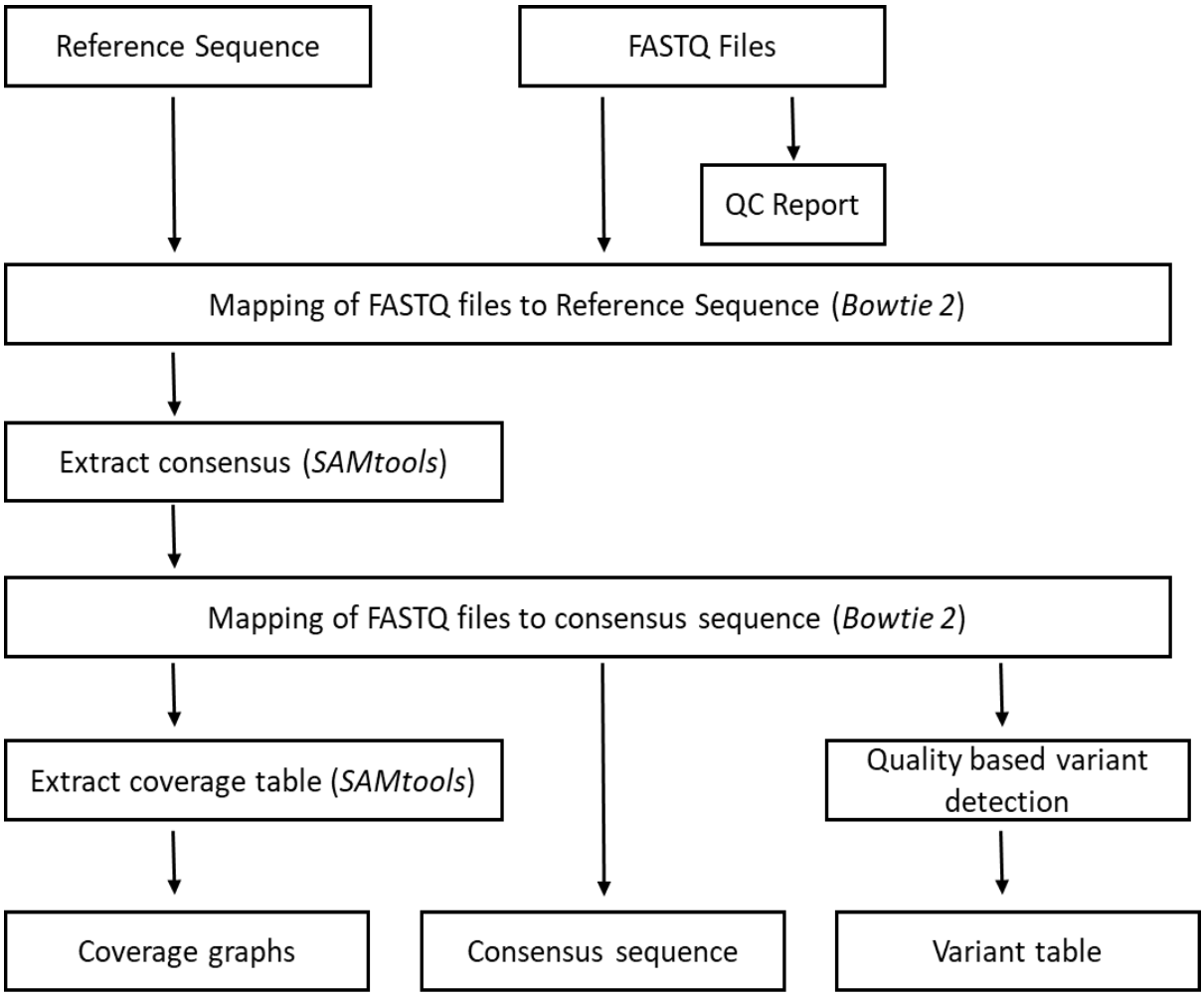
To monitor selection of resistant variants to AB-729, samples from all subjects undergoing AB-729 treatment will be collected at baseline and different timepoints during treatment and follow-up. Where feasible, next-generation sequencing will be conducted to assess the AB-729 HBV target site sequence in subjects at baseline or who experience a non-response to AB-729 treatment (defined as <0.3 $\log_{10}$ reduction in HBsAg after 24 weeks of dosing) or who show a rebound in either HBsAg (defined as ≥0.5 $\log_{10}$ IU/mL change above nadir) or HBV DNA (defined as ≥1 log10 IU/mL change above nadir or quantifiable HBV DNA after being undetectable) during repeat dosing of AB-729. Viral nucleic acids will be isolated from plasma K_2EDTA samples and used as a template for polymerase chain reaction (PCR) amplification using primers flanking the AB-729 target site, located at least 100 base pairs away from the 5′ and 3′ ends of the AB-729 target site (i.e. spanning at least positions 1479 to 1679 of the HBV genome as referenced by accession no. EU939600.1; the EcoRI restriction enzyme site is designated as position 1). The resulting amplicons will be used to prepare Nextera XT libraries and sequenced using the Illumina MiSeq platform. Summary statistics reports for each sequence run will be generated that will include the total number of reads, quality scores, average read length as well as trimming parameters. A detailed NGS protocol, including sample preparation and performance characteristics of the Illumina platform, will be communicated to the Food and Drug Administration (FDA) prior to submission of sequencing data.

An overview of the bioinformatics sequence analysis approach is depicted in Figure 3.

Mapping of sequence reads will be conducted to representative HBV genomic sequences for each genotype (Table 16).

Sequence variant calling will be determined by comparison to the fully matched AB-729 target site (defined as complementary to antisense strand positions 2-18). Frequency tables of AB-729 target site nucleotide variants identified at ≥1% sequence-read frequency and raw NGS data in FASTQ format will be submitted to the FDA. Summary tables identifying AB-729 target site nucleotide variants identified at ≥15% sequence-read frequency will be provided. The procedures for submission of NGS data to the FDA will be as referenced in the published FDA guidance.[38] Further details of the bioinformatics sequence analysis pipeline, including algorithm parameter settings and rationale, will be included in submissions of sequence data to the FDA.

Figure 3. Overview of Bioinformatics Sequence Analysis Approach



Abbreviation: QC = quality control.

Table 16. HBV Genotype Reference Sequences for Read Mapping

HBV Genotype	Genbank Accession No.
A	X02763
B	AB219428
C	GQ924620
D	AF121240
E	AB106564
F	AY090458
G	AF160501
H	FJ356716
I	EU833891

Elecsys HBsAg II quant II

cobas®

REF		Σ	SYSTEM
07027443119	07027443500	100	**cobas e** 801

English

System information

Short name	ACN (application code number)
HBSAGQ2	10055

Intended use

Immunoassay for the in vitro quantitative determination of hepatitis B surface antigen (HBsAg) in confirmed HBsAg positive human serum and plasma. Assay results, in conjunction with HBV DNA quantification and clinical information, may be used as an aid to monitor treatment of individuals with chronic hepatitis B.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the **cobas e** 801 immunoassay analyzer.

Note

Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit catalogue number: 07027443190 for the Elecsys HBsAg II quant II. The last 3 digits -190 have been replaced by -119 for logistic purposes.

Summary

The hepatitis B surface antigen (HBsAg), a polypeptide of varying size, is a component of the external envelope of the hepatitis B virus (HBV) particle.[1,2] In addition to the intact infectious viral particles, the blood of persons infected with HBV contains large amounts of non-infectious particles which consist only of an outer coat containing HBsAg.[3] After infection, HBsAg is the first immunological marker detectable in serum and is usually present weeks to months before the onset of clinical symptoms and the appearance of other biochemical markers.[4] In the case of acute HBV infection with recovery, HBsAg is detectable in serum for up to 6 months after its appearance.[4] If HBsAg persists for more than 6 months after acute hepatitis, the presence of chronic hepatitis B (CHB) infection must be assumed.

Classifying the stage of CHB infection is essential for identifying patients who require treatment and monitoring, as well as assessing the likelihood of responding to treatment and risk of progression to more severe liver disease.[5,6,7] A CHB patient with elevated aminotransferase levels, high HBV DNA viral load, and histological abnormalities will be considered for therapy and two different treatment strategies are applicable: treatment of finite duration with pegylated interferon alpha or long-term treatment with nucleoside/nucleotide analogs (NUCs).[5] Monitoring HBsAg levels, in addition to HBV DNA, before[8,9] and during pegylated interferon-based therapy can help physicians to predict the likely response and implement the response-guided therapy algorithms, as recommended in the guidelines, to achieve the optimal outcome, which is sustained HBsAg loss with or without seroconversion to anti-HBs.[5,6,8,9,10,11] There is also some evidence suggesting that HBsAg quantification may have value for monitoring response to NUC therapy and identifying patients able to achieve a sustained response after terminating treatment.[3,12,13,14,15] This is based on the suggestion that HBsAg levels decline during antiviral therapy with NUCs reflecting an improvement in the degree of host immune control of the virus, with lower HBsAg levels at end of treatment being associated with continued remission.[11,16,17] However, further studies in larger cohorts are required.

For patients in the immune clearance phase of CHB, HBV DNA levels have traditionally been used to determine the disease progression risk. However, HBsAg monitoring can provide additional information and distinguish true inactive carriers (HBV DNA < 2000 IU/mL and HBsAg < 1000 IU/mL), who are at the lowest risk of progression from those at a higher risk of developing cirrhosis or hepatocellular carcinoma (HCC). An HBsAg level ≥ 1000 IU/mL in hepatitis B 'e' antigen negative patients with HBV DNA < 2000 IU/mL has been identified as an independent risk factor for progression to HCC.[5,6,11,18,19,20]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, two biotinylated monoclonal anti-HBsAg antibodies, and a mixture of monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack (M, R1, R2) is labeled as HBSAGQ2.

M Streptavidin-coated microparticles, 1 bottle, 6.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-HBsAg-Ab~biotin, 1 bottle, 7.2 mL:
Two biotinylated monoclonal anti-HBsAg antibodies (mouse) > 0.5 mg/L; phosphate buffer 100 mmol/L, pH 7.5; preservative.

R2 Anti-HBsAg-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 6.3 mL:
Monoclonal anti-HBsAg antibody (mouse), polyclonal anti-HBsAg antibodies (sheep) labeled with ruthenium complex > 1.5 mg/L; phosphate buffer 100 mmol/L, pH 8.0; preservative.

HBSAGQ2 Cal1	Negative calibrator 1, 2 bottles of 1.3 mL each: Human serum, buffered, pH 6.5; preservative.
HBSAGQ2 Cal2	Positive calibrator 2, 2 bottles of 1.3 mL each: HBsAg approximately 50 IU/mL in human serum, buffered, pH 6.5; preservative.
HBSAGQ2 Dil HepB	**cobas e** pack with 2 bottles of 12.1 mL each and 1 bottle of 21 mL: Human serum negative for HBsAg and anti-HBs, buffered, pH 6.5; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

All human material should be considered potentially infectious.

The calibrators and HBSAGQ2 Dil HepB have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg (HBSAGQ2 Cal1 and HBSAGQ2 Dil HepB only) and antibodies to HCV and HIV.

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing HBsAg (HBSAGQ2 Cal2) was inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[21,22]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents (M, R1, R2, Dil HepB) in the kit are ready-for-use and are supplied in **cobas e** packs.

Calibrators

The calibrators are supplied ready-for-use in bottles compatible with the system.

Unless the entire volume is necessary for calibration on the analyzer, transfer aliquots of the ready-for-use calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C for later use.

Elecsys HBsAg II quant II

cobas®

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the **cobas e** packs (M, R1, R2, Dil HepB):	
unopened at 2-8 °C	up to the stated expiration date
on the **cobas e** 801 analyzer	16 weeks

Stability of the calibrators:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	16 weeks
on the **cobas e** 801 analyzer at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, Na-heparin, K_2-EDTA and Na-citrate plasma.

Criterion: Slope 0.9‑1.1 + intercept within ≤ ± 0.5 IU/mL + coefficient of correlation ≥ 0.95.

Stable for 6 days at 20-25 °C, 14 days at 2-8 °C, 6 months at ‑20 °C (± 5 °C). The samples may be frozen 6 times.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat‑inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- REF 07143745190, PreciControl HBsAg II quant II, 15 x 1.3 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- **cobas e** 801 analyzer

Accessories for the **cobas e 801** analyzer:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cups, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. **Pre-dilution of samples is mandatory according to the test algorithm (see "Dilution" section).** Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibrators:

Place the calibrators in the sample zone.

Read in all the information necessary for calibrating the assay.

Calibration

Traceability: This method has been standardized against the NIBSC standard (code number: 00/588; WHO Second International Standard for HBsAg, subtype adw2, genotype A; IU/mL).

The predefined master curve is adapted to the analyzer using HBSAGQ2 Cal1 and HBSAGQ2 Cal2.

Calibration frequency: Calibration must be performed once per reagent lot using HBSAGQ2 Cal1, HBSAGQ2 Cal2 and fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl HBsAg II quant II.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration (IU/mL) based on the measurement of HBSAGQ2 Cal1 and HBSAGQ2 Cal2. In case of a manual pre-dilution, the dilution factor needs to be accounted for manual calculation of the final result.

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 684 µmol/L or ≤ 40 mg/dL
Hemoglobin	≤ 0.311 mmol/L or ≤ 500 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 164 nmol/L or ≤ 40 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
Albumin	≤ 7.0 g/dL

Criterion: For concentrations of 0.05-1 IU/mL the deviation is ≤ 0.2 IU/mL. For concentrations of 1-130 IU/mL the recovery is 80-120 %.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No high-dose hook effect was found with the Elecsys HBsAg II quant II assay up to a concentration of 250000 IU/mL when samples were analyzed according to instructions for use (predilution 1:900).

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special drugs used in hepatitis B therapy were tested. No interference with the assay was found.

Special drugs

Drug	Concentration tested mg/L
Peginterferon alfa-2a	≤ 0.036
Peginterferon alfa-2b	≤ 1.6
Lamivudine	≤ 300
Adefovir	≤ 10
Entecavir	≤ 1.0
Telbivudine	≤ 600
Tenofovir	≤ 245

In rare cases, interference due to extremely high titers of antibodies to analyte‑specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

Measuring range for pre-diluted samples:

45-117000 IU/mL for 900-fold diluted samples.
Values below the measuring range are reported as < 45 IU/mL.
Values above the measuring range are reported as > 117000 IU/mL.

1350-3510000 IU/mL for 27000‑fold diluted samples.
Values below the measuring range are reported as < 1350 IU/mL.
Values above the measuring range are reported as > 3510000 IU/mL.

Measuring range for undiluted samples:

0.05-130 IU/mL (defined by the Limit of Detection and the maximum of the master curve).
Values below the Limit of Detection are reported as < 0.05 IU/mL.
Values above the measuring range are reported as > 130 IU/mL.

Lower limits of measurement

Limit of Blank and Limit of Detection

Limit of Blank = 0.03 IU/mL

Limit of Detection = 0.05 IU/mL

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

Dilution

Initial onboard dilution of 1:900 with HBSAGQ2 Dil HepB is mandatory for every sample. Therefore every sample should first be run with a dilution step of 1:900 ordered by the user and performed by the analyzer.

After dilution by the analyzer, the software automatically takes the dilution into account when calculating the sample concentration.

If a result is found within the measuring range 45-117000 IU/mL for 900‑fold diluted samples no further dilution is necessary.

If a result is found < 45 IU/mL for 900-fold diluted samples, the sample has to be run undiluted and should be found within 0.05-130 IU/mL.

If a result is found > 117000 IU/mL for 900-fold diluted samples the sample has to be run with a dilution step 1:27000 ordered by the user and performed by the analyzer.

A dilution algorithm can be performed automatically (see section "**cobas e** flows").

cobas e flows

cobas e flows are procedures programmed into the system to enable a fully automated sequence of measurements and the calculation of assay combinations to perform decision algorithms.
A **cobas e** flow is available to automatically perform an initial 1:900 sample dilution and calculate the assay result. In case the result is found above the extended measuring range, another dilution of the sample is carried out (1:27000) and another result is calculated. In case the initial result is found below the extended measuring range, another measurement is carried out without dilution of the sample and the result is reported.

Expected values

Note: Where indicated, data have been generated using the Elecsys HBsAg II quant assay. Since the reagents (M, R1, R2) of the Elecsys HBsAg II quant assay are the same as those of the Elecsys HBsAg II quant II assay (only the controls and calibrators have been modified) the data generated with the Elecsys HBsAg II quant assay are transferable to the Elecsys HBsAg II quant II assay and no new data needed to be generated.

From 611 samples obtained from a multicenter evaluation the following values have been reported with the Elecsys HBsAg II quant assay.

IU/mL	MCE (n = 611)	% of total
< 1	17	2.78
1-< 10	20	3.27
10-< 100	35	5.73
100-< 1000	127	20.8
1000-< 10000	239	39.1
10000-< 100000	147	24.1
100000-< 1000000	26	4.26

The final result was determined from the first measurement in 70.0 % of the samples with 1:100 dilution and 86.5 % of the samples with a 1:400 dilution.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 801 analyzer					
		Repeatability[b)]		**Intermediate precision**[c)]	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
Human serum 1	0.103	0.002	2.0	0.003	2.6
Human serum 2	44.0	0.433	1.0	0.927	2.1
Human serum 3	304	5.37	1.8	8.84	2.9
PC[d)] HBsAg II quant II 1	3.33	0.035	1.1	0.072	2.2
PC HBsAg II quant II 2	78.5	0.747	1.0	1.69	2.2
PC HBsAg II quant II 3	72.1	1.69	2.3	2.11	2.9

b) Repeatability = within-run precision

c) Intermediate precision = between-run precision

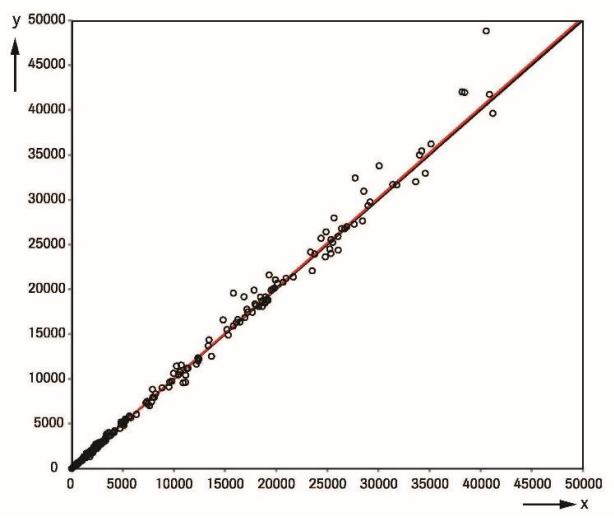
d) PC = PreciControl

Elecsys HBsAg II quant II

cobas®

Method comparison

A comparison of the Elecsys HBsAg II quant II assay (y) with the Elecsys HBsAg II quant assay (x) using 288 serum samples gave the following correlations:



x: Elecsys HBsAg II quant assay

y: Elecsys HBsAg II quant II assay

Passing/Bablok[23]

y = 1.01x - 0.00

τ = 0.997

The sample concentrations were between 0 and 50000 IU/mL.

A comparison of the Elecsys HBsAg II quant II assay REF 07027443190 (**cobas e** 801 analyzer; y) with the HBsAgII quant II assay, REF 07143737190 (**cobas e** 601 analyzer; x) gave the following correlations (IU/mL):

Number of serum samples measured: 214

Passing/Bablok[23]	Linear regression
y = 1.02x + 0.202	y = 0.987x + 297
τ= 0.975	τ = 0.996

The sample concentrations were between 0.000 and 122022 IU/mL.

Quantitation of potentially cross reactive samples

1285 samples containing potentially interfering substances were tested with the Elecsys HBsAg II quant assay comprising specimens:

- containing antibodies against HAV, HCV, HIV, HTLV, CMV, EBV, HSV, Rubella, Parvo virus, VZV, Toxoplasma gondii, Treponema pallidum
- containing autoantibodies (ANA, SLE), elevated titers of rheumatoid factor or HAMA antibodies
- positive for Mumps, Measles, Malaria
- after vaccination against HBV and influenza
- from patients with monoclonal gammopathy and multiple myeloma/lymphoma, patients undergoing dialysis or patients suffering from alcoholic liver disease
- from pregnant women

No results were found ≥ 0.05 IU/mL.

Quantitation of HBV mutants

A total of 50 samples comprising different HBsAg mutations were tested with the Elecsys HBsAg II quant assay. Results of observed concentrations are displayed.

Mutant panel	Elecsys HBsAg II quant (IU/mL)[e)]
Native mutant panel (strains displaying amino acid substitutions either linked to vaccine resistance, resistance to therapy with human HB immunoglobulin or impaired HBsAg reactivity)	< 0.05 (n = 2) 0.05-324 (n = 17)
Recombinant mutant panel	> 0.05-6.9 (n = 31)

e) Observed concentrations with HBV mutants might differ compared to competitor assays and are a characteristic of the individual assays.

Seroconversion panels

18 seroconversion panels were analyzed with the Elecsys HBsAg II quant assay. In all panels the Elecsys HBsAg II quant assay showed a significant increase in concentration upon seroconversion correlated to the shift as it is detectable in qualitative screening assays. Observed concentrations ranged from < Limit of Detection for negative samples, and 0.058 - 92300 IU/mL for conversion (confirmed positive) samples.

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
⟶	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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www.roche.com

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Performance characteristics of the high sensitivity Alinity i & ARCHITECT HBsAg Next Qualitative/Confirmatory assays

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ABSTRACT

Despite improvement in vaccinations, Hepatitis B remains a major health concern due to the difficulty of prevention even in low endemic areas such as Europe. In this report we describe the performance characteristics of the new HBsAg Next Qualitative and HBsAg Next Confirmatory assays designed for blood screening and diagnostic purposes on the Alinity i and ARCHITECT fully automated systems.
The new assays were evaluated in comparison to the ARCHITECT HBsAg Qualitative II and Confirmatory assays on seroconversion, analytical sensitivity, and mutant panels along with testing of over 400 clinical positive samples demonstrating excellent improvements in sensitivity. Additionally, specificity was shown to be improved with testing of over 6000 donors and 240 diagnostic specimens. Overall, the Alinity i & ARCHITECT HBsAg Next assays have taken a step forward in improving the detection of Hepatitis B virus.

1. Introduction

Although vaccination programs for Hepatitis B have been available since 1982 (WHO Hepatitis B key facts, 2019), viral hepatitis is still one of the 10 main causes of death, with Hepatitis B and C causing 96% of all hepatitis mortality (Global Hepatitis Report, 2017).

Transmission of hepatitis B virus (HBV) has been close to impossible to prevent mainly in high endemic areas, where it occurs either perinatally from mother to child at birth or through infected blood. Detection of infected mothers is of major importance as children infected during the first year of life have the highest incidence of developing chronic infection, i.e. 80-90% (Hyams, 1995), potentially leading to severe outcomes. Even in low endemic areas like Europe the incidence of acute HBV infection is increasing due to increased immigration and mass population influxes (Coppola et al., 2015; Hampel et al., 2016).

Hepatitis B surface antigen (HBsAg) is the first and most important antigenic marker for early and late acute as well as chronic infection detection (Kuhns et al., 2019a; Yang et al., 2016).

Although assays for HBsAg detection have been constantly improved (Enjalbert et al., 2014; Mortensen et al., 2016; Candotti and Laperche, 2018; Gerlich et al., 2018), there is still a need for higher sensitivity assays, to avoid false negatives in the diagnostic as well as transfusion settings, due to escape mutants, new genotypes, reactivation and occult infection.

Sensitivity is often obtained at the cost of diminished specificity. Especially in the blood bank setting higher sensitivity should not compromise specificity, to avoid time-consuming and expensive confirmatory testing, as well as delays in reporting results and unnecessary donor deferrals (Popp et al., 2011). NAT testing is more sensitive but also brings with it higher costs and the need for specialized equipment/facilities that are frequently prohibitive in developing countries. Therefore, sensitive HBsAg assays approaching mini-pool NAT sensitivity (Lou et al., 2018) could be an alternative especially in developing countries due to cost-effectiveness or in low prevalence areas.

In this report, we describe the performance characteristics of the new HBsAg Next Qualitative and HBsAg Next Confirmatory assays designed for blood screening and diagnostic purposes on the Alinity i and ARCHITECT fully automated random-access analyzers in comparison to the current on-market ARCHITECT HBsAg Qualitative II/ Confirmatory assays.

2. Materials and methods

2.1. Testing sites

Testing was performed at one external site, i.e. Sanquin National Screening Laboratory, Amsterdam, Netherlands and internally at the research laboratories of Abbott GmbH, in Wiesbaden, Germany

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2.1.1. Assay principle

The HBsAg Next assay is a one-step chemiluminescent microparticle immunoassay (CMIA) assay that has been built to run on both the Abbott Alinity i and the ARCHITECT i Systems.

The Alinity i system is part of a family of next generation Abbott automated analyzer systems for use with immunoassays. The Abbott ARCHITECT i analyzer system is the predecessor of the Alinity i system. The assay detects the qualitative presence of HBsAg and is calibrated/traceable to the World Health Organization (WHO) Second International Standard for HBsAg (subtype adw2, genotype A, NIBSC Code 00/588). Seventy-five microliters of the sample (serum or plasma) is mixed with antibody coated microparticles, specimen diluent and an Acridinium labeled conjugate in the first step of the assay. A series of washes including the use of an ancillary wash buffer ensues after an eighteen-minute incubation at 37 °C. Following the wash cycles, pre-trigger and trigger are added and the chemiluminescent signal is measured in relative light units (RLUs). The assay has a time to first result of 29 minutes and a throughput of up to 200 tests per hour on the ARCHITECT and Alinity i systems.

The ARCHITECT and Alinity HBsAg Next Confirmatory assay both consist of two single tests (referred to as C1 and C2) that are one-step pre-treatment immunoassays.

C1: Sample and Pre-Treatment 1 diluent are combined and incubated. The HBsAg present in the sample is neutralized by a Sheep anti-HBs in Pre-Treatment 1 diluent. An aliquot of the pretreated sample, anti-HBs coated paramagnetic microparticles, assay specific diluent and anti-HBs acridinium-labeled conjugate are combined and incubated. Any remaining non-neutralized HBsAg present binds to the anti-HBs coated microparticles and to the anti-HBs acridinium labeled conjugate. The neutralized HBsAg is blocked from forming a sandwich with acridinium-labeled anti-HBs conjugate and anti-HBs coated microparticles. Following a wash cycle, pre-trigger and trigger solutions are added. The resulting chemiluminescent reaction is measured as RLUs. There is a direct relationship between the amount of non-neutralized HBsAg in the sample and the RLUs detected by the ARCHITECT i or Alinity i System optics.

C2: Sample and Pre-Treatment 2 diluent are combined. Pre-Treatment 2 diluent does not contain Sheep anti-HBs and will not neutralize HBsAg present in the sample. Otherwise the C2 reaction follows a similar flow as C1. There is a direct relationship between the amount of HBsAg in the sample and the RLUs detected by the ARCHITECT or Alinity i System optics.

If the signal for the non-neutralized sample (incubated with Pre-Treatment 2 diluent) result is greater than or equal to the cutoff of 0.70 S/CO and the RLU of the neutralized sample (incubated with Pre-Treatment 1 diluent) is reduced by at least 50% compared to the non-neutralized sample, the sample is considered confirmed positive for HBsAg.

2.2. Specimens and Testing procedures

2.2.1. Seroconversion sensitivity

Seroconversion sensitivity was evaluated on a panel consisting of consecutive bleeds from 32 seroconverting donors obtained from commercial vendors. Testing was performed on Alinity i HBsAg Next Qualitative and ARCHITECT HBsAg Qualitative II.

2.2.2. Analytical sensitivity

Analytical Sensitivity of the ARCHITECT and Alinity i HBsAg Next Qualitative and Confirmatory assays was evaluated on the 2nd and 3rd WHO International standards for HBsAg, subtype adw2, genotype A, NIBSC code: 00/588 and NIBSC code 12/226, respectively.

The standards were reconstituted with distilled water and diluted with recalcified normal human plasma to a target stock concentration of 1000 mIU/mL. Further dilutions were prepared by combining the stock and recalcified normal human plasma to the target concentrations of 0, 3, 5, 8, 10, 15, 20, and 40 mIU/mL. Dilutions where tested on multiple reagent lots and instruments and a minimum of 4 replicates each. For each instrument and reagent lot combination, the mean sample to cut-off (S/CO) values were calculated for each dilution. A least squares linear regression analysis was performed by regressing the mean S/CO values versus the target concentrations across the dilutions.

2.2.3. Clinical sensitivity

Clinical sensitivity was evaluated, by testing 450 samples known to be HBsAg positive and HBV NAT positive from acute ($n = 12$) and chronic ($n = 94$) phases of infection as well as from undefined infection stage ($n = 344$), all sourced from a variety of commercial suppliers.

Testing was performed on ARCHITECT and Alinity i HBsAg Next Qualitative and Confirmatory compared to ARCHITECT HBsAg Qualitative II and Confirmatory. However, testing could not be completed for all specimens on Alinity i HBsAg Next due to sample depletion (total $n=444$).

2.2.4. Mutant panel

Further sensitivity testing was completed on a total of 167 mutant panel specimens consisting of 71 recombinant mutant samples, 2 wild type controls and 94 native mutant samples collected through Abbott's Global Viral Surveillance Program (Harris et al., 2017; Rodgers et al., 2017).

All recombinant HBsAg mutant samples had amino acid sequences representing native mutants of hepatitis B surface antigen (Lou et al., 2018). Only 4 of the 165 mutant samples shared the same mutation pattern. 151 of these samples contained at least one substitution or insertion in the region spanning aa 120–145 within the 'a' determinant of the s antigen. 42 of the samples had single substitutions, 32 had double substitutions, 86 had 3 to a maximum of 18 substitutions or insertions, and 6 had insertions following aa 122 or 123 of the s antigen. Thirty-nine samples contained mutants Gln-129-His, Met-133-Leu, Asp-144-Ala, Gly-145-Arg, and Thr-123-Ala or insertion mutants 122NT, 122RA, P142L+G145R, P142S+G145R. The mutants had been diluted to a target of about 2 S/CO in the ARCHITECT HBsAg Qualitative II assay using negative human plasma.

2.2.5. Clinical specificity

Clinical specificity of the ARCHITECT HBsAg Next Qualitative assay in a blood bank population was evaluated on 6618 donor specimens (collected in 3300 serum and 3318 plasma tubes) in comparison to ARCHITECT HBsAg Qualitative II.

The Alinity i HBsAg assay was evaluated in a separate study using 6718 donor specimens (collected in 3181 serum and in 3537 plasma tubes). These specimens were obtained from 3 blood banks located in Amsterdam, Netherlands; München, Germany; and Mainz, Germany. Clinical specificity in a diagnostic setting was evaluated on a population of 240 serum and plasma specimens from hospitalized patients obtained from a commercial supplier.

2.2.6. Within assay sample carryover

Within sample carryover was evaluated by testing a high positive specimen at a concentration ≥125,000 IU/mL followed by a high negative specimen testing at ~0.76 S/CO. The testing was carried out for 10 iterations.

2.3. Instrument configuration

Induction Heating, a system enhancement technology to preserve sample integrity, must be installed on the ARCHITECT i2000SR and the Alinity i Systems to run the Alinity i & ARCHITECT HBsAg Next Qualitative/ Confirmatory assays.

3. Results

3.1. Seroconversion sensitivity

In 25 of 32 panels (78%) the number of days to the first repeat reactive and confirmed result was less for Alinity i HBsAg Next Qualitative compared to ARCHITECT HBsAg Qualitative II. For the remaining 7 panels, the investigational and comparator assay showed no difference in the number of days to the first repeatedly reactive and confirmed result. For Alinity i HBsAg Next Qualitative, the mean number of days to the first repeat reactive and confirmed result was 31.8 versus 38.3 for ARCHITECT HBsAg Qualitative II. For HBV NAT, the mean number of days to the first repeat reactive and confirmed result was 23.9 (Table 1). A second calculation was performed excluding panels 6272 and 11000, since they are known to be probable vaccine breakthrough seroconverters (Keating et al., 2014) and thus not representative of typical seroconversion in primary acute infection. Without these panels, the mean number of days to the first repeat reactive and confirmed result is 32.2 for Alinity i HBsAg Next Qualitative, 36.8 for ARCHITECT HBsAg Qualitative II and 25.5 for HBV ID-NAT.

Table 1
Alinity i HBsAg Next Qualitative seroconversion sensitivity.

		Days until first detection (n members detected)		
Panel ID	Total n panel members	HBV NAT	Alinity i	ARCHITECT
6271	5	0 (5)	0 (5)	7 (3)
6272	25	0 (25)	51 (14)	94 (7)
6273	6	3 (5)	14 (3)	14 (3)
6275	7	0 (7)	0 (7)	7 (5)
6277	11	21 (9)	26 (8)	33 (6)
6278	10	0 (10)	4 (9)	8 (8)
6279	7	12 (6)	19 (4)	26 (2)
6281	12	0 (10)	7 (8)	13 (7)
6282	14	0 (14)	12 (11)	14 (10)
6284	19	36 (11)	43 (9)	50 (7)
6285	16	31 (10)	38 (8)	40 (7)
6286	9	22 (8)	29 (6)	29 (6)
6290	12	0 (12)	14 (8)	14 (7)
9074	20	52 (8)	66 (5)	70 (4)
11000	9	0 (9)	3 (8)	26 (3)
11001	8	33 (6)	44 (4)	44 (4)
11002	6	0 (6)	0 (6)	7 (4)
11003	8	133 (5)	133 (5)	142 (3)
11006	17	19 (12)	35 (7)	42 (6)
11007	14	15 (10)	29 (6)	34 (5)
11008	18	51 (10)	62 (7)	69 (5)
11012	6	0 (6)	8 (4)	18 (3)
11013	35	230 (11)	239 (9)	244 (8)
11014	12	35 (5)	37 (4)	51 (3)
11017	14	25 (9)	34 (7)	40 (6)
11026	16	25 (9)	36 (6)	39 (5)
11029	13	21 (9)	32 (6)	35 (5)
13867/3482	30	0 (15)	0 (14)	0 (13)
1807/3463	25	0 (21)	4 (19)	4 (18)
43527/3453	26	0 (24)	0 (24)	0 (21)
26022/14518	30	0 (9)	0 (10)	7 (7)
0994/3457	23	0 (23)	0 (23)	4 (22)
Mean days	N/A	23,9	31,8	38,3
n Panel members detected of total		339/483	274/483	214/483

The days until detection of first reactive bleed of each panel is provided in the table above. The total number of bleeds detected of a panel is outlined in brackets. Although first bleed detection might be the same, this number of bleeds detected can be different between assays, e.g. panel 13867/3482, as early and late bleeds are counted.

3.2. Analytical sensitivity

For Alinity i and ARCHITECT HBsAg Next Qualitative analytical sensitivity values of 4.50 to 5.97 mIU/mL and 4.62 to 6.14 mIU/mL, respectively, have been obtained when using the WHO 2nd International Standard across reagent lots. The Alinity i and ARCHITECT HBsAg Next Qualitative assays were about 4-fold more sensitive compared to ARCHITECT HBsAg Qualitative II with an analytical sensitivity of 17.00 to 22.00 mIU/mL across reagent lots.

Using the WHO 3rd International Standard, the analytical sensitivity was assessed to be between 4.15 and 5.11 mIU/mL for ARCHITECT HBsAg Next Qualitative and between 4.35 and 5.24 mIU/mL for Alinity i HBsAg Next Qualitative across reagent lots. For the ARCHITECT HBsAg Qualitative II assay, analytical sensitivity on the 3rd WHO International Standard was not measured in this study.

3.3. Clinical sensitivity

The clinical sensitivity was determined to be 100.00% for both investigational Alinity i and ARCHITECT HBsAg Next Qualitative assays for the three sample categories tested. In comparison, the ARCHITECT HBsAg Qualitative II assay achieved an overall sensitivity of 99.78% (Table 2). This is due to one discordant HBsAg NAT positive specimen, which was found to be repeat reactive and confirmed on ARCHITECT HBsAg Next with an initial S/CO result of 4.99 and also repeat reactive and confirmed on Alinity i with an initial S/CO results of 5.57, but nonreactive on the comparator assay ARCHITECT HBsAg Qualitative II with an S/CO result of 0.77.

3.4. Mutants

Mutant specimens were better detected with the Alinity i and ARCHITECT HBsAg Next Qualitative assay. S/CO results of more than 100 were observed for single panel members that were detected at about 2 S/CO in the comparator assay with an on average 7-fold increase in S/CO values over the ARCHITECT HBsAg Qualitative II assay (Fig. 1). The new HBsAg Next Confirmatory assay showed a more efficient neutralization of mutant specimens as compared to the respective ARCHITECT HBsAg Qualitative II confirmatory assay (Fig. 2).

3.5. Clinical specificity on blood donors and diagnostic specimens

The ARCHITECT and Alinity i HBsAg Next Qualitative assays were assessed to have comparable specificity of 99.95% and 99.96%, respectively, on blood donations, and both 100% specificity on a diagnostic population. The predecessor assay ARCHITECT HBsAg Qualitative II showed a slightly lower specificity of 99.92% on blood donors, but also 100% on diagnostic specimens (Table 3). The S/CO distribution in the specificity populations is outlined in Table 4.

3.6. Within assay sample carryover

Alternating high positive specimen and elevated nonreactive specimens were tested for 10 iterations. The difference between the protected sample (nonreactive specimen not exposed to replicates of the positive sample prior to testing) and the unprotected sample (run immediately after an extremely high positive HBsAg specimen) had an absolute bias of 0.08 S/CO (confidence interval: 0.02 to 0.13) for a high nonreactive sample running at 0.76 S/CO.

4. Discussion

Although current HBsAg assays exhibit relatively high sensitivities there is a continuous need to improve sensitivity of these HBV assays, to avoid false negative results on early and late acute specimens, and to enable earlier detection of vaccine breakthrough mutants, occult

Table 2
Comparison of Clinical Sensitivity of ARCHITECT/ Alinity i HBsAg Next Qualitative and ARCHITECT HBsAg Qualitative II

Assay	Category	N	RR	Sensitivity %	95% Confidence interval
ARCHITECT HBsAg Next Qualitative	Acute HBV Infection	12	12	100.00 (12/12)	(73.54–100.00)
	Chronic HBV Infection	94	94	100.00 (94/94)	(96.15–100.00
	HBsAg Positive	344	344	100.00 (344/344)	(98.93–100.00)
	Overall	450	450	100.00 (450/450)	(99.18–100.00)
ARCHITECT HBsAg Qualitative II (Comparator)	Acute HBV Infection	12	12	100.00 (12/12)	(73.54–100.00)
	Chronic HBV Infection	94	94	100.00 (94/94)	(96.15–100.00)
	HBsAg Positive	344	343	99.71 (343/344)	(98.39–99.99)
	Overall	450	449	99.78 (449/450)	(98.77–99.99)
Alinity i HBsAg Next Qualitative	Acute HBV Infection	11	11	100.00 (11/11)	(71.51–100.00)
	Chronic HBV Infection	92	92	100.00 (92/92)	(96.07–100.00)
	HBsAg Positive	341	341	100.00 (341/341)	(98.92–100.00)
	Overall	444*	444	100.00 (444/444)	(99.17–100.00)

RR = Repeatedly Reactive
* 6 samples could not be evaluated on the Alinity platform due to insufficient sample volume.

infections and viral reactivation (Kuhns et al., 2019a). Improved acute infection detection is especially important in regions where NAT evaluation is not available and/or affordable (Martin et al., 2012; Stramer et al., 2012; Vermeulen et al., 2013) to further reduce the detection window during acute HBV infection.

Since the introduction of HBsAg assays, the challenge has been to provide the highest possible sensitivity without compromising specificity. Maintaining this balance is critical in the blood bank setting, as high specificity is needed to avoid time-consuming confirmatory testing, as well as to eliminate delays in reporting and releasing results and avoiding unnecessary donor deferrals as well as uncertainty for donors, resulting in stress (Jonas et al., 2005). Specific care was given to reducing non-specific background and noise levels in the new HBsAg Next assays by introducing new rare reagents and buffer systems as well as upgrading and improving hardware systems to complement the heightened sensitivity of the assays.

As a result of these modifications to the reagents and instrument systems the analytical sensitivity of the new HBsAg Next assays on the Alinity i / ARCHITECT was increased about 4-fold compared to the on-market already very sensitive ARCHITECT HBsAg Qualitative II assay. The increased analytical sensitivity enables better clinical diagnostic and screening sensitivity, mutant detection and seroconversion sensitivity. During clinical sensitivity testing, one incremental pick up was observed in one HBsAg NAT positive specimen, which was detected at 5.57 S/CO (Alinity i HBsAg Next) and 4.99 S/CO (ARCHITECT HBsAg Next) but was high non-reactive at 0.77 S/CO on ARCHITECT HBsAg Qualitative II.

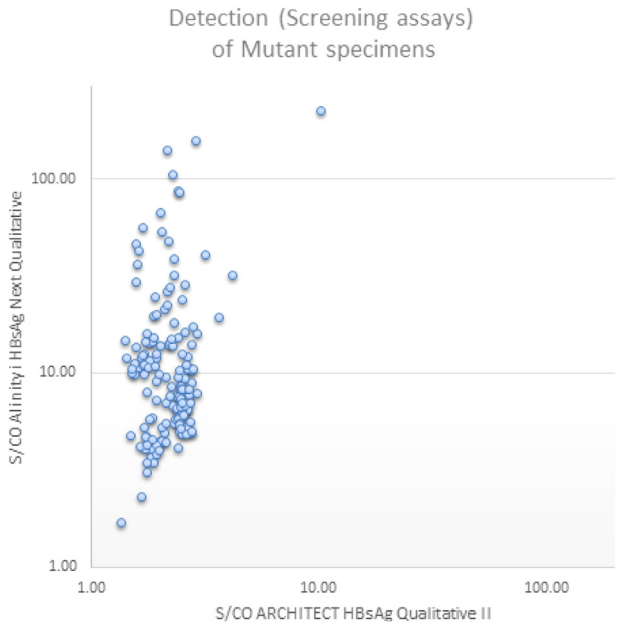

Fig. 1. Comparison of mutant specimen detection (S/CO results) between Alinity i HBsAg Next Qualitative and ARCHITECT HBsAg Qualitative II. Results ranged from 1.69 to 225.07 S/CO in the Alinity I HBsAg Next Qualitative assay and from 1.35 to 10.13 S/CO in the ARCHITECT HBsAg Qualitative II

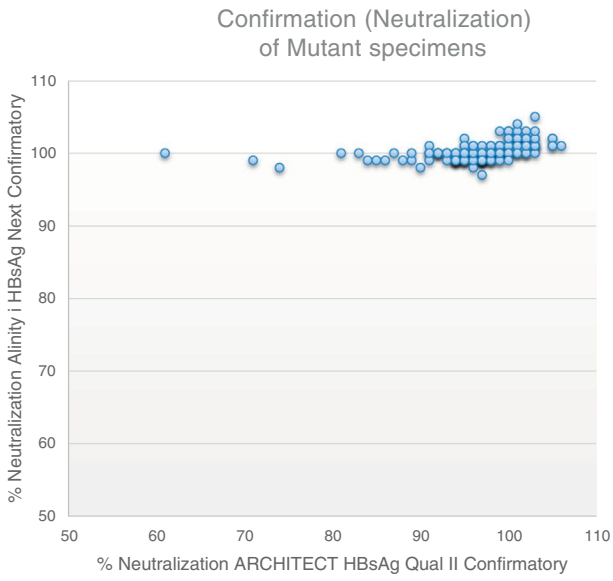

Fig. 2. Comparison of mutant neutralization capacity between Alinity i HBsAg Next Qualitative and ARCHITECT HBsAg Qualitative II.

Table 3
Comparison of Clinical Specificity of ARCHITECT/ Alinity i HBsAg Next Qualitative and ARCHTECT HBsAg Qualitative II

Assay	Category	N	IR (% of Total)	RR (% of Total)	Conf. positive (% of RR)	Specificity %	95% Confidence interval
ARCHITECT HBsAg Next Qualitative	Blood Donors Serum	3300	6 (0.18)	1 (0.03)	0 (0.00)	99.97 (3299/3300)	(99.83–100.00)
	Blood Donors Plasma	3318	10 (0.30)	2 (0.06)	0 (0.00)	99.94 (3316/3318)	(99.78–99.99)
	Total BD	6618	16 (0.24)	3 (0.05)	0 (0.00)	99.95 (6615/6618)	(99.87–99.99)
	Diagnostics	240	2 (0.83)	1 (0.42)	1 (100.00)	100.00 (239/239)	(98.47–100.00)
ARCHITECT HBsAg Qualitative II	Blood Donors Serum	3300	3 (0.09)	0 (0.00)	0 (0.00)	100.00 (3300/3300)	(99.89–100.00)
	Blood Donors Plasma	3318	8 (0.24)	5 (0.15)	0 (0.00)	99.85 (3313/3318)	(99.65–99.95)
	Total BD	6618	11 (0.17)	5 (0.08)	0 (0.00)	99.92 (6613/6618)	(99.82–99.98)
	Diagnostics	240	1 (0.42)	1 (0.42)	1 (100.00)	100.00 (239/239)	(98.47–100.00)
Alinity i HBsAg Next Qualitative	Blood Donors Serum	3181	4 (0.13)	3 (0.09)	0 (0.00)	99.91 (3178/3181)	(99.72–99.98)
	Blood Donors Plasma	3537	2 (0.06)	2 (0.06)	2 (100.00)	100.00 (3535/3535)	(99.90–100.00)
	Total BD	6718	6 (0.09)	5 (0.07)	2 (40.00)	99.96 (6713/6716)	(99.87–99.99)
	Diagnostics	240	1 (0.42)	1 (0.42)	1 (100.00)	100.00 (239/239)	(98.47–100.00)

IR = Initially Reactive, RR = Repeatedly Reactive, Conf. positive = Number of confirmed positive specimens by the corresponding Confirmatory assays, BD = blood donors.

Seventy-one recombinant mutant samples and 94 native mutant samples were evaluated, including mutants that specifically occur in regions with known mutational propensity. The mutants tested included thirty-nine samples with well documented mutations such as Gln-129-His, Met-133-Leu, Asp-144-Ala, Gly-145-Arg, and Thr-123-Ala or insertion mutants 122NT, 122RA, P142L+G145R, P142S+G145R. Furthermore, mutations at each of AA 121 through 124 and AA 112 through 117 are represented in the panel, as well as point mutations for more than half of AAs 187 to 207 mutations. Overall, mutants were detected on average about 7-fold higher with ARCHITECT / Alinity i HBsAg Next versus the ARCHITECT HBsAg Qual II assay.

Vermeulen et al. reported the first known case of transfusion-transmitted HBV infection by blood screened using individual-donation (ID)-NAT (Vermeulen et al., 2012). There are three stages where high assay sensitivity is needed: the first window period (pre-HBsAg & NAT), the second window period (post-HBsAg & NAT) and third window is in occult infection, as these donations are infectious. Alinity i HBsAg Next Qualitative seroconversion sensitivity was improved on average by approximately 6.5 days over the ARCHITECT HBsAg Qualitative II assay (maximum 43 days in one case) and was on average 7.9 days behind HBV ID-NAT. Excluding vaccine breakthrough panels 6272 and 11000, seroconversion sensitivity is improved on average by approximately 4.6 days (maximum 14 days in one case) compared to ARCHITECT HBsAg Qual II and is 6.7 days behind ID-NAT. Nevertheless, the HBsAg Next assays exhibit sensitivity approaching that of mini-pool NAT (Lou et al., 2018). As already published by Kuhns et al., 2019a, the HBsAg Next Qualitative assays exhibit improved early acute seroconversion sensitivity but also for late acute specimens, as exhibited by the testing of panels 13867-3482, 1807-3463, 26022-14518, and 43527-3453. These assays also demonstrate improvements in detection of HBsAg in vaccine breakthrough cases as exemplified by Panel 6272 and 11000 (Kuhns et al., 2019b). In both panels, the Alinity i HBsAg Next assay has significantly improved sensitivity, despite the presence of anti-HBs antibodies which frequently mask the presence of infection.

Assays launched so far exhibit either increased sensitivity or better specificity thus compromising one or the other. Specificity numbers for HBsAg assays vary. Package Insert data show initial/repeat specificity numbers of 99.51/ 99.91% for Siemens ADVIA Centaur Hepatitis B surface Antigen II (Atellica IM Hepatitis B surface Antigen II (HBsII) (Siemens) Package Insert: Rev. 02, 2017-12), 99.91/ 99.98% for Roche Cobas Elecsys HBsAg II (Elecsys HBsAg II (Roche Cobas) Package Insert: Rev. 2018-06, V 1.0) and 99.89/ 99.91% for ARCHITECT HBsAg Qualitative II (ARCHITECT HBsAg Qualitative II Package Insert: G3-4444/R05, 2013) respectively for blood donor specimen testing. The initial/ repeat specificity

Table 4
S/CO distribution in specificity populations.

Assay	Category	N	SD	Mean S/CO
ARCHITECT HBsAg Next Qualitative	Blood Donors Serum	3300	0.074	0.26
	Blood Donors Plasma	3318	0.064	0.23
	Total BD	6618	0.070	0.25
	Diagnostics	240	0.068	0.25
ARCHITECT HBsAg Qualitative II	Blood Donors Serum	3300	0.032	0.16
	Blood Donors Plasma	3318	0.030	0.16
	Total BD	6618	0.031	0.16
	Diagnostics	240	0.034	0.18
Alinity i HBsAg Next Qualitative	Blood Donors Serum	3181	0.084	0.32
	Blood Donors Plasma	3537	0.079	0.30
	Total BD	6718	0.082	0.31
	Diagnostics	240	0.069	0.32

BD= blood donors S/CO= Sample to Cut-off SD= standard deviation.

numbers obtained for ARCHITECT and Alinity i HBsAg Next Qualitative were at 99.76/ 99.95% and 99.94/ 99.96% and indicate that increased sensitivity of these assays does not impact specificity. Diagnostic specificity as outlined in the package inserts was at 100% for Siemens ADVIA Centaur, 99.88% for Roche Cobas Elecsys, and 99.93% for ARCHITECT HBsAg Qualitative II. In our study the new HBsAg Next Qualitative assays exhibited 100% specificity.

Furthermore, specificity of these high sensitive assays is not impacted as seen by the within assay sample carryover results due to using heat induction wash technology on both platforms.

Overall, the Alinity i/ ARCHITECT HBsAg Next Qualitative assays showed significantly improved sensitivity compared to the ARCHITECT HBsAg Qualitative II assay without compromising specificity. The parallel improvement of sensitivity and neutralization capacity of the Alinity i and ARCHITECT HBsAg Next Confirmatory assays allows laboratories the use of common reagents without having to rely on alternate methods (e.g. PCR) to confirm HBsAg positive specimens. The improved sensitivity of the HBsAg Next Qualitative / Confirmatory assays significantly improves detection and confirmation of HBsAg early and late infection, ang Qualitative II Package Insert: Gd in a variety of mutants, occult infections and vaccine breakthrough cases even in the presence of protective levels of Anti-HBs antibodies.

With the improved sensitivity and specificity, the HBsAg Next Qualitative / Confirmatory assays can be used in both the blood screening as well as in the diagnostic setting, to permit safe and effective detection of HBV.

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Declarations of interest

All authors of this publication are employees of Abbott Laboratories.

Credit Author contribution statement

E. Sickinger: Conceptualization; Data curation; Methodology; Formal analysis; Supervision; Writing – original draft; Writing – review & editing. **H.B. Braun**: Investigation; Data curation; Formal analysis; Writing – original draft; Writing – review & editing. **T. Meyer**: Data curation; Investigation; Formal analysis; Writing – original draft; Writing – review & editing. **K. Schmid**: Investigation; Data curation; Formal analysis; Writing – review & editing. **D. Daghfal**: Formal analysis; Writing – original draft; Writing – review & editing. **M. Oer**: Formal analysis; Writing – review & editing; Supervision; Project administration. **J. Schultess**: Formal analysis; Writing – review & editing.

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March 2020

RUO

Read this insert carefully before performing the assay and make sure you are using the most recent version of the package insert.
The reliability of assay procedures other than those described in this package insert cannot be guaranteed.

CL0008 R02

For research use only.
Not for use in diagnostic procedures.

READ HIGHLIGHTED CHANGES: Revised March 2020

Chemiluminescent Enzyme Immunoassay Reagent

Lumipulse® G HBcrAg

Immunoreaction Cartridges set

- **NAME**

Lumipulse G HBcrAg Immunoreaction Cartridges set
(Also referred to as 'HBcrAg Cartridges set' in this package insert)

- **INTENDED USE**

For Research Use Only, with the LUMIPULSE G System for the quantitative detection of Hepatitis B virus core-related antigen in serum or plasma.

- **SUMMARY AND EXPLANATION OF THE ASSAY**

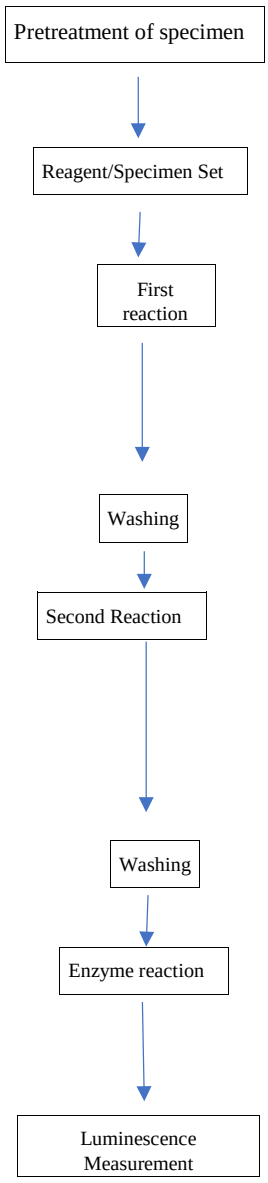
Acute hepatitis B and chronic hepatitis B are conditions directly caused by HBV infection. The amount of HBV is significantly altered in the natural course of chronic hepatitis B. Furthermore, the amount of HBV correlates with its infectivity and patient prognosis. To measure HBV in liver tissue, detection of HBeAg, HBsAg or HBV-DNA in blood is commonly used. However, these methods contain methodological limitations. HBeAg cannot be detected when HBeAg forms immune complex with HBeAb. HBV-DNA detection methods are more cumbersome and time-consuming procedures. Antiviral agents such as lamivudine, adefovir dipivoxil or entecavir; inhibit DNA synthesis and release of enveloped virus particles, containing HBV-DNA. Therefore, HBV-DNA assays may show negative results even when HBV is present in liver tissue. The method of cccDNA (covalently closed circular DNA) analysis in liver biopsy specimens is effective, but as this is highly invasive it is not always preferred.

Lumipulse G HBcrAg assay is a novel CLEIA for the quantification of HBV. This assay is easy-to-use, does not use an invasive sample, has a wide measurement range and a high sensitivity. Three HBV correlated proteins are detected with this kit: 1)HBeAg, 2)HBcAg and 3)HBV pre-core protein (also known as p22cr). Different reports[3-7] suggest that these HBV core-related proteins are present in blood of patients under treatment with antiviral agents and can be used to measure the amount of HBV in liver tissue as a complementary tool in the follow-up of patients with hepatitis B for the monitoring and possible discontinuation of treatment.

- **PRINCIPLES OF THE PROCEDURE**

Lumipulse G HBcrAg is an assay system, including a set of immunoassay reagents, for the quantitative detection of HBcrAg in specimens based on CLEIA technology by a two-step immunoassay method on the LUMIPULSE G System.

<Reaction protocol; 2-step mode>



AMPPD: 3-(2'-spiroadamantane)-4-methoxy-4-(3'-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt

- **MATERIALS PROVIDED**

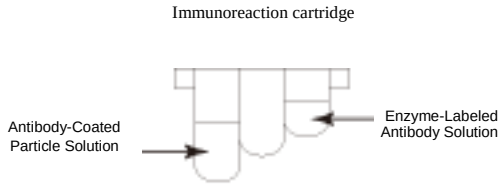
Lumipulse G HBcrAg Immunoreaction Cartridges set:
IRC SET REF 231715

1. Lumipulse G HBcrAg Immunoreaction Cartridges: IRC
3 x 14 Tests

1)Antibody-Coated Particle Solution
(Liquid when used, 230 µL/Immunoreaction Cartridge)
Contains anti-HBcrAg monoclonal antibody (HB44, HB61, HB114)-coated particles, protein stabilizers (bovine) and chemical stabilizers in 0.2 M sodium chloride/Tris buffer. This solution contains gelatin and turns into gel at 15°C or lower.
Preservative: sodium azide.

2)Enzyme-Labeled Antibody Solution
(Liquid, 350 µL/1mmunoreaction Cartridge)
Contain alkaline phosphatase (ALP)-labeled anti-HBcrAg monoclonal antibody (HB91), ALP-labeled anti-HBcrAg monoclonal antibody (HB 110) and protein stabilizers

(bovine and rabbit) and chemical stabilizers in 0.037 M sodium chloride/Tris buffer. Preservative: sodium azide



2. Lumipulse G HBcrAg Calibrators: CAL Liquid,
1 x 2 Concentrations
HBcrAg Calibrator 0 kU/mL (1 x 1.5 mL)
HBcrAg Calibrator 10000 kU/mL (1 x 1.5 mL)
3. Lumipulse G HBcrAg Pretreatment solution: Liquid, 1 x 8 mL

- **MATERIALS REQUIRED BUT PROVIDED SEPARATELY**

1. **Lumipulse G Substrate Solution**: Liquid,
6 x 50 mL REF 231166
6 x 100 mL REF 231197
Contains 0.2 mg/mL AMPPD as a substrate in diethanolamine buffer with a chemical stabilizer. Preservative: sodium azide.
2. **Lumipulse G Wash Solution**: Concentrate,
1 x 1000 mL REF 231173
3. **Sampling tips for LUMIPULSE SYSTEM** (rack packed):
10 x 96 tips REF 231579
Ready to use for G1200.
4. **Soda lime for LUMIPULSE SYSTEM**:
6 x 2 tubes REF 231562
Ready to use.

- **MATERIALS REQUIRED BUT NOT PROVIDED**

1. Purified water
2. Control materials; refer to •QUALITY CONTROL
3. Micropipettes
4. Recommended sample cups: refer to the LUMIPULSE G System Operation Manual and SPECIMEN COLLECTION AND PREPARATION section.
5. Plastic microcup (Maximum volume of 0.3 mL or more).
6. Incubator for pre-treatment: pre-treatment reaction is processed at 56-64 ºC.

- **WARNINGS AND PRECAUTIONS**

For research use only.

1. SAFETY PRECAUTIONS

Please refer to the Safety Data Sheet (SDS) and product labelling for information on potentially hazardous components. The most recent SDS version is available on the website www.fujirebio.com.

Warning:
Pretreatment Solution

Hazard statements
H315 Causes skin irritation.
H319 Causes serious eye irritation.
EUH032 Contact with acids liberates very toxic gas.
EUH210 Safety data sheet available on request.

Precautionary statements

P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313	If skin irritation occurs: Get medical advice/attention.
P337+P313	If eye irritation persists: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

2. PRECAUTIONS FOR HANDLING

1) No given tests can completely guarantee that infected materials are absent. To avoid risk of infection when using human specimens, wear disposable gloves to avoid direct contact to them, do not perform pipetting by mouth and follow appropriate biosafety practice.
2) The following reagents contain sodium azide as a preservative. Avoid contact with skin, eyes, or mouth.
Wash Solution: 1.0% (w/v) (before dilution)
Substrate Solution: 0.05% (w/v)
Antibody-Coated Particle Solution: 0.1 % (w/v)
Enzyme-Labeled Antibody Solution: 0.1 % (w/v)
Calibrators: 0.1% (w/v)
3) Lumipulse G Substrate Solution is an alkaline solution (pH 10). Handle carefully to avoid contact with skin, eyes, or mouth.
4) In the event of accidental contact of any reagent with skin, eyes, or mouth immediately rinse thoroughly with water and seek medical attention if necessary.

3. PRECAUTIONS FOR USE

1) Read through this package insert and the LUMIPULSE G System Operation Manual. Follow their instructions. Incorrect use of the reagents, instrument, or other consumables may result in unreliable data or accident hazard.
2) The HBcrAg Cartridges set (HBcrAg Cartridges/ HBcrAg Calibrators), Substrate Solution and Wash Solution are packed separately.
3) The HBcrAg Cartridges can be stored on-board the LUMIPULSE G System for a maximum of 30 days.
4) Do not use expired reagent.
5) To ensure measurement accuracy, always use freshly purified water.
6) Avoid using the reagents which may have been stored in an improper way.
7) Use new sampling tips and new sample cups as specified by the LUMIPULSE G System. Refer to the LUMIPULSE G System Operation Manual.
a. If large amounts of bubbles are present in the HBcrAg Calibrators, use a new calibrator bottle. Bubbles in a sample tube may cause sampling errors.
b. Bring the calibrators to room temperature (15-25 ºC). Mix the calibrators by gentle inversion.
c. Start the HBcrAg assay immediately after sample setup to avoid evaporation of the specimen or HBcrAg Calibrators.
d. Avoid removing the Substrate Solution until it is time to replace it. The Substrate Solution cannot be used if it is contaminated with ALP. Use new gloves when replacing the Substrate Solution.

e. Replace the soda lime on the LUMIPIJLSE G System according to the LUMIPLJLSE G System Operation Manual.

4. PRECAUTIONS FOR WASTE

1) The reagents contain sodium azide as a preservative, as previously described (See 2. PRECAUTIONS FOR HANDLING). Follow any applicable regulations for disposal. If flushing down the drain, use generous amount of water when discarding to prevent the formation of explosive metal azides.

2) Handle any medical waste produced by this assay in compliance with waste-related regulations in each country or region.

3) When any liquid such as specimen or assay waste is splashed, wipe and disinfect the whole area with an appropriate disinfectant such as sodium hypochlorite or glutaric aldehyde.

▪ STORAGE INSTRUCTIONS

Store the following reagents at 2-10 ºC.

DO NOT FREEZE.

1. Lumipulse G HBcrAg Immunoreaction Cartridges set
2. Lumipulse G Substrate Solution
3. Lumipulse G Wash Solution

When stored and handled properly, reagents remain stable until the expiration date. Refer to the expiration date shown on the immediate container labels.

▪ INSTRUMENT

The reagents are designed for a fully automated chemiluminescent enzyme immunoassay (CLEIA) on the LUMIPULSE G System. Refer to the LUMIPULSE G System Operation Manual for further information.

▪ REAGENT PREPARATION

1. Lumipulse G HBcrAg Cartridges

HBcrAg Cartridges are filled with Antibody-Coated Particle Solution and Enzyme-Labeled Antibody Solution. Peel off the transparent film from the Immunoreaction Cartridge case before installing the case for measurement.

2. Lumipulse G HBcrAg Calibrators

Bring to room temperature (15-25 ºC). Mix the calibrators by gentle inversion. Pipette 200µL of calibrators into plastic tube and proceed to the manual pre-treatment step. Dispense the required amount of pre-treated calibrators into the sample cup, taking into account the dead volume which is at least 100 µL when using recommended sample cups with the LUMIPULSE G System.

3. Lumipulse G Substrate Solution

Use as is. Refer to the LUMIPULSE G System Operation Manual for installing into the LUMIPULSE G System and the package insert of Lumipulse G Substrate Solution for general information. Ensure the substrate cap seal is properly installed to avoid air entering the system.

4. Lumipulse G Wash Solution

Wash solution is concentrated. When using, dilute 10 x with purified water and mix thoroughly. The diluted Wash Solution shall be brought to room temperature (15-25 ºC). Refer to the LUMIPULSE G System Operation Manual for loading into the LUMIPULSE G System and the package insert of Lumipulse G Wash Solution for general information.

5. HBcrAg pre-treatment solution

Bring to room temperature (15-25 ºC) until deposition of detergent have been disappeared completely. Mix the pre-treatment solution evenly and confirm deposition is not precipitating at the bottom of the bottle. If mixing is not complete, deposition will be attached to the bottom of the bottle. Pre-treatment solution with this deposition cannot be used.

▪ SPECIMEN COLLECTION AND PREPARATION

1. Specimens are brought to room temperature before pre-treatment. Dispense 150µL of specimens into plastic tubes. Add 150µL of HBcrAg Pre-Treatment solution to the plastic tubes and mix. Incubate at 60 +/-4 ºC for 30 minutes and then leave the tube at room temperature for approximately 5minutes. After this measure the sample with Lumipulse G1200.
Do not mix pre-treated specimens. Detergent in pre-treatment solution is likely to form bubbles and cause sampling error.
2. HBcrAg Calibrators are brought to room temperature before pretreatment. Dispense 200µL of Calibrators into plastic tubes. Add 200µL of HBcrAg Pre-Treatment solution to the plastic tubes and mix. Incubate at 60 +/-4 ºC for 30 minutes and then leave the tube at room temperature for approximately 5minutes. After this measure the sample with Lumipulse G.
Do not mix pre-treated calibrators. Detergent in pre-treatment solution is likely to form bubbles and cause sampling error.
3. It is recommended to use fresh specimens.
4. Specimens may be stored at -20 ºC.
5. Avoid successive freezing and thawing of specimens. Do not perform freeze/thaw cycle 6 or more times.
6. Measurements may be affected by erythrocytes, fibrin, or other unspecified precipitates or debris contained in specimens. Centrifuge specimens and/or remove these to ensure accurate results.
7. Avoid using heat-inactivated specimens.
8. Handle specimens carefully to avoid cross-contamination.
9. Human serum or plasma collected in dipotassium EDTA, sodium citrate, sodium heparin anticoagulant tubes may be used in the Lumipulse G HBcrAg. Follow the manufacturer's instructions for use. If the blood collection tubes other than the above are used, each laboratory should validate their applicability to the Lumipulse G HBcrAg.
10. Dispense pre-treated serum or plasma to the sample containers. Refer to the LUMIPULSE G System Operation Manual for the recommended sample cups and sample tubes.
11. The Lumipulse G HBcrAg uses 130 µL of pre-treated specimen. The dead volume is at least 100 µL and 250 µL when using recommended sample cups and sample tubes with the LUMIPULSE G System respectively. Therefore, the total sample volume required per assay is over 230 µL for sample cups and 380 µL for sample tubes. The size of the sample tubes for the LUMIPULSE G System is described in the LUMIPULSE G System Operation Manual.
12. When shipped, package and label specimens in compliance with any applicable regulations for the transport of clinical specimens and infectious substances.

▪ ASSAY PROCEDURE

1. Refer to the LUMIPULSE G System Operation Manual and place the specimens and reagents needed for measurement in the specified locations.
2. Enter the assay requests of HBcrAg Calibrators and specimens.
3. Before starting the assay, confirm that the required amounts of HBcrAg Cartridges, Substrate Solution, diluted Wash Solution and Sampling tips are set on the LUMIPULSE G System.
4. Press the START key to start the measurement.

▪ CALIBRATION

1. Required Calibrators

Lumipulse G HBcrAg Calibrators.

2. Calibration Procedure

Refer to 'REAGENT PREPARATION. For subsequent procedures follow the LUMIPULSE G System Operation Manual.

3. When to calibrate

Calibration is performed in the following cases:

1) On the first assay of the Lumipulse G HBcrAg
2) When the HBcrAg Cartridges or substrate solutions are replaced with a different lot.
3) When quality control results fall out of the range.
4) When 30 days have elapsed since the previous calibration. Update the calibration data whenever needed.

4. The calibrator must be tested in duplicate.

▪ RESULTS

The HBcrAg concentration of a specimen is automatically calculated from the luminescent signal, which is also automatically created from the calibration data.

1. Unit calculation

The result is indicated with the unit of kU/mL and LogU/mL in the LUMIPULSE G System. Substitute the value into LogU/mL. 1kU/mL is equal to 3LogU/mL

2. Measurement Range

3.0 LogU/mL - 7.0 LogU/mL (1.0 - 10000.0 kU/mL) Be careful that the LUMIPULSE G System may indicate down to 0.1 kU/mL of HBcrAg, which is out of the measurement range.

▪ QUALITY CONTROL

1. Quality Control Material Preparation

   Use control materials with at least two levels (e.g. negative or nonreactive and positive or reactive).

2. Quality Control Procedure

   Refer to the package insert of control materials. Refer to Internal Quality Control Testing for further information! It is recommended to conduct Quality Control Testing at least once every 24 hours.

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Q² Solutions®

Valencia, CA, USA

Assay Validation Report

Validation of an Electro-chemiluminescence Immunoassay by Roche for the Evaluation of Quantitative Hepatitis Be Antigen (HBeAg) in Serum on the Roche Cobas e411 Analyzer (S/N. AE1066-19)

This validation has been reviewed and performance of this method considered acceptable for patient testing

	Name and Title	Signature	Date
Authored by	David Strickler Ph.D. Senior Scientist Technical Services		17-SEP-2019
Reviewed by	Charlie Fix Global Director Scientific Harmonization		17Sep2019
Reviewed by	Nasrin Ramazani Director Laboratory Operations	Nasrin Ramazani	19-Sept-2019
Reviewed by	Sona Manoukian Manager Chemistry/Special Chemistry		17Sep 2019
Approved by	Marc Edwards, MD, MBA Senior Medical Director North America		19-Sept-2019
QA Review by	Héctor Alberto Repetto, BSc, CQA, MBA Manager Quality Assurance		

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Approved by	Marc Edwards, MD, MBA Senior Medical Director North America		
QA Review by	Héctor Alberto Repetto, BSc, CQA, MBA Manager Quality Assurance		11SEP2018

Q2CL_AP_280255VD.100.1

Table of Contents

1. Objective

The objective of this study was to validate the performance characteristics of a Roche Electro-chemiluminescence Immunoassay for the quantitative determination of Hepatitis Be Antigen (HBeAg) in Serum at Q² Solutions Valencia on the Roche Cobas e411 Analyzer (S/N. AE1066-19).

2. Assay Principle

The HBeAg is an electrochemiluminescence immunoassay and uses monoclonal anti-HBe antibodies (mouse) for the HBeAg determination. This assay employs a sandwich principle with a total duration of 18 minutes.

1st incubation: HBe antigen from 35 µL sample, a biotinylated monoclonal HBeAg specific antibody, and a monoclonal HBeAg-specific antibody labelled with a ruthenium complex form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M/ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cut-off value previously obtained by calibration. The generated measuring signals in the form of cut-off index (COI) corresponds to the HBeAg concentration. HBeAg Quantitative is reported in IU/mL. The COI result from analyzer will be converted to a result in IU/mL

3. Materials and Method

3.1. Sample/Specimen Description

For the purpose of the Precision studies, one level of Roche Elecsys HBeAg Negative and one level of Roche Elecsys HBeAg Positive controls (Negative Lot# 370148, Positive Lot# 370149 Exp. 31-Jul-20) and one level of BioRad VIROTROL HBeAg (Lot# 113590 Exp. 28-Feb-20) control were used on the Roche Cobas e411 Analyzer (S/N. AE1066-19).

For the purpose of the Patient Correlation studies, forty anonymized serum samples (numbered C1 – C40) spanning the AMR as much as possible were prepared from anonymized remnant samples known to be positive for HBeAg, collected in the Quest Diagnostics Nichols Institute San Juan Capistrano laboratory and run using the Roche Elecsys HBeAg reagent. The samples were split into two aliquots at the Q² Solutions Valencia laboratory and one set was shipped to the Q² Solutions Singapore laboratory with both sets stored at or below -60°C.

For the purpose of the Analytical Measurement Range (AMR) Studies, a high unknown patient sample was used and assigned a target value by analyzing the unknown in duplicate. Serial dilutions were performed down to 100 – 150% of the manufacturer's low AMR limit of 0.142 IU/mL. The concentrations of quantitative serum HBeAg were 41.037, 20.519, 8.207, 4.104, 0.410, 0.205, and 0.137 IU/mL.

3.2. Materials

The materials used during the assay validation are given Table 1.

Table 1: Materials

Material	Preparation	Producer	Storage	Stability
Validation Reagent: Roche Elecsys HBeAg Catalog#: 11820583122 Lot: 33448505 Exp: 30-Nov-19	Ready for Use	Available from Roche Diagnostics UK	Refrigerated (2-8°C)	**Unopened:** (Up to expiration date at 2-8°C) **On-system:** (8 weeks at 2-8°C)
Calibrator: Roche Anti-HBeAg CalSet Levels 1 and 2 Catalog#: 11820583122 Lot: 33448505 Exp: 30-Nov-19	Ready for Use	Available from Roche Diagnostics UK	Refrigerated (2-8°C)	**Unopened:** (Up to expiration date at 2-8°C) **Opened:** (8 weeks at 2 - 8°C) **On-system:** (Up to 5 hours at 20 - 25°C)

Production Controls: PreciControl HBeAg (Positive and Negative) Catalog#: 11876376122 Lot: **Negative** 370148 **Positive** 370149 Exp: 31-Jul-20	Ready for Use	Available from Roche Diagnostics UK	Refrigerated (2-8°C)	**Unopened:** (Up to expiration date at 2-8°C) **Opened:** (8 weeks at 2 to 8°C) **On-system:** (Up to 6 hours at 20 to 25°C)
Production Control: BioRad VIROTROL HBeAg Catalog#: 00144 Lot: 11350 Exp: 28-Feb-20	Ready for Use	BioRad Laboratories	Refrigerated (2-8°C)	**Unopened:** (Up to expiration date at 2-8°C) **Opened:** (Up to 60 days at 2 to 8°C)

3.3. Equipment

Roche Cobas e411 Analyzer (S/N. AE1066-19).

3.4. FDA Status

Laboratory Developed Test (LDT).

3.5. Method

The method used in the validation was competitive chemiluminescent immunoassay, and assay manufacturer was Roche Diagnostics.

The validation studies listed below was performed in accordance with the guidelines listed in the Q² Solutions Standard Operating Procedures:

- Q² Solutions "Validation of Analytical Methods for Research Use" - Q² Solutions eSOP Q2CL_OP_240032 Revision 3.
- Q² Solutions "HBeAg Quantitative Assay on Roche Cobas E Analyzers" eSOP# Q2CL_AP_280255 Version 2 – September 2019.

3.6. Reagent Preparation

Materials were prepared as per manufacturer package insert and summarized Table 1.

4. Assay Validation Parameters

The purpose of this assay validation is to describe the process and requirements of an infectious disease assay for serum quantitative HBeAg validation on the Roche Cobas e411 Analyzer (S/N. AE1066-19). The following studies were performed to evaluate precision, accuracy, and analytical measuring range (AMR).

The current Q² Solutions method for serum quantitative HBeAg assay on the Roche Cobas e411 Analyzer utilizes an 10.0% "Maximum Allowable Error". Based on these guidelines, a Maximum Allowable Error of 10.0% is recommended for this assay and will be used as acceptance criteria to accept the studies performed in the validation.

4.1. Precision

One level of Roche Elecsys HBeAg Negative and one level of Roche Elecsys HBeAg Positive controls and one level of BioRad VIROTROL HBeAg control (listed in Table 1) intended for use in production was used for precision and accuracy (Section 4.2) studies.

Precision was considered acceptable if the CV (%) is less than, or equal to the Maximum Allowable Error of 10.0%.

4.1.1 1x20 Intra-assay Precision

One level of Roche Elecsys HBeAg Negative, one level of Roche Elecsys HBeAg Positive, and one level of BioRad VIROTROL HBeAg controls were measured twenty times each for serum quantitative HBeAg in a single run (n = 20) on the Roche Cobas e411 Analyzer (S/N. AE1066-19).

Intra-assay precision was considered acceptable if the intra-assay %CV is within the Maximum Allowable Error of 10.0% stated in the Technical Method for quantitative HBeAg for established methods.

4.1.2 5x5 Inter-assay Precision

One level of Roche Elecsys HBeAg Negative, one level of Roche Elecsys HBeAg Positive, and one level of BioRad VIROTROL HBeAg controls were measured five times each for serum quantitative HBeAg in five separate runs (n = 25) on the Roche Cobas e411 Analyzer (S/N. AE1066-19) over 5 days.

Targets and 2 SD ranges were compared against the manufacturers targets for the Roche Elecsys HBeAg assay for acceptance of each analytical run.

Inter-assay precision was considered acceptable if the inter-assay %CV falls within the Maximum Allowable Error of 10.0% stated in the Technical Method for the quantitative HBeAg assay for established methods.

4.2. Accuracy

4.2.1 Recovery of QC Samples

Achieved control means (combined results from Intra and Inter Precision (n=45)) were compared against the manufacturers targets. Accuracy is demonstrated if recovery is within the Maximum Allowable Error (10.0%) stated in the Technical Methods for the quantitative HBeAg assay for established methods.

4.2.2 Matrix Specific Correlation Studies

Forty serum correlation samples evenly distributed over the AMR were measured in singlet in batches of 10 over 4 independent analytical runs (minimum of 2 days) for serum quantitative HBeAg on the Roche Cobas e411 Analyzer (S/N. AE1066-19). Resulting data for correlation samples (described Section 3.1) from the Q² Solutions Valencia Roche Cobas e411 Analyzer (S/N. AE1066-19) were compared to data from the Q² Solutions Singapore Roche Cobas e411 Analyzer (S/N. 091617). The following statistical analysis and acceptance criteria were used:

1. Least squares linear regression analysis (Microsoft Excel).
2. Coefficient of correlation (r) ≥0.975
3. Average absolute bias.
4. Average percent bias.

The correlation study was considered acceptable if the average %bias calculated from the Linear Regression Formula was less than, or equal to, the Maximum Allowable Error of 10.0%.

4.3. AMR Studies

4.3.1 AMR/Calibration Validation

The Q² Solutions global stated AMR for the serum quantitative HBeAg assay is 0.142-50.00 IU/mL. To verify the claimed AMR on the Roche Cobas e411 Analyzer (S/N. AE1066-19), a high unknown patient sample was used and assigned a target value by analyzing the unknown in duplicate. Serial dilutions were performed down to 100 – 150% of the manufacturer's low AMR limit of 0.142 IU/mL. Seven levels of diluted and undiluted high patient sample were each measured in duplicate and the achieved results compared to the corresponding assigned target values by linear regression. Where possible the lowest non-zero standard had a concentration within 100 – 150% of the lower AMR and the highest calibrator had a concentration within 80 – 120% of the upper AMR.

The mean value, SD, %CV, Absolute Bias and %Bias versus expected were calculated for each standard.

The AMR/Calibration Validation study will be considered acceptable if the achieved values for the standards used fall within 2x Maximum Allowable Error of the target values (Based on the recommended Maximum Allowable Error for the control value closest to the calibration validation material). The achieved value for the verifiers near the low end of the reportable range (LLOQ) can be greater than 2x Maximum Allowable Error from this limit but cannot exceed 40%.

4.3.2 Linearity of Dilution

To verify that accurate results can be obtained on diluted samples for serum quantitative HBeAg on the Roche Cobas e411 Analyzer (S/N. AE1066-19), a manual dilution study was performed. Four serum samples were prepared using previously analyzed patient samples with concentrations values of approximately 40 – 49 IU/mL.

Per the Q² Solutions eSOP (Q2CL_AP_280255), samples with quantitative HBeAg concentrations above the upper AMR limit of 50.00 IU/mL will be run manually diluted (1:80) on the Roche Cobas e411 Analyzer (S/N. AE1066-19). The upper CRR for the Roche Elecsys Quantitative HBeAg assay on the platform is 4000.00 IU/mL.

All four serum samples were manually diluted (1:80) and were tested along with undiluted samples in duplicate. Achieved results on the manually diluted samples were corrected for the dilution and values compared against the undiluted result of each sample to calculate the mean value, Absolute Bias, and %Bias versus expected (undiluted) sample. Results were considered acceptable if the bias observed was less than or equal to the proposed Maximum Allowable Error of 10.0%.

4.4 Reference Range Verification

HBeAg is an infectious disease and therefore the expected result would be "<0.142 IU/mL" for the quantitative HBeAg immunoassay.

4.5 Stability

Per the information provided by Roche Diagnostics, Quantitative HBeAg is stable in stored serum for the following time periods:

7 days at 20 to 25°C
11 days at 2 to 8°C
3 months at or below -15°C

4.6 Carryover

A carryover study was not performed. The Roche Cobas e411 instrument uses disposable pipette tips and sample cups, however the sample is aspirated into a common measuring cell from where potential carryover could occur. The Roche Cobas e411 (S/N. AE1066-19) was previously evaluated for potential carryover from samples containing unusually high levels of Troponin T. No carryover was observed. From this carryover study, it can be assumed that the instrument's ability to prevent any significant carryover applies to other similar assays using the same instrument.

4.7 Exceptional Conditions

There were no exceptional conditions that occurred during the course of the assay validation execution.

5. Documentation and Archiving

Raw data was recorded in daily worksheets. The Senior Medical Director (or designee(s)) will have final review before this validation report is issued.

6. Responsibilities

The assay validation experiments were performed by Allison Gray, Senior Laboratory Associate. Data was reported by David Strickler, Senior Scientist. Review and approval were performed by Charlie Fix, Global Director Scientific Harmonization, Nasrin Ramazani, Laboratory Operations Director, and Sona Manoukian, Automated Chemistry Manager, and Héctor Alberto Repetto, QA Manager. Final review and approval were performed by Marc Edwards MD, MBA, Senior Medical Director.

7. Results

This assay validation document was prepared upon completion of the assay validation study. The raw data are documented in this Assay Validation Report as well as all of the performance characteristics, statistical analyses, summary and conclusions for quantitative HBeAg quantification in serum using the Roche Cobas e411 Analyzer (S/N. AE1066-19).

Results are reported in IU/mL throughout the Assay Validation Report.

7.1 Precision

7.1.1 1x20 Intra-assay Precision

One level of Roche Elecsys HBeAg Negative control, one level of Roche Elecsys HBeAg Positive control, and one level of BioRad VIROTROL HBeAg control material (given in Table 1) were each analyzed twenty times within one run for serum quantitative HBeAg on the Roche Cobas e411 Analyzer (S/N. AE1066-19). Data and achieved %CVs are given in Table 2.

Table 2: *1x20 Intra-assay Precision Data

Intra-assay Precision (Within-Run)			
Replicate	Roche Elecsys HBeAg Negative (IU/mL)	Roche Elecsys HBeAg Positive (IU/mL)	BioRad VIROTROL HBeAg (IU/mL)
1	0.027	2.82	0.240
2	0.027	2.97	0.244
3	0.029	2.86	0.240
4	0.029	2.86	0.226
5	0.026	2.93	0.218
6	0.024	2.81	0.233
7	0.031	2.83	0.238
8	0.028	2.87	0.240
9	0.028	2.85	0.233
10	0.027	2.78	0.226
11	0.028	2.78	0.238
12	0.026	2.85	0.238
13	0.029	2.81	0.238
14	0.027	2.81	0.235
15	0.028	2.81	0.218
16	0.028	2.74	0.235
17	0.029	2.85	0.242
18	0.030	2.79	0.233
19	0.029	2.85	0.242
20	0.028	2.81	0.235
n	20	20	20
Mean	0.028	2.83	0.235
SD	0.002	0.05	0.007
CV%	5.5%	1.8%	3.1%
Acceptance Criteria	10.0%	10.0%	10.0%
Pass/Fail	Pass	Pass	Pass

*Analysis date: 04-Sep-2019

The serum HBeAg results on the Roche Cobas e411 Analyzer (S/N. AE1066-19) were acceptable for the intra-precision study, with observed imprecision (CV%) for the Roche Elecsys HBeAg Negative, Roche Elecsys HBeAg Positive and BioRad VIROTROL HBeAg QC levels within the Maximum Allowable Error acceptance criteria of 10.0%.

7.1.2 5x5 Inter-assay Precision

One level of Roche Elecsys HBeAg Negative control, one level of Roche Elecsys HBeAg Positive control, and one level of BioRad VIROTROL HBeAg control material given in Table 1 were analyzed five times each in five unique runs for serum quantitative HBeAg on the Roche Cobas e411 Analyzer (S/N. AE1066-19). Data and achieved %CVs are given for the Roche Elecsys HBeAg Negative, Roche Elecsys HBeAg Positive, and BioRad VIROTROL HBeAg QC Levels, in Tables 3 through Table 5 respectively.

Table 3: * Roche Elecsys HBeAg Negative Inter-Assay Precision

Negative Inter-assay Precision (Between-Run)						Summary	
Replicate/Run	1	2	3	4	5	Max Allowable Error	10.0%
1	0.031	0.029	0.028	0.029	0.028	Grand Mean	0.030
2	0.030	0.029	0.030	0.032	0.030	Pooled Intra-SD	0.001
3	0.031	0.029	0.032	0.032	0.032	Pooled Intra-CV%	4.7%
4	0.030	0.030	0.032	0.030	0.028	Pass/Fail	Pass
5	0.027	0.029	0.030	0.030	0.029	Overall Mean	0.030
Mean	0.030	0.029	0.030	0.031	0.029	Overall SD	0.001
Intra-SD	0.002	0.000	0.002	0.002	0.002	Overall %CV	4.7%
%CV	5.7%	1.4%	5.7%	5.2%	5.5%	Pass/Fail	Pass

*Analysis dates: Run 1, 16-Jul-2019; Run 2, 17-Jul-2019; Run 3, 18 -Jul-2019; Run 4, 29-Jul-2019; Run 5, 30-Jul-2019

Table 4: * Roche Elecsys HBeAg Positive Inter-Assay Precision

Positive Inter-assay precision (Between-Run)						Summary	
Replicate/Run	1	2	3	4	5	Max Allowable Error	10.0%
1	3.05	2.91	2.92	3.16	2.93	Grand Mean	3.00
2	3.00	2.93	2.95	3.08	3.07	Pooled Intra-SD	0.09
3	2.59	2.99	3.01	2.99	3.03	Pooled Intra-CV%	3.1%
4	3.08	3.01	3.10	3.03	3.18	Pass/Fail	Pass
5	3.00	2.98	2.98	3.02	3.07	Overall Mean	3.00
Mean	2.94	2.97	2.99	3.06	3.06	Overall SD	0.11
Intra-SD	0.20	0.04	0.07	0.07	0.09	Overall %CV	3.7%
%CV	6.9%	1.4%	2.3%	2.1%	3.0%	Pass/Fail	Pass

*Analysis dates: Run 1, 16-Jul-2019; Run 2, 17-Jul-2019; Run 3, 18 -Jul-2019; Run 4, 29-Jul-2019; Run 5, 30-Jul-2019

Table 5: * BioRad VIROTROL HBeAg Inter-Assay Precision

VIROTROL Inter-assay precision (Between-Run)						Summary	
Replicate/Run	1	2	3	4	5	Max Allowable Error	10.0%
1	0.224	0.222	0.222	0.226	0.229	Grand Mean	0.228
2	0.224	0.224	0.222	0.233	0.224	Pooled Intra-SD	0.005
3	0.226	0.229	0.231	0.233	0.222	Pooled Intra-CV%	2.0%
4	0.229	0.224	0.233	0.229	0.226	Pass/Fail	Pass
5	0.229	0.224	0.222	0.244	0.238	Overall Mean	0.228
Mean	0.226	0.225	0.226	0.233	0.228	Overall SD	0.005
Intra-SD	0.002	0.002	0.006	0.007	0.006	Overall %CV	2.4%
%CV	1.0%	1.1%	2.4%	2.9%	2.6%	Pass/Fail	Pass

*Analysis dates: Run 1, 29-Aug-2019; Run 2, 30-Aug-2019; Run 3, 02 -Sep-2019; Run 4, 03-Sep-2019; Run 5, 04-Sep-2019

The serum HBeAg results on the Roche Cobas e411 Analyzer (S/N. AE1066-19) were acceptable for the inter-precision study, with observed imprecision (CV%) for the Roche Elecsys HBeAg Negative, Roche Elecsys HBeAg Positive and BioRad VIROTROL HBeAg QC levels within the Maximum Allowable Error acceptance criteria of 10.0%.

The serum HBeAg results on the Roche Cobas e411 Analyzer (S/N. AE1066-19) for the Roche Elecsys HBeAg Negative, Roche Elecsys HBeAg Positive, and BioRad VIROTROL HBeAg Controls were acceptable for the precision study, with intra- and inter-imprecision within the Maximum Allowable Error acceptance criteria of 10.0%. The results of the precision studies were acceptable.

7.2 Accuracy

7.2.1 Recovery of QC Samples

Combined means for the Roche Elecsys HBeAg Negative, Roche Elecsys HBeAg Positive, and BioRad VIROTROL HBeAg Control material listed in Table 1, obtained from the intra- and inter-precision studies (n=45) were compared to the manufacturer's or Q² Solutions acceptability targets. For the BioRad VIROTROL HBeAg control the target mean was established by including all 25 data point values obtained from the inter-assay precision study and calculating the mean and 2 standard deviation control range (HBeAg Quantitative Assay on Roche Cobas E Analyzers) eSOP #Q2CL_AP_280255 Revision 2)). The achieved mean and bias calculated on the Roche Cobas e411 Analyzer (S/N. AE1066-19) for each QC level are summarized Table 6.

Table 6: *QC Recovery Data

Overall-assay Precision & Accuracy			
Levels	Roche Elecsys Negative (IU/mL)	Roche Elecsys Positive (IU/mL)	BioRad VIROTROL HBeAg (IU/mL)
n	45	45	45
Manufacturer's or Q² Solutions Target Mean	0.031	3.13	0.228
Q² Solutions Valencia Overall Mean	0.029	2.93	0.231
%Bias	**-6.5%**	**-6.4%**	**1.3%**
Acceptance criteria	**10.0%**	**10.0%**	**10.0%**
Pass/Fail	**Pass**	**Pass**	**Pass**

*Analysis dates: **Roche** 06-Jul-2019 – 30-Jul-2019 **BioRad** 29-Aug-2019 – 03-Sep-2019

For the evaluation of accuracy using the Roche Elecsys HBeAg Negative, Roche Elecsys HBeAg Positive, and BioRad VIROTROL HBeAg Control material, mean values achieved in Q² Solutions Valencia for all three levels of QC materials for the serum quantitative HBeAg assay on the Roche Cobas e411 Analyzer (S/N. AE1066-19) % bias vs. established manufacturer's targets were all within the Maximum Allowable Error acceptance criteria of 10.0%. Acceptable recovery of the Roche Elecsys HBeAg Negative, Roche Elecsys HBeAg Positive, and BioRad VIROTROL HBeAg QC material was demonstrated.

7.2.2 Matrix Specific Correlation Studies

Forty serum samples were assayed for quantitative HBeAg in singlicate on both the Q² Solutions Valencia Roche Cobas e411 Analyzer (S/N. AE1066-19) and on the Q² Solutions Singapore Roche Cobas e411 Analyzer (S/N. 091617). Results obtained from this correlation study (n = 40) are summarized in Tables 7 through 8. Because of the large amount of data above the AMR, the data was re-examined using the patient samples with results above the AMR (>50.00 IU/mL).

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Table 7: All Correlation Data

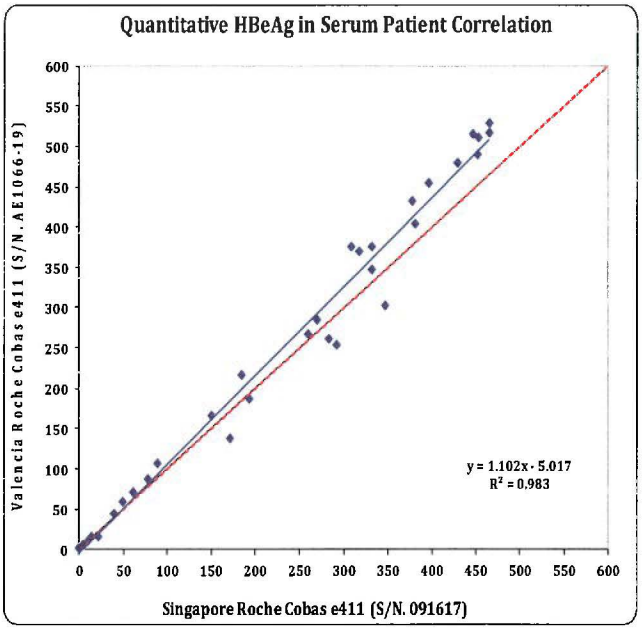
ALL DATA				
Specimen	X Singapore Roche Cobas e411 (S/N. 091617) (IU/mL)	Y Valencia Roche Cobas e411 (S/N. AE1066-19) (IU/mL)	Y-X Bias	%(Y-X)/X %Bias
C1	61.61	69.80	8.19	13.3%
C2	1.53	1.62	0.10	6.4%
C3	465.31	516.82	51.50	11.1%
C4	1.23	1.60	0.37	29.7%
C5	307.47	374.96	67.49	21.9%
C6	88.98	105.41	16.43	18.5%
C7	317.02	369.19	52.17	16.5%
C8	4.34	5.20	0.86	19.8%
C9	428.90	479.52	50.62	11.8%
C11	380.95	402.71	21.76	5.7%
C12	9.74	8.76	-0.98	-10.0%
C15	446.89	514.37	67.49	15.1%
C16	14.15	15.91	1.76	12.4%
C18	465.09	527.69	62.60	13.5%
C19	331.22	375.18	43.96	13.3%
C20	346.76	302.36	-44.40	-12.8%
C21	151.00	164.95	13.94	9.2%
C22	39.32	43.67	4.35	11.1%
C23	451.77	489.07	37.30	8.3%
C24	331.45	347.43	15.98	4.8%
C25	3.37	3.78	0.42	12.4%
C26	48.80	57.90	9.10	18.7%
C27	0.88	0.98	0.10	11.3%
C28	21.87	15.92	-5.94	-27.2%
C29	171.58	137.55	-34.03	-19.8%
C30	77.61	86.54	8.92	11.5%
C31	0.50	0.56	0.05	10.1%
C32	291.04	252.68	-38.36	-13.2%
C33	396.49	453.04	56.55	14.3%
C34	184.33	216.67	32.35	17.5%
C35	282.38	260.15	-22.23	-7.9%
C36	259.07	266.04	6.97	2.7%
C37	269.73	284.86	15.13	5.6%
C38	193.85	185.77	-8.08	-4.2%
C39	453.55	510.92	57.37	12.7%
C40	377.84	430.81	52.97	14.0%
n	36	36	Avg. %Bias	
Mean	213.27	230.01	7.9%	
SD	170.67	189.66	PASS	
Slope	1.102			
Intercept	-5.017			
r	0.992			

Total Allowable Error	15.0%
AMR	0.142-50.00 IU/mL

Sample ID	Dates of Analysis	
	Singapore	Valencia
C1 - C10	28-Aug-19	9-Aug-19
C11 - C20	28-Aug-19	12-Aug-19
C21 - C30	29-Aug-19	13-Aug-19
C31 - C40	30-Aug-19	14-Aug-19

Samples below the Lower AMR <0.142 IU/mL

C10	0.04	0.03	-0.01	-14.40%
C13	0.02	0.03	0.01	67.40%
C14	0.02	0.04	0.02	98.00%
C17	0.02	0.03	0.01	26.20%



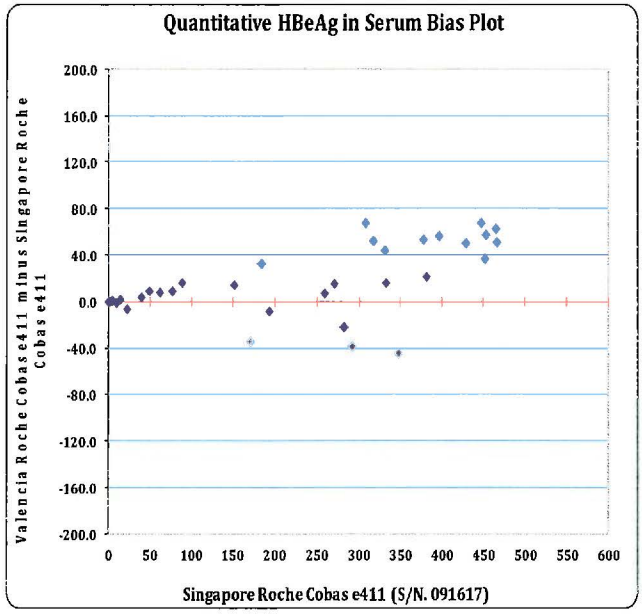


Table 8: Data Within The AMR

Within The AMR				
	X	Y	Y-X	%(Y-X)/X
Specimen	Singapore Roche Cobas e411 (S/N. 091617) (IU/mL)	Valencia Roche Cobas e411 (S/N. AE1066-19) (IU/mL)	Bias	%Bias
C2	1.53	1.62	0.10	6.4%
C4	1.23	1.60	0.37	29.7%
C8	4.34	5.20	0.86	19.8%
C12	9.74	8.76	-0.98	-10.0%
C16	14.15	15.91	1.76	12.4%
C22	39.32	43.67	4.35	11.1%
C25	3.37	3.78	0.42	12.4%
C26	48.80	47.90	-0.90	-1.8%
C27	0.88	0.98	0.10	11.3%
C28	21.87	25.92	4.05	18.5%
C31	0.50	0.56	0.05	10.1%
n	11	11	Avg. %Bias	
Mean	13.25	14.17	7.0%	
SD	16.75	17.44	PASS	
Slope	1.036			
Intercept	0.443			
r	0.995			

Total Allowable Error	15.0%
AMR	0.142-50.00 IU/mL

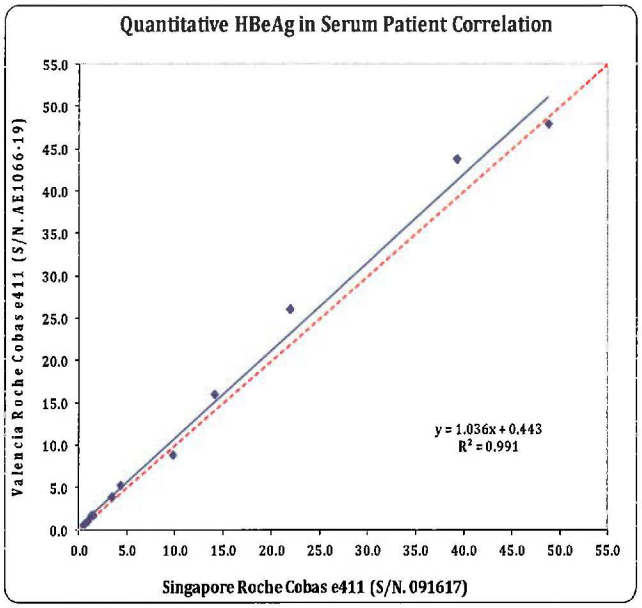
Sample ID	Dates of Analysis	
	Singapore	Valencia
C1 - C10	28-Aug-19	9-Aug-19
C11 - C20	28-Aug-19	12-Aug-19
C21 - C30	29-Aug-19	13-Aug-19
C31 - C40	30-Aug-19	14-Aug-19

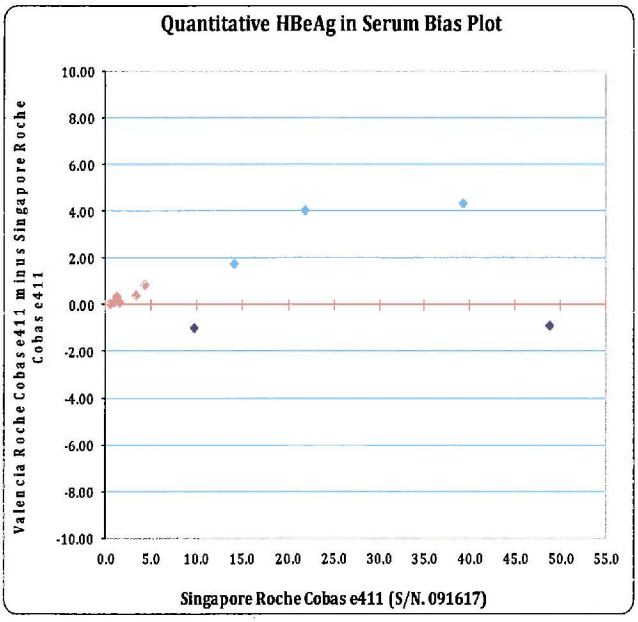
Sample above the Upper AMR >50.00 IU/mL

C1	61.61	69.80	8.19	13.30%
C3	465.31	516.82	51.5	11.10%
C5	307.47	374.96	67.49	21.90%
C6	88.98	105.41	16.43	18.50%
C7	317.02	369.19	52.17	16.50%
C9	428.90	479.52	50.62	11.80%
C11	380.95	402.71	21.76	5.70%
C15	446.89	514.37	67.49	15.10%
C18	465.09	527.69	62.6	13.50%
C19	331.22	375.18	43.96	13.30%
C20	346.76	302.36	-44.4	-12.80%
C21	151.00	164.95	13.94	9.20%
C23	451.77	489.07	37.3	8.30%
C24	331.45	347.43	15.98	4.80%
C29	171.58	137.55	-34.03	-19.80%
C30	77.61	86.54	8.92	11.50%
C32	291.04	652.68	361.64	124.30%
C33	396.49	653.04	256.54	64.70%
C34	184.33	216.67	32.35	17.50%
C35	282.38	560.15	277.77	98.40%
C36	259.07	266.04	6.97	2.70%
C37	269.73	384.86	115.13	42.70%
C38	193.85	185.77	-8.08	-4.20%
C39	453.55	810.92	357.38	78.80%
C40	377.84	830.81	452.97	119.90%

Samples below the Lower AMR <0.142 IU/mL

C10	0.04	0.03	-0.01	-14.40%
C13	0.02	0.03	0.01	67.40%
C14	0.02	0.04	0.02	98.00%
C17	0.02	0.03	0.01	26.20%





Results for the serum quantitative HBeAg assay from the Q² Solutions Valencia Roche Cobas e411 Analyzer (S/N. AE1066-19) vs. Q² Solutions Singapore Roche Cobas e411 Analyzer (S/N. 091617) (All Data) at Q² Solutions Valencia demonstrated acceptable agreement between the two instrument platforms. An R^2 value of 0.983 and an average bias of +7.9% was achieved which met the acceptance criteria of 10.0%.

Results for the serum quantitative HBeAg assay from the Q² Solutions Valencia Roche Cobas e411 Analyzer (S/N. AE1066-19) vs. Q² Solutions Singapore Roche Cobas e411 Analyzer (S/N. 091617) (Data Within The AMR) at Q² Solutions Valencia demonstrated acceptable agreement between the two instrument platforms. An R^2 value of 0.991 and an average bias of +7.0% was achieved which met the acceptance criteria of 10.0%.

The Q² Solutions Valencia laboratory Roche Cobas e411 Analyzer (S/N. AE1066-19) has demonstrated acceptable agreement of serum quantitative HBeAg results when compared against the Q² Solutions Singapore Roche Cobas e411 Analyzer (S/N. 091617). The acceptance criteria for Accuracy were met.

7.3 AMR Studies

7.3.1 AMR/Calibration Validation

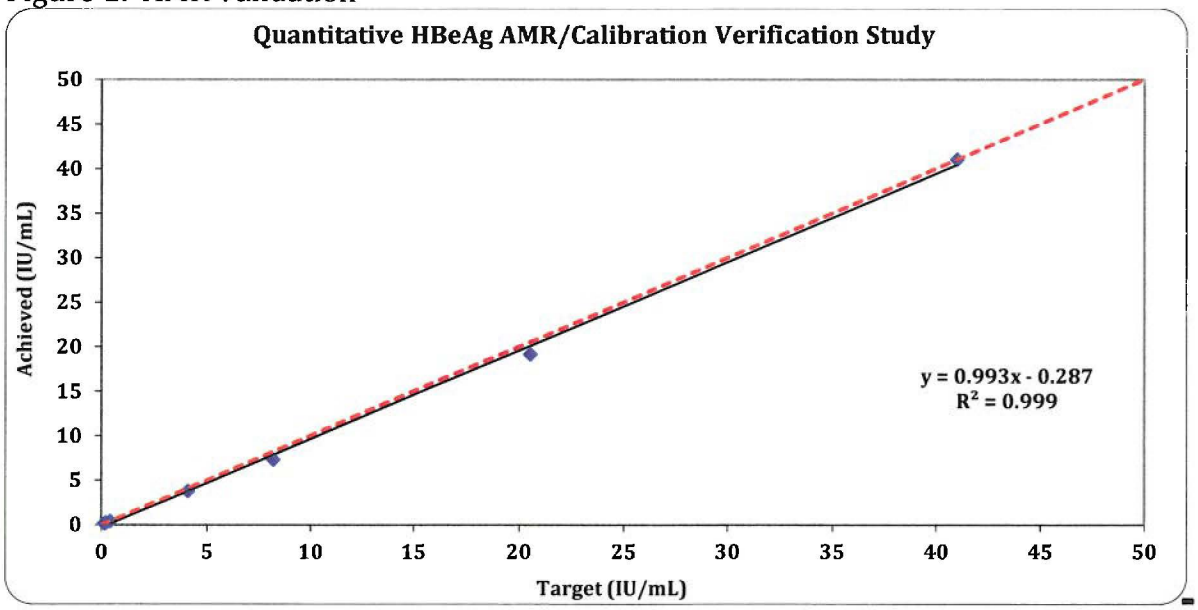
A high unknown patient sample was used and assigned a target value by analyzing the unknown in duplicate. Serial dilutions were performed down to 100 – 150% of the manufacturer's low AMR limit of 0.142 IU/mL. Seven levels of diluted and undiluted high patient sample given in Table 1 were each measured in duplicate within one run (for each, n = 2) on the Roche Cobas e411 Analyzer (S/N. AE1066-19) at Q² Solutions Valencia. Data and achieved Mean, %CV, absolute, and % biases are summarized in Table 9 and Figure 1.

Table 9: *Analytical Measurement Range (AMR)

Level	Target	Rep 1	Rep 2	Mean	CV%	Absolute Bias	% Bias	Acceptance (± 2x Max Error %)	Pass/Fail
Neat	41.037	41.514	40.559	41.037	1.6%	0.000	0.0%	20.0%	Pass
1:2	20.519	19.219	19.125	19.172	1.6%	-1.347	-6.6%	20.0%	Pass
1:5	8.207	7.419	7.206	7.313	1.6%	-0.895	-10.9%	20.0%	Pass
1:10	4.104	3.836	3.770	3.803	1.2%	-0.301	-7.3%	20.0%	Pass
1:100	0.410	0.404	0.420	0.412	2.7%	0.002	0.5%	20.0%	Pass
1:200	0.205	0.218	0.218	0.218	0.0%	0.013	6.3%	20.0%	Pass
1:300	0.137	0.151	0.149	0.150	0.9%	0.013	9.5%	20.0%	Pass

*Analysis date: 22-Aug-2019

Figure 1: AMR Validation



The serum quantitative HBeAg assay performed on the Roche Cobas e411 Analyzer (S/N. AE1066-19) produced acceptably accurate and linear results throughout the Q² Solutions AMR (0.142 – 50.00 IU/mL) with all results meeting the acceptance criteria when compared to the expected target. Linear regression analysis showed acceptable agreement for Roche Cobas e411 Analyzer ($y = 0.993x - 0.287$, $R^2 = 0.999$). The achieved bias at all levels was within acceptable limits.

The Q² Solutions Valencia laboratory was able to demonstrate acceptable linearity throughout the Q² Solutions AMR of 0.142 – 50.00 IU/mL for the serum quantitative HBeAg assay on the Roche Cobas e411 Analyzer (S/N. AE1066-19).

7.3.2 Linearity of Dilution

To verify that accurate results can be obtained on diluted samples, a manual dilution study was performed using four serum samples with endogenous serum quantitative HBeAg levels with concentrations values of approximately 40 – 49 IU/mL. The four samples were diluted 1:80 and tested with undiluted (neat) sample in duplicate.

The achieved results were calculated by multiplying by the 1:80 dilution factor and compared against the undiluted result for the respective sample to calculate the % Bias as shown in Table 10. Results were considered acceptable if the observed %Bias is within the acceptance criteria of 20.0%.

Table 10: *Linearity of Dilution

Sample ID	Dilution	Rep 1	Rep 2	Mean	% Bias	PASS/FAIL
S1	1 (Neat)	44.6	44.1	44.4	N/A	N/A
	80 (Manual)	49.6	51.2	50.4	**13.6%**	**PASS**
S2	1 (Neat)	48.8	44.7	46.8	N/A	N/A
	80 (Manual)	50.4	54.4	52.4	**12.1%**	**PASS**
S3	1 (Neat)	40.8	42.8	41.8	N/A	N/A
	80 (Manual)	39.2	39.2	39.2	**-6.2%**	**PASS**
S4	1 (Neat)	46.5	46.0	46.3	N/A	N/A
	80 (Manual)	42.4	44.0	43.2	**-6.6%**	**PASS**

*Analysis date: 22-Aug-2019

Linearity of dilution was demonstrated for achieved %Biases within the Acceptance Criteria (±20.0%) for maximum dilutions up to 1:80 for the quantitative HBeAg assay.

Samples testing above the upper AMR of 50.00 IU/mL for serum quantitative HBeAg may be diluted up to a maximum dilution of 1:80 and results as high as 4000.00 IU/mL reported after multiplying by the dilution factor.

AMR = 0.142 – 50.00 IU/mL

CRR = 0.142 – 4000.00 IU/mL

7.4 Summary

Table 11: Summary

Parameter	Acceptance Criteria	Observed Result
1x20 Intra-assay Precision	**Roche Elecsys HBeAg Negative, Roche Elecsys HBeAg Positive and BioRad VIROTROL HBeAg** Intra-assay within the Maximum Allowable Error of 10.0% stated in the Technical Method for the serum quantitative HBeAg assay.	**Roche Cobas e411 Analyzer (S/N. AE1066-19)** %CV Roche Elecsys HBeAg Negative Control: **5.5%** %CV Roche Elecsys HBeAg Positive Control: **1.8%** %CV BioRad VIROTROL HBeAg Control: **3.1%**
5x5 Inter-assay Precision	**Roche Elecsys HBeAg Negative, Roche Elecsys HBeAg Positive and BioRad VIROTROL HBeAg** Inter-assay within the Maximum Allowable Error of 10.0% stated in the Technical Method for the serum quantitative HBeAg assay.	**Roche Cobas e411 Analyzer (S/N. AE1066-19)** %CV Roche Elecsys HBeAg Negative Control: **4.7%** %CV Roche Elecsys HBeAg Positive Control: **3.7%** %CV BioRad VIROTROL HBeAg Control: **2.4%**

Recovery of QC Samples	**Roche Elecsys HBeAg Negative, Roche Elecsys HBeAg Positive and BioRad VIROTROL HBeAg** %Bias within the Maximum Allowable Error of 10.0% stated in the Technical Method for the serum quantitative HBeAg assay.	**Roche Cobas e411 Analyzer (S/N. AE1066-19)** %CV Roche Elecsys HBeAg Negative Control: **-6.5%** %CV Roche Elecsys HBeAg Positive Control: **-6.4%** %CV BioRad VIROTROL HBeAg Control: **+1.3%**
Correlation Study	Slope = 1.00 +/- 0.15 r ≥0.975 Average %Bias ≤10.0%	**Valencia Roche Cobas e411 Analyzer (S/N. AE1066-19) vs. Singapore Roche Cobas e411 Analyzer (S/N. 091617) (All Data)** Slope: 1.102 r: 0.992 Average Bias: +7.9% **Valencia Roche Cobas e411 Analyzer (S/N. AE1066-19) vs. Singapore Roche Cobas e411 Analyzer (S/N. 091617) (Within the AMR)** Slope: 1.036 r: 0.995 Average Bias: +7.0%
AMR/ Calibration Validation	Each calibrator %Bias should be ±2x Maximum Allowable Error of 10.0% of the target except at the LLOQ where ≤40% is acceptable stated in the Technical Method for the serum quantitative HBeAg assay.	**Roche Cobas e411 Analyzer (S/N. AE1066-19)** %Bias Neat: 0.0% at 41.037 IU/mL %Bias 1:2: -6.6% at 20.519 IU/mL %Bias 1:5: -10.9% at 8.207 IU/mL %Bias 1:10: -7.3% at 4.104 IU/mL %Bias 1:100: +0.5% at 0.410 IU/mL %Bias 1:200: +6.3% at 0.205 IU/mL %Bias 1:300: +9.5% at 0.137 IU/mL **AMR = 0.142-50.00IU/mL**
Linearity of Dilution	Absolute %Bias ≤20%	**Roche Cobas e411 Analyzer (S/N. AE1066-19) Manual Dilution** Dilution 1 %Bias: +13.6% Dilution 2 %Bias: +12.1% Dilution 3 %Bias: -6.2% Dilution 4 %Bias: -6.6% Maximum Dilution Verified = 1:80 **CRR = 0.142-4000.00 IU/mL**

The assay validation for serum quantitative HBeAg demonstrated acceptable performance for precision, accuracy, and analytical measurement range studies on the Roche Cobas e411 Analyzer (S/N. AE1066-19) at Q² Solutions Valencia in accordance with the Assay Validation Plan.

7.5 Deviations/Exceptional Conditions

No deviations/Exceptional conditions from the validation plan were encountered during the course of the validation execution.

7.6 Recommendations

It is recommended that the Roche Cobas e411 Analyzer (S/N. AE1066-19) be used when analyzing levels of quantitative HBeAg in serum at Q² Solutions Valencia.

The results obtained during this validation study demonstrate that there are no clinically or statistically significant differences in serum quantitative HBeAg results produced on Roche Cobas e411 Analyzer (S/N. AE1066-19) at Q² Solutions Valencia when compared to the Q² Solutions Singapore Roche Cobas e411 Analyzer (S/N. 091617) at Q² Solutions Valencia.

The method can be used for pharmaceutical studies requiring the testing of patient samples for quantitative HBeAg in serum at Q² Solutions Valencia.

8. Definitions and Abbreviations

8.1 Definitions

Accuracy Closeness of the mean test results obtained by the method to the known true value of the analyte expressed as percent accuracy.

Accuracy (%): measured value/nominal value X 100

Analytical Measuring Range (AMR): The range of results produced by a test system that a method can directly measure on the specimen without any dilution, concentration or any other pretreatment not part of the usual assay process, producing results that lie within the lowest limit of quantitation (LLOQ) and the upper linearity limits. Also known as the Direct Reportable Range or Linear Range or Dynamic Range.

Analytical Method: The methodology or assay being verified to demonstrate it is suitable for its intended purpose.

Bias: Deviation of a value from the nominal value

Calibration Standard: A sample for which the analyte concentration is known.

Calibration Validation: A study evaluating at least three levels of known (assayed or diluted) standards across the AMR for that analyte to verify the accuracy of a test.

Critical Reagents, Materials, or Instrumentation: Specific components necessary to execute the method that, if substituted with similar components, may significantly alter the relevant performance characteristics of the method. Equivalency of substituted components must be demonstrated as acceptable through a documented qualification study or through re-validation of the method using "Validation of Analytical Methods" Q2CL_OP_240032.

Exceptional Condition: Any impact/event that occurs during the course of the validation that impacts the validation outcome. This may be, but is not limited to, impact deviations from the approved method, validation acceptance criteria failures, multiple invalid results indicating a systematic problem.

Maximum Allowable %CV: the maximum %CV that is to be used for QC acceptance criteria when both verifying new platforms for existing methods and new lots of QC material. Also used as "Maximum Allowable Error" for acceptance of bias in various accuracy studies.

Method Validation: The process to confirm an Analytical Method performs as expected in the laboratory environment and is comparable to manufacturer claim(s). The primary location/originating laboratory must perform accuracy and precision over a period of time. Secondary locations must perform a split sample correlation with the primary location using "Technology Transfer of Analytical Methods" Q2CL_OP_240031.

Precision: the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous samples under the same prescribed conditions. Note: precision is not typically represented by a numerical value but is expressed quantitatively in terms of imprecision: the SD or %CV of the results in a set of replicate measurements. Also referred to as imprecision where the higher the imprecision, the higher the SD or %CV.

Quality Control (QC): Quality Control sample is a neat or spiked sample in biological matrix which is used to monitor the performance of a bioanalytical method. The QC is adopted to assess the integrity and validity of the results of the samples analyzed in any batch.

Standard Deviation: a statistic used to describe the distribution or spread in the data in a population (that is shown to have the shape of a normal Gaussian curve). SD squared gives a term called variance.

Maximum Allowable Error: the amount of error that can be tolerated without compromising the clinical utility of the analytical result, or the maximum amount of error allowed for successful performance in proficiency testing.

8.2 Abbreviations

AMR	Analytical Measurement Range
CV	Coefficient of Variation
FDA	Food and Drug Administration
QC	Quality Control
SD	Standard Deviation
SOP	Standard Operating Procedure

9 References:

Product insert and relevant documentation regarding this assay validation summary.

9.1 Q² Solutions "Validation of Analytical Methods for Research Use" - Q² Solutions eSOP Q2CL_OP_240032 Revision 3.

9.2 Roche Diagnostics HBeAg Reagent (HBeAg) Kit Insert 2019-02 V1.0.

9.3 Q² Solutions "HBeAg Quantitative Assay on Roche Cobas E Analyzers" eSOP# Q2CL_AP_280255 Version 2 – September 2019.

0675 HBV surface antigen large and middle isoform composition are proportional to total HBsAg

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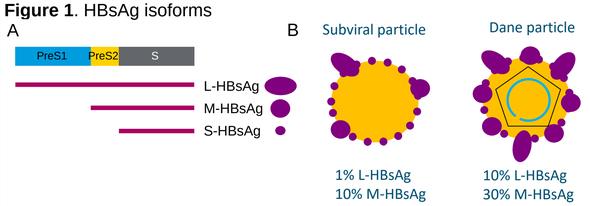
Background

Diagnostic markers are needed to stratify HBV patients to determine an appropriate treatment plan. The relative abundances of isoforms of HBV surface antigen (HBsAg) have been proposed as novel biomarkers for staging infections and monitoring patients on therapy (Pfefferkorn et al, 2018). To evaluate the utility of HBsAg isoforms as HBV biomarkers, we developed prototype assays that specifically detect L-HBsAg (large) and M-HBsAg (middle) products of the S gene and determined the isoform composition of clinical specimens.

Figure 1. HBsAg isoforms

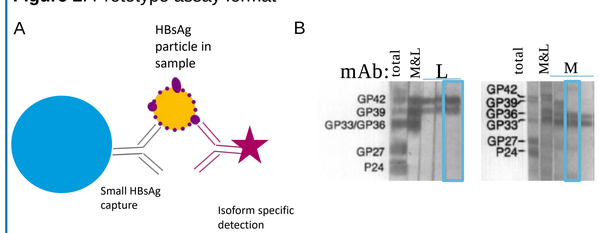


The PreS1, PreS2, and S open reading frames encode for HBV L-HBsAg, M-HBsAg, and S-HBsAg as depicted in panel A. The isoform compositions of non-infectious subviral particles and infectious Dane particles is shown in panel B. These ratios were established by a silver stained gel of particles purified from a single donor in 1984 (Heerman et al, 1984).

Methods

Prototype HBsAg isoform assays were developed on a fully automated chemiluminescent immunoassay instrument. Antibodies directed against the "a" determinant loop shared by all isoforms were coated on the solid phase to capture HBsAg-containing particles. Total (S-HBsAg), L-HBsAg, and M-HBsAg were detected with acridinium conjugated isoform-specific antibodies. Antibody specificity was confirmed by Western blot and assay specificity was confirmed using recombinant S-HBsAg and purified Dane particles. Three seroconversion panels, serial samples from HBeAg positive patients in the US on nucleoside analog (NA) therapy, and 284 HBsAg positive plasma donations from Cameroon, Spain, and the USA were evaluated with the prototype assays to determine isoform composition.

Figure 2. Prototype assay format



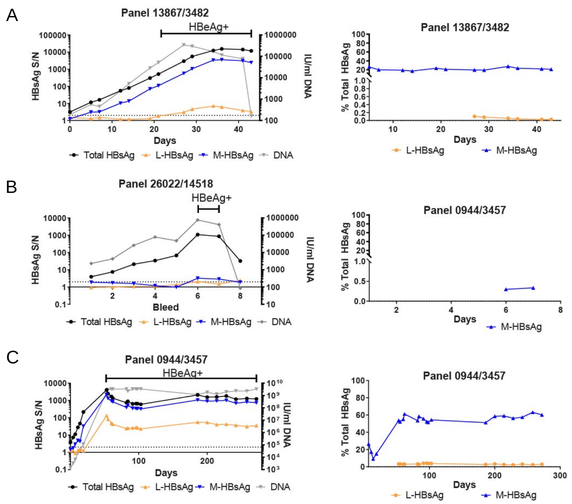
The format is shown in panel A and the isoform specificity of the detection antibodies was previously confirmed by immunoblots (Mimms et al, 1990) and is reproduced in panel B. Clones used in this study are in blue boxes.

Results

Table 1. Specificity controls

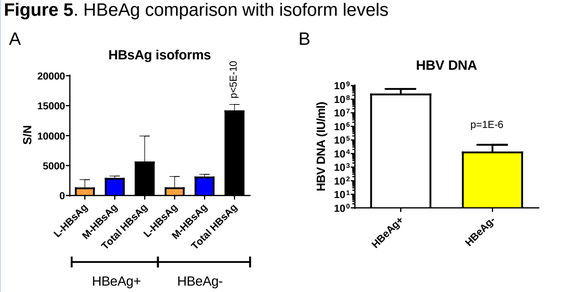
	L-HBsAg			M-HBsAg			Total HBsAg		
	RLU	S/N	% total	RLU	S/N	% total	RLU	S/N	IU/ml
Recombinant S-HBsAg	53	0.96	NA	50	0.96	NA	366859	6551	53
Purified Dane virions	733	13.33	0.15	183	3.52	0.04	488113	8716	81
Normal human plasma	55	1	NA	52	1	NA	56	1	0

Figure 3. Seroconversion panels



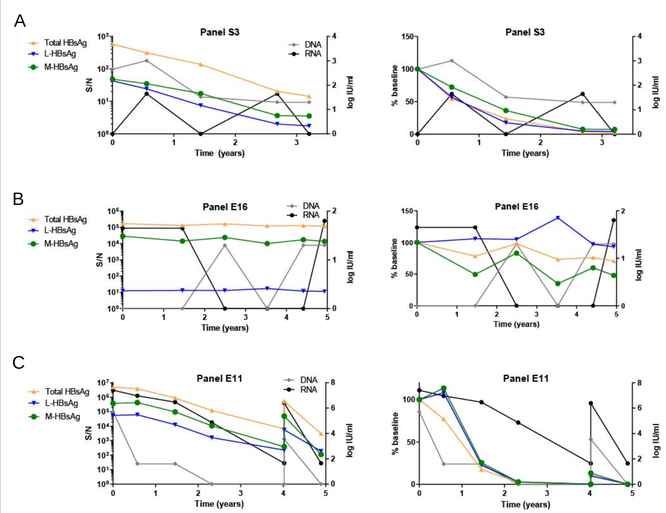
HBsAg positive specimens from acute/resolving (a and b) and chronic (c) seroconversion panels are shown. Dashed lines indicate 2.0 S/N, the assay cutoff for L-HBsAg, M-HBsAg, and Total HBsAg. Data are presented as signal to noise (S/N, left) or percentage of total HBsAg S/N (right) for timepoints with positive isoform S/N.

Figure 5. HBeAg comparison with isoform levels



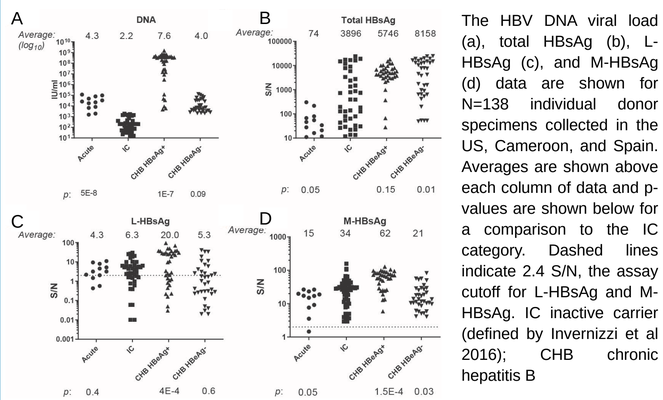
Single timepoint specimens were categorized as HBeAg positive or negative and tested for HBsAg isoform levels (a) and DNA viral load (b).

Figure 4. Isoform composition of serial bleeds from patients on NA therapy.



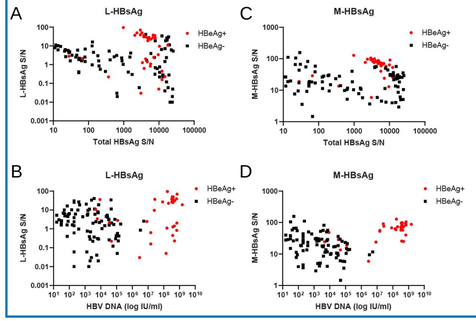
The S/N (left) and percent baseline S/N (right) for total HBsAg, L-HBsAg, and M-HBsAg are plotted on the left y-axis and nucleic acid loads (RNA and DNA) are plotted on the right y-axis for three patients on NA therapy (a-c). Patient S3 became HBsAg negative after the last timepoint plotted and was HBeAg positive for all plotted timepoints. Patient E16 was HBeAg negative for the entire timecourse and E11 became HBeAg negative on bleed 5.

Figure 6. Single timepoint specimens



The HBV DNA viral load (a), total HBsAg (b), L-HBsAg (c), and M-HBsAg (d) data are shown for N=138 individual donor specimens collected in the US, Cameroon, and Spain. Averages are shown above each column of data and p-values are shown below for a comparison to the IC category. Dashed lines indicate 2.4 S/N, the assay cutoff for L-HBsAg and M-HBsAg. IC inactive carrier (defined by Invernizzi et al 2016); CHB chronic hepatitis B

Figure 7. HBsAg isoform ratios



The L-HBsAg (A) and M-HBsAg (C) S/N for single timepoint specimens are compared to total HBsAg levels and DNA levels (B and C). HBeAg positive specimens are shown as red circles and HBeAg negative specimens are shown as black squares.

Conclusions

- Automated HBsAg isoform specific research assays were developed.
- The most reliable unit of measure was signal to noise (S/N). An isoform specific standard will be required for quantitative detection and inter-assay comparisons.
- The levels of L-HBsAg and M-HBsAg were proportional to total HBsAg in longitudinal samples from seroconversion panels and patients on NA therapy, despite fluctuations in other markers.
- Overall, the levels of isoforms followed the expected pattern: total > M > L
- A wide variability in isoform composition between different patients was observed in all sample types tested.
- L-HBsAg and M-HBsAg do not appear to provide an additional diagnostic benefit beyond the total HBsAg test for disease staging or monitoring response to treatment in routine clinical settings.

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Financial Disclosures

This study was funded in part by Abbott Laboratories. MAR, AV, JG, MCK, TPL, GD, and GAC are employees and shareholders of Abbott Laboratories.

P13

Increasing the performance of a fully automated quantitative assay for the detection of circulating HBV pregenomic RNA

Mark Anderson,[1] Michael Stec,[1] Danijela Lucic,[2] Ka-Cheung Luk,[1] Anita McClernon,[3] Daniel McClernon,[3] Gavin Cloherty[1]

1. Background

Hepatitis B virus pregenomic RNA (HBV pgRNA) has been proposed as a potential circulating biomarker for the activity of covalently closed circular DNA (cccDNA) that is present in infected hepatocytes of HBV patients. HBV RNA is of particular interest in patients who are on nucleos(t)ide analog therapy as HBV DNA is generally low or undetectable in these patients [1]. There are an increasing number of studies [2-4] showing the utility of HBV RNA quantitation in monitoring the effectiveness of both experimental and standard of care therapies and it is being investigated as an endpoint for clinical trial effectiveness and therapy removal. We have previously reported on the development of a fully automated dual-target, quantitative assay for the measurement of HBV pgRNA (v1.0) with a lower limit of quantitation (LLOQ) of 1.65 log U/mL (~152 copies/mL) [5]. Here we report on a modified assay (v2.0) with increased overall precision, sensitivity (15-fold), and a limit of detection of ~10 HBV RNA copies/mL. Quantitated HBV RNA levels with the v2.0 assay were indistinguishable from v1.0.

2. Methods

- A Research Use Only (RUO) fully automated real-time PCR assay for the detection and quantitation of HBV RNA (v1.0) was developed for the Abbott m2000 (Abbott Molecular Diagnostics, Des Plaines, IL, USA) platform and previously described [5]. Briefly, targets in conserved regions of the HBV x and core genes are used to ensure robust detection in the presence of mutations, and the assay is standardized against a WHO secondary DNA standard. An internal control is included to detect PCR interference. Assay LLOQ was measured by Probit analysis to be 1.65 log U/mL (~152 copies/mL) using a 0.2 mL sample volume input and 95% detection threshold.
- Modifications were made to the reagent formulation, cycling parameters, and sample input volumes (0.6 mL) which improve analytical performance. Performance (linearity, sensitivity, standard deviation, and concordance) of the new assay (v2.0) was compared to v1.0. A patient sample with high levels of HBV RNA was selected from which a panel of 11 serial dilutions into negative human plasma was made. Target HBV RNA concentrations ranged from 1.00E6 log U/mL (~3.41E6 copies/mL) down to 3.13 U/mL (~10-11 copies/mL) and either 3 or 20 replicates at each dilution were tested with both assays. Longitudinal samples from 3 on-therapy patients were also tested.

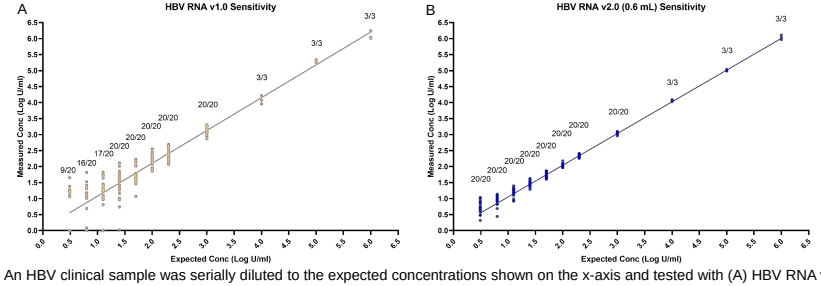
Table 1: Comparison of precision and reported results with v1.0 and v2.0

Expected Concentration (log U/mL)	v1.0 Stdev (log U/mL)	v2.0 Stdev (log U/mL)	Δv1.0-v2.0 Reported Result (log U/mL)
6.00	0.13	0.07	0.05
5.00	0.04	0.03	0.25
4.00	0.13	0.04	-0.05
3.00	0.13	0.03	0.01
2.30	0.20	0.04	-0.05
2.00	0.21	0.06	0.00
1.70	0.28	0.09	-0.14

Standard deviation is lower with v2.0 and reported results are within v1.0 standard deviation

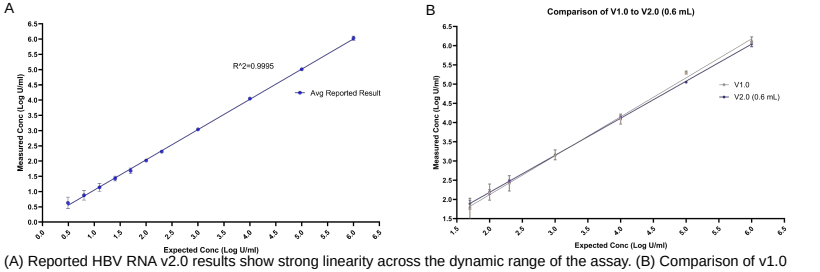
3. Results

Figure 1. HBV RNA serial dilution panel shows increased sensitivity of v2.0 compared to v1.0



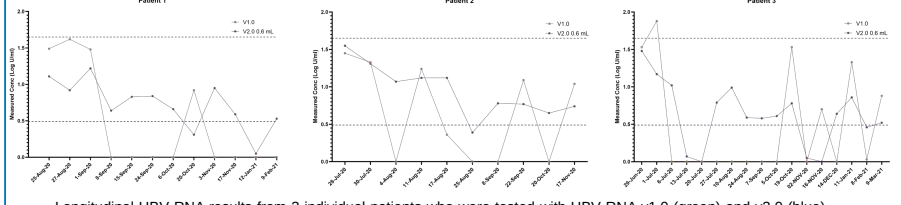
An HBV clinical sample was serially diluted to the expected concentrations shown on the x-axis and tested with (A) HBV RNA v1.0 and (B) HBV RNA v2.0 with the measured concentrations reported on the y-axis. Numbers above each concentration indicate the number of positive detections at each tested concentration.

Figure 2. HBV RNA v2.0 Linearity and comparison of reported results from v1.0 and v2.0



(A) Reported HBV RNA v2.0 results show strong linearity across the dynamic range of the assay. (B) Comparison of v1.0 and v2.0 quantitated HBV RNA results showing reported results are indistinguishable between the two versions.

Figure 3. HBV RNA v2.0 enhances detection and increases granularity in low copy longitudinal samples



Longitudinal HBV RNA results from 3 individual patients who were tested with HBV RNA v1.0 (green) and v2.0 (blue). Horizontal lines indicate v1.0 and v2.0 limits of detection.

4. Conclusions

- HBV RNA v2.0 is ~15 fold more sensitive than v1.0 and detected 100% (20/20) of tested replicates at 10 copies/mL concentration.
- Reported results are linear (R^2=0.9995) across the dynamic range of the v2.0 assay.
- Results reported by v2.0 are within the standard deviation of those reported by v1.0 showing good concordance between assay versions.
- Increased sensitivity of the HBV RNA v2.0 (0.6 mL) assay yields tangible increases in detected and quantifiable results in low RNA copy samples from patients on therapy.
- Increased precision of HBV RNA v2.0 leads to more granular visibility into HBV RNA changes in longitudinal samples.

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Financial Disclosures

This study was funded in part by Abbott Laboratories. MA, MS, LD, KCL, and GC are employees and shareholders of Abbott Laboratories.

AM and DM are employees and owners of bioMONTR® Labs

Appendix 11 Protocol Amendment History

Clinical Study Protocol Amendment 1 – Summary of Changes

AB-729-201

AB-729 Subcutaneous Injection

A RANDOMIZED, OPEN-LABEL, MULTICENTER STUDY INVESTIGATING AB-729, NUCLEOS(T)IDE ANALOGUE AND PEGYLATED INTERFERON ALFA-2A TREATMENT IN SUBJECTS WITH CHRONIC HEPATITIS B INFECTION

Initial Protocol; Version 1.0: 30 June 2021
Amendment 1; Version 2.0: 01 November 2021

Confidential Information of Arbutus Biopharma Corporation

PROTOCOL AMENDMENT SUMMARY OF CHANGES

All revisions from the original protocol (Version 1 dated 30 June 2021) to this protocol amendment 1 (Version 2.0 dated 01 November 2021) are described below. The corresponding specific changes to the protocol text are shown in a tracked-changes version of the protocol.

REVISION NO. 1:	Revised the Medical Monitor as well as the Sponsor's signatory.
General Description of Changes and Rationale:	The Executive Director (Karen Sims, MD, PhD) title changed and is now Vice President, Clinical Development. The following text replaced the original wording: Name: Karen Sims, MD, PhD Title: Vice President, Clinical Development Arbutus Biopharma Corporation
Protocol Section Affected:	Sponsor Approval Signatures page
REVISION NO. 2:	Clarified the discontinuation criteria for discontinuation of NA.
General Description of Changes and Rationale:	The discontinue NA treatment criteria has been revised as below: • Alanine aminotransferase (ALT) <2 × upper limit normal (ULN), and • Undetectable HBV DNA, and • At least one of the following: o HBsAg undetectable (via conventional assay) for at least 24 weeks after the ~~end of~~ *last dose of AB-729* treatment o HBsAg <100 IU/mL at two consecutive visits at least 24 weeks after the ~~end of~~ *last dose of AB-729* treatment o HBsAb positive for at least 24 weeks after the ~~end of~~ *last dose of AB-729* treatment
Protocol Section Affected:	Synopsis; 4.1 Overall Design; 4.4 Nucleos(t)ide Discontinuation Criteria.
REVISION NO. 3:	Clarified that PBMC collections are preferred to be prior to administration of AB-729 or Peg-IFNα-2a.

General Description of Changes and Rationale:	The Schedule of Activities was revised to include the following: *All laboratory assessments including clinical laboratory tests (safety assessments), virology, biomarker and PBMC collections should be drawn pre-dose (AB-729 or Peg-IFNα-2a) when applicable*
Protocol Section Affected:	1.3 Schedule of Activities.
REVISION NO. 4:	Clarified the follow-up visit schedules for early termination prior to Week 24
General Description of Changes and Rationale:	The Schedule of Activity tables were revised to include Early Termination visits, as well as a new Appendix added for the Schedule of Activities for Early Termination for the Lead-in Period.
Protocol Section Affected:	1.3 Schedule of Activities, Appendix 8.
REVISION NO. 5:	The upper limit of BMI was increased from ≤35 kg/m^2 to ≤38 kg/m^2 for inclusion into the study.
General Description of Changes and Rationale:	The inclusion criteria below for BMI was increased from ≤35 kg/m^2 to ≤38 kg/m^2 given prior experience with AB-729 and to allow more participant access to the study. 1. Body mass index (BMI) ≥18 kg/m^2 and ≤38 kg/m^2.
Protocol Section Affected:	5.1 Inclusion Criteria
REVISION NO. 6:	An exclusion criterion was added for central nervous system disease per the Peg-IFNα-2a label.
General Description of Changes and Rationale:	Subjects will be excluded with known epilepsy or central nervous system dysfunction.
Protocol Section Affected:	5.2 Exclusion criteria

REVISION NO. 7:	The exclusion criterion for any known or suspected hypersensitivity or previous severe reactions was revised to include biologics such as vaccines.
General Description of Changes and Rationale:	The exclusion criterion was revised as follows: Any known or suspected hypersensitivity or previous severe reactions to any of the constituents of AB-729 or Peg-IFNα-2a, *to biologics such as vaccines*, or a history of previous severe hypersensitivity reactions (i.e., anaphylaxis, Stevens-Johnson Syndrome, toxic epidermal necrolysis).
Protocol Section Affected:	5.2 Exclusion criteria
REVISION NO. 8:	The exclusion criterion for poorly controlled diabetes mellitus subjects was clarified
General Description of Changes and Rationale:	The exclusion criterion was revised as follows: Poorly controlled diabetes mellitus with whole blood hemoglobin A1c (HbA1c) ≥7.5% ~~or~~ *and/or* Screening fasting plasma glucose of ≥126 mg/dL, confirmed by repeat
Protocol Section Affected:	5.2 Exclusion criteria
REVISION NO. 9:	The exclusion criterion for any known or suspected hypersensitivity or previous severe reactions was revised to include biologics such as vaccines.
General Description of Changes and Rationale:	The exclusion criterion was revised as follows: Previous *serious adverse events or Grade 4 adverse events due to prior* treatment with Peg-IFNα-2a are exclusionary.
Protocol Section Affected:	5.2 Exclusion criteria
REVISION NO. 10:	Assessments and study visits were clarified for subjects who restart NA therapy

General Description of Changes and Rationale:	The table of Schedule of Activities for Subjects who Restart NA Therapy was revised to clarify study assessments and study visits.
Protocol Section Affected:	Appendix 9

REVISION NO.11:	Corrections for typographical errors and omissions.
General Description of Changes and Rationale:	The purpose of the changes is to correct typographical errors and to correct minor omission in the text.

Clinical Study Protocol Amendment 2 – Summary of Changes

AB-729-201

AB-729 Subcutaneous Injection

A RANDOMIZED, OPEN-LABEL, MULTICENTER STUDY INVESTIGATING AB-729, NUCLEOS(T)IDE ANALOGUE AND PEGYLATED INTERFERON ALFA-2A TREATMENT IN SUBJECTS WITH CHRONIC HEPATITIS B INFECTION

Initial Protocol; Version 1.0: 30 June 2021
Amendment 1; Version 2.0: 01 November 2021
Amendment 2; Version 3.0: 17 June 2022

Confidential Information of Arbutus Biopharma Corporation

PROTOCOL AMENDMENT SUMMARY OF CHANGES

All revisions from the protocol amendment 1 (Version 2.0 dated 01 November 2021) to this protocol amendment 2 (Version 3.0 dated 17 June 2022) are described below. The corresponding specific changes to the protocol text are shown in a tracked-changes version of the protocol.

REVISION NO. 1:	The visit schedule for Cohorts A1 and A2 was corrected to coincide with the Week 124 duration and the visit schedule for Cohorts B1 and B2 was also corrected to coincide with the Week 112 duration.
General Description of Changes and Rationale:	The visit number was corrected for Cohorts A1 and A2 to the following: Subjects randomized to Cohorts A1 and A2 will have between 19 (through Week 100) and ~~28~~ *32* (through Week 124) visits depending on whether they qualify for NA discontinuation. The visit number was corrected for Cohorts B1 and B2 to the following: Subjects randomized to Cohorts B1 and B2 will have between 16 (through Week 88) and ~~25~~ *29* (through Week 112) visits depending on whether they qualify for NA discontinuation.
Protocol Section Affected:	Synopsis, Section 4.1.2
REVISION NO. 2:	The criteria for resuming NA therapy during the second follow-up period was revised.
General Description of Changes and Rationale:	The following revisions were made to the criteria for resuming NA therapy: Resumption of NA therapy during the second follow-up period should occur ~~in~~ *if any of* the following ~~situations~~ *scenarios occur* after discussion with the Sponsor Medical Monitor: • Persistent ALT elevations ≥2 × baseline, AND ≥2 – 5 × ULN, AND HBV DNA >2000 IU/mL for 12 weeks • Persistent ALT elevations ≥2 × baseline, AND ≥5 – 10 × ULN, AND HBV DNA >2000 IU/mL for 4 weeks • *HBV DNA > 20,000 IU/mL regardless of ALT level, confirmed by repeat* • ALT > 10 x ULN confirmed by repeat • ALT > baseline and > ULN, AND:

- increased direct *or total* bilirubin ≥*2 × ULN and* ≥*2 ×* ~~>1.5 mg/dL (or >25 µmol/L)~~ from baseline confirmed by repeat, OR
- i~~ncreased~~ INR ~~from~~ *increase of* ≥0.5 from baseline, confirmed by repeat.

Protocol Section Affected:	Section 4.4, Section 8.6, and Appendix 9
REVISION NO. 3:	The requirement for HAV and HEV antibodies at screening were removed.
General Description of Changes and Rationale:	Virology screening parameters for HAV and HEV are no longer required due to the occurrence of false-positives in cases if low clinical suspicion and confounding of the HAVAb test by prior vaccination and both tests by prior/resolved infection. Assessment of acute HAV or HEV infection will be made via clinical assessment of symptoms and liver function testing.
Protocol Section Affected:	Table 1, Section 8.5.5.1, and Table 12
REVISION NO. 4:	Two inclusion criteria were revised. The first was that the age was increased to include up to and including 60 years of age. Secondly, the NA therapy was revised to include switching criteria for TDF and TAF prior to Screening.
General Description of Changes and Rationale:	Criterion #1 was revised to: Subjects must be 18 (or other appropriate age of consent) to ~~50~~ *60* years of age, inclusive. Criterion #8 was revised to: Subjects must have been receiving either TAF, TDF (or equivalent), or ETV consistently for ≥12 months prior to Day 1 and are willing to continue with the same NA treatment through the final study visit *(switching from TDF to TAF or vice versa at least 28 days prior to dosing Day 1 is permitted).* ~~Switching of NA therapy during this window (between 12 months prior to Day 1 and the final study visit) is not permitted.~~
Protocol Section Affected:	Section 5.1
REVISION NO. 5:	Two exclusion criteria were revised. The first was streamlining laboratory data for ANA, ASMA, and anti-LKM1 titers. The second was to revise AFP to >10 ng/mL from >100 ng/mL

General Description of Changes and Rationale:	Criterion #15 was revised to: Laboratory data *(such as positive ANA plus positive ASMA and/or anti-LKM1, confirmed by repeat) and/or clinical signs or symptoms* suggestive of autoimmune hepatitis., ~~confirmed by repeat:~~ ~~a. ANA titer ≥ 1:80 AND~~ ~~b. ASMA titer ≥ 1:80, OR~~ ~~c. Anti-LKM1 titer ≥ 1:40.~~ Criterion #16 was revised to: AFP >10~~0~~ ng/mL. ~~For subjects with AFP results of 50 to 100 ng/mL, historical documentation of a liver ultrasound, computed tomography scan, or magnetic resonance imaging scan performed within 3 months prior to the first dose of study treatment to rule out HCC or other malignancy or liver abnormality must be provided~~
Protocol Section Affected:	Section 5.2
REVISION NO. 6:	The complete and targeted physical examination schedule was added to coincide with the Schedule of Activities
General Description of Changes and Rationale:	A complete physical examination is required at Screening. A targeted physical examination can occur after Day 1 and will be based on any changes to the subject's health since the last visit.
Protocol Section Affected:	Table 1, Section 8.5.1
REVISION NO. 7:	Quantitative HBV DNA testing was clarified to include both local and central laboratory testing.
General Description of Changes and Rationale:	Quantitative HBV DNA testing can be performed locally in addition to central laboratory testing to facilitate more rapid results.
Protocol Section Affected:	Table 14
REVISION NO. 8:	A reference was updated to the Resistance Monitoring Plan.
General Description of Changes and Rationale:	The reference for the HBV RNA marker was updated to include a more recent citation.
Protocol Section Affected:	Appendix 11

REVISION NO. 9:	Resistance samples and PBMC samples are required.
General Description of Changes and Rationale:	The optional language was removed for resistance samples and PBMC samples since they are required.
Protocol Section Affected:	Table 1, Table 2, Table 3, Table 4, Table 5, and Table 13
REVISION NO. 10:	Additional secondary endpoints were added to the following objective that evaluates the proportion of subjects who experience clinical and/or viral relapse in the follow-up period after discontinuing NA therapy.
General Description of Changes and Rationale:	The following 2 secondary endpoints were added to the following objective that evaluates the proportion of subjects who experience clinical and/or viral relapse in the follow-up period after discontinuing NA therapy • Proportion of subjects who have HBV DNA <LLOQ at each timepoint after discontinuation of NA therapy • Proportion of subjects who have HBsAg <100 IU/mL or <10 IU/mL at each timepoint after discontinuation of NA therapy
Protocol Section Affected:	Synopsis, Section 3
REVISION NO. 11:	Corrections for typographical errors and omissions.
General Description of Changes and Rationale:	The purpose of the changes is to correct typographical errors and to correct minor omission in the text.

INVESTIGATOR SIGNATURE PAGE

Study Protocol Number: AB-729-201

Study Title: A Randomized, Open-Label, Multicenter Study Investigating AB-729, Nucleos(t)ide Analogue and Pegylated Interferon Alfa-2a Treatment in Subjects with Chronic Hepatitis B Infection

INVESTIGATOR'S STATEMENT

I understand that all information concerning the product supplied to me by Arbutus Biopharma Corporation (Arbutus) or their designee, in connection with this study and not previously published, is confidential information. This information includes the Investigator's Brochure, protocol (and applicable amendments), Case Report Forms, Pharmacy Manual, Study's PK/Laboratory Manual, and basic scientific data.

I will conduct the study per the protocol and I understand that any changes to the protocol must be approved in writing by Arbutus or their designee and by the IRB/EC before implementation, except where necessary to eliminate apparent immediate hazards to the study participants.

I confirm that I will report all adverse events following the regulations referenced in the protocol.

I confirm that I will conduct this study in conformance with the principles of the Declaration of Helsinki, Good Clinical Practices, and local law and regulations.

I confirm that I am informed of the need for records retention and that no data will be destroyed without the written consent of Arbutus.

By my signature below, I hereby attest that I have read, understood, and agree to abide by all conditions, instructions, and restrictions contained in this protocol (version as per page footer).

_____

Investigator's Name (printed)

_____

Investigator's Signature

_____

Date