2 SYNOPSIS

Study Title	A Multi-center, Open-label, Long-term Extension Study of ABI-H0731+Nucleos(t)ide as Finite Treatment for Chronic Hepatitis B Patients
Protocol Number	ABI-H0731-211
Study Phase	2
Drug	Vebicorvir (VBR; formerly ABI-H0731)
Indication	Chronic hepatitis B virus infection (cHBV)
Study Sponsor	Assembly Biosciences
Number of Study Site(s) and Countries	This study was conducted at a total of 24 sites located in the following countries: United States of America, Canada, Hong Kong, United Kingdom, and New Zealand
Publications	• Sulkowski MS, Agarwal K, Fung SK, et al. Continued Therapy with ABI-H0731 + NrtI Results in Sequential Reduction/Loss of HBV DNA, HBV RNA, HBeAg, HBcrAg and HBsAg in HBeAg-Positive Patients (**Poster Presentation; AASLD 2019**) • Yuen MF, Agarwal K, Ma X, et al. Antiviral activity and safety of the hepatitis B core inhibitor ABI-H0731 administered with a nucleos(t)ide reverse transcriptase inhibitor in patients with HBeAg-positive chronic hepatitis B infection in a long-term extension study (**Poster Presentation; EASL 2020**) • Cai D, Connelly E, Kumar R, et al. Amino Acid Substitutions in the Inhibitor Binding Pocket of HBV Core Protein Confer Differential Changes in Susceptibility to Three Generations of HBV Core Inhibitors (**Poster Presentation; AASLD 2020**). • Jacobson IM, Ma X, Nguyen T, et al. Analysis of the longer-term safety profile of the hepatitis B virus core inhibitor ABI-H0731 in an open-label extension study (**Poster Presentation; AASLD 2020**) • Sulkowski M, Agarwal K, Li Y, et al. Changes in Viral Antigens are More Strongly Associated with HBV pgRNA than HBV DNA in Studies of Vebicorvir and NrtI in Treatment-naïve Patients with Chronic HBV Infection (**Poster Presentation; AASLD 2020**) • Cai D, Evanchik M, Yan R, et al. Second-generation hepatitis B virus core inhibitors ABI-H2158 and ABI-H3733 have enhanced potency and target coverage for both antiviral inhibition and covalently closed circular DNA establishment activities (**Oral Presentation; EASL 2021**) • Yuen MF, Locarnini S, Revill P, et al. No emergent core inhibitor resistance in patients with chronic hepatitis B virus infection treated with vebicorvir in combination with a nucleos(t)ide reverse transcriptase inhibitor (**Poster Presentation; EASL 2021**) • Gane E, Sulkowski M, Ma X, et al. Viral response and safety following discontinuation of treatment with the core inhibitor vebicorvir and a nucleos(t)ide reverse transcriptase

	inhibitor in patients with HBeAg positive or negative chronic hepatitis B virus infection. (**Poster Presentation; EASL 2021**) • Yuen MF, Ma X, Hassanein T, et al. HBV pgRNA and DNA both rebound immediately following discontinuation of the core inhibitor vebicorvir despite continued NrtI treatment in patients with HBeAg positive chronic hepatitis B virus infection: Findings from a Phase 2 open label study. (**Oral Presentation; AASLD 2021**)
Study Period	20 Dec 2018 to 26 Apr 2021
Rationale	Study ABI-H0731-211 was designed to assess the safety of extended treatment with VBR (ie, up to 148 weeks) when administered in combination therapy with a standard-of-care (SOC) nucelos(t)ide reverse transcriptase inhibitor (NrtI) in subjects previously participating in 1 of 2 parent studies, ie, Study ABI-H0731-201 (Parent Study 201), a Phase 2a study evaluating VBR+NrtI in virologically-suppressed subjects with hepatitis B "e" antigen (HBeAg) positive and negative cHBV; and Study ABI-H0731-202 (Parent Study 202), a Phase 2a study evaluating VBR with entecavir (ETV) in treatment-naïve subjects with HBeAg positive cHBV. The study would also evaluate off-treatment virologic response rates following cessation of treatment in subjects meeting specific criteria. Additionally, the study would assess the effect of the combination regimen on serum biomarkers such as HBV deoxyribonucleic acid (DNA), quantitative and qualitative reduction in HBeAg, hepatitis B core-related antigen (HBcrAg), and hepatitis B surface antigen (HBsAg), as well as exploratory biomarkers such as reduction in circulating hepatitis B virus (HBV) pregenomic ribonucleic acid (pgRNA).
Objectives and Endpoints	**The primary objective(s) of this study was:** • To evaluate the potential for combination therapy with VBR+NrtI to increase SVR rates in subjects who have cHBV **The secondary objectives of this study were:** • To evaluate the longer-term safety and tolerability of VBR added to SOC NrtI therapy • To evaluate improvement in transaminases in subjects on treatment and post-treatment • To evaluate the durability of changes in viral antigen and viral DNA after discontinuation of combination therapy **The exploratory objectives of this study were:** • To evaluate the kinetics of and absolute changes from Baseline in biomarkers of transcriptionally active covalently closed circular DNA (cccDNA) (HBeAg and HBsAg) • To assess the relationship between exploratory viral biomarkers, such as changes in viral pgRNA and hepatitis B core-related antigen (HBcrAg), and outcome • To evaluate potential emergence of HBV resistance associated variants (RAVs), if any, to VBR in combination with an SOC NrtI • To evaluate the durability of virologic response between 24 weeks post-treatment discontinuation and 36 months post-treatment discontinuation

	• For subjects who have provided an optional pharmacogenomic sample in parent studies (eg, ABI-H0731-201 or ABI-H0731-202), to evaluate the potential contribution of host genomics to outcomes • To assess steady state plasma levels of VBR and SOC NrtI for possible correlation with markers of safety and efficacy **The primary endpoint(s) of this study was:** • Proportion of subjects with SVR at 24 weeks off treatment. **The secondary endpoints of this study were:** • Incidence of AEs, premature discontinuations due to AEs, abnormal safety laboratory results, electrocardiogram, or vital signs • Incidence of subjects with abnormal alanine aminotransferase (ALT) at Baseline who have normal ALT at end of treatment (EOT) and end of study (EOS) • Incidence of subjects with suppression/loss of viral antigen/DNA on combination treatment whose viral antigens rebound off therapy **The exploratory endpoints of this study were:** • Mean change from Baseline in $\log_{10}$ serum HBeAg • Mean change from Baseline in $\log_{10}$ serum HBsAg • Incidence of subjects with loss or change in $\log_{10}$ HBsAg or $\log_{10}$ HBeAg (< 0.5, ≥ 0 .5 to 1.0, or > 1.0 in viral antigens) at EOT and end of follow-up • Incidence of subjects with HBsAg seroconversion (loss of HBsAg and appearance of HBsAg antibody) or HBeAg seroconversion (loss of HBeAg and appearance of HBeAg antibody) • Incidence of subjects with “detectable” HBV DNA by polymerase chain reaction at Baseline whose HBV DNA becomes “target not detected” • Quantitative changes from Baseline in viral RNA on treatment and through end of follow-up • Quantitative changes in serum HBcrAg levels on treatment and through end of follow-up • Incidence of HBsAg or HBeAg seroreversion in subjects up to 3 years off therapy • Incidence of subjects requiring retreatment following SVR through 3 years off therapy • Incidence of subjects with emergence of HBV resistance associated variants (RAVs) • If differences are seen in outcomes/AEs between racial or ethnic groups: Pharmacogenomics correlation will be performed with clinical outcomes in subjects who

	have provided an optional informed consent and sample in Study ABI-H0731-201 or Study ABI-H0731-202 • Quantitative levels of VBR and NrtI in plasma
Methodology	Study 211 was an open-label, multi-center, long-term extension study evaluating the safety and efficacy of VBR in combination with a SOC NrtI in subjects with cHBV, who completed 24 weeks of treatment in either of the Parent Studies 201 or 202. It was anticipated that approximately 100 male or female subjects from the parent studies would be enrolled. The planned duration of treatment with VBR+NrtI was up to 148 weeks; the actual duration of treatment for each subject was based on their respective HBV treatment history (ie, virologically suppressed or treatment naïve) and HBeAg status (ie, HBeAg positive or HBeAg negative) at Baseline in the parent study, and their individual virologic response in Study 211. Based on assessment of these factors at specific study visits, each subject was evaluated for virologic response and assigned to 1 of the following 3 treatment actions, ie, discontinue both VBR+NrtI; or discontinue VBR only and continue NrtI alone; or continue both VBR+NrtI for up to 148 weeks. Study 211 was terminated early by the Sponsor based upon the goals of the study, the predetermined study endpoints, and preliminary data generated. The decision for early termination of the study was not related to a safety concern observed in this study or in the overall VBR clinical program.
Number of Subjects Planned	Up to 100 were planned.
Main Criteria for Inclusion and Exclusion	To be eligible to participate in the study, subjects were required to provide informed consent, had been previously enrolled in a study of VBR (Study 201 or 201) and completed the treatment period, with demonstrated compliance in the opinion of the Investigator, and agreed to comply with all required study procedures, including adherence to contraceptive requirements, lifestyle considerations, and ability to take oral medication. Subjects who had evidence of RAVs or lack of compliance on a previous study of VBR or had a treatment-emergent AE or laboratory abnormalities deemed clinically significant and possibly or probably related to study drug during their participation in Parent Study 201 or 202, that in the opinion of the Investigator or the Sponsor made the subject unsuitable for Study 211, were ineligible for study participation.
Study Interventions, Dose, Mode of Administration, and Batch Number(s)	The investigational product in Study 211 was VBR. Vebicorvir tablets were manufactured according to Good Manufacturing Practice and contained approximately 100 mg VBR (Batch No: VBR: ZZBV, CBZNP, CBYNP, CCSST, CDMHH; ETV: CM0472A, CM0737A). All subjects self-administered VBR orally, by mouth (PO) as three 100 mg tablets once daily (QD), after a meal, at approximately the same time each day. The NrtI was taken PO QD, and as per package insert.
Planned Duration of Study Treatment	The planned duration of treatment was up to 148 weeks.
Summary of Results and Conclusions	
Disposition, Demographics, and Baseline Disease	A total of 92 subjects (69 from Parent Study 201; 23 from Parent Study 202) were enrolled at 24 sites in the United States. All 92 subjects were treated with 300 mg VBR and NrtI. A total of 70% (64/92) of subjects completed study drug treatment; the most common reasons for discontinuation of study drug were Study Terminated by Sponsor (14/92; 15%) and Withdrawal by Subject (8/92; 9%). A total of 51% (47/92) of subjects completed the study;

Characteristics Results	the most common reasons for discontinuation study were Study Terminated by Sponsor (28/92; 30%) and Withdrawal by Subject (10/92; 11%). Three subjects discontinued study drug treatment and withdrew from the study due to an AE. A majority of subjects were < 50 years of age (67%; 62/92) and Asian (87%; 80/92). Overall, the mean (SD) self-reported years of HBV positivity was longer among virologically-suppressed, HBeAg positive and negative subjects of Parent Study 201 (ie, 11.8 [7.80] and 17.1 [10.99] years, respectively) than treatment-naïve HBeAg positive subjects from Parent Study 202 (ie, 10.8 [8.77] years). As expected, levels of baseline virologic parameters (ie, HBV DNA and HBV pgRNA) were higher among treatment-naïve subjects than virologically-suppressed subjects, particularly among treatment-naïve subjects treated with placebo+ETV in Parent Study 202.
Efficacy Results	**Summary of Efficacy – On-Treatment Phase** Changes from Baseline in efficacy parameters are relative to the respective Baseline values at the start of Study 211. Consistent with the initial findings in the parent study, subjects belonging to the virologically-suppressed population from Parent Study 201 showed evidence of deep viral suppression with VBR+NrtI treatment in Study 211, as supported by the following: a) HBV DNA: There was an overall increase in the proportion of subjects achieving HBV DNA TND at end of treatment/last observation. By the Cobas TaqMan Assay (LOD 10 IU/mL), the proportions of subjects with HBV DNA TND increased from Baseline to end of treatment/last observation for HBeAg negative subjects (ie, 73% [19/26] to 81% [21/26]), but not for HBeAg positive subjects (57% [24/43] to 37% [16/43]). By the more sensitive Assembly Assay (LOD 5 IU/mL), the proportions of subjects with HBV DNA TND increased from Baseline to end of treatment/last observation for both the HBeAg negative subjects (82% [18/22] to 100% [14/14]) and HBeAg positive subjects (53% [20/38] to 86% [36/42]). b) HBV pgRNA and HBV TNA: There was an overall 0.5 $\log_{10}$ U/mL reduction in HBV pgRNA and 0.4 $\log_{10}$ U/mL reduction in HBV TNA at Week 48 among HBeAg positive subjects. In the virologically-suppressed population who had lower levels of viral antigens at Baseline, there were no notable changes in HBeAg, HBsAg, or HBcrAg in Study 211. No subjects lost HBsAg and 1 subject lost HBeAg; none had HBsAg or HBeAg seroconversion. Consistent with the initial findings in the parent study, subjects belonging to the treatment-naïve population from Parent Study 202 showed evidence of deep viral suppression with VBR+ETV treatment in Study 211, as supported by the following: a) HBV DNA: There was an overall increase in the proportion of subjects achieving HBV DNA TND at end of treatment/last observation. By the Cobas TaqMan Assay (LOD 10 IU/mL), the proportions of subjects with HBV DNA TND increased from Baseline to end of treatment/last observation (ie, 0 [0/23] to 26% [6/23]). By the more sensitive Assembly Assay (LOD 5 IU/mL), the proportions of subjects with HBV DNA TND increased more notably from Baseline to end of treatment/last observation (ie, 0 [0/23] to 62% [8/13]).

	b) HBV pgRNA: There was an overall 1.7 log_{10} U/mL reduction in mean HBV pgRNA levels at end of treatment/last observation. c) ALT Normalization: Of subjects who started with abnormal ALT at Baseline, 71% had normal ALT at end of treatment. d) HBV Antigens: There were overall reductions in the mean levels of HBeAg (0.5 log_{10} IU/mL), HBsAg (0.4 log_{10} IU/mL), and HBcrAg (0.7 log_{10} kU/mL) from Baseline to end of treatment/last observation. No subjects lost HBsAg and 1 subject lost HBeAg; none had HBsAg or HBeAg seroconversion. It is noted that greater reductions were observed for the treatment-naïve subjects in Parent Study 202 on VBR+ETV than on placebo+ETV after 24 weeks of treatment (ie, 5.3 vs 4.2 log_{10} IU/mL decrease in HBV DNA [p=0.0084], 2.3 vs 0.6 log_{10} U/mL decrease in HBV pgRNA [p < 0.0001], and 100% (4/4) vs 50% (2/4) of subjects with ALT normalization at end of treatment for subjects who started with abnormal ALT at Baseline). These differences at the end of treatment in the parent study led to subjects with different levels of viral parameters at Baseline in Study 211. As such, the greater reductions observed in Study 211 in viral parameters among subjects on placebo+ETV in Parent Study 202, compared to those on VBR+ETV in Parent Study 202 (ie, 2.1 vs 0.9 log_{10} IU/mL decrease in HBV DNA, 2.9 vs 0.7 log_{10} U/mL decrease in HBV pgRNA, and 83% [5/6] vs 0% [0/1] of subjects with ALT normalization at end of treatment for subjects who started with abnormal ALT at Baseline) are attributable to their entering Study 211 with higher Baseline levels of viral parameters, and receiving treatment with VBR+ETV thereafter. There were 9 subjects in Study 211 who experienced on-treatment virologic rebound, of whom 8 subjects had no evidence of resistance-associated variants at any timepoint, as assessed by Sanger sequencing. One subject had a T109I resistance-associated substitution detected in the core gene during virologic rebound. Five of the 9 subjects had virologic rebound in a setting of noncompliance with study drug, including the subject with T109I detected in the core gene. **Summary of Efficacy – Following Implementation of Treatment Action** Of 43 virologically-suppressed subjects (20 HBeAg positive and 23 HBeAg negative) who met the Treatment Action criteria to discontinue treatment with both VBR and NrtI, all subjects experienced virologic relapse with mean HBV DNA levels increasing among HBeAg negative subjects by 3.6 log_{10} IU/mL, HBV TNA by 2.6 log_{10} U/mL, and HBcrAg by 1.1 log_{10} kU/mL. Among HBeAg positive subjects, mean HBV DNA levels increased by 4.7 log_{10} IU/mL, HBV TNA by 4 log_{10} U/mL, HBeAg by 1.4 log_{10} IU/mL, and HBcrAg by 1.7 log_{10} kU/mL. There was no notable change in HBsAg levels off treatment. Following NrtI restart, there was evidence of viral suppression with reductions in HBV DNA, HBV pgRNA, HBV TNA, HBeAg, and HBcrAg, as applicable, across the populations. Among subjects who continued NrtI/ETV only in the Off-Treatment Phase, there was evidence of a decreased level of viral suppression following discontinuation of VBR. Among treatment-naïve HBeAg positive subjects, HBV DNA increased by 1.4 log_{10} IU/mL, HBV pgRNA by 1.5 log_{10} U/mL, and HBV TNA by 1.4 log_{10} U/mL. Among virologically-suppressed HBeAg positive subjects, HBV pgRNA, and HBV TNA increased by 2 log_{10} U/mL from Off-Treatment Baseline to Last Visit/observation. These data provide further evidence of the deeper level of viral suppression achieved following the addition of VBR to NrtI therapy.

Pharmacokinetic Results	The Week 48 predose geometric mean concentrations for VBR, ETV, and TFV were in general in the same range as those observed in Parent Studies 201 and 202. Collectively, PK results from Parents Studies 201 and 202 and Study 211 support lack of significant drug-drug interaction between VBR and NrtIs following longer term administration.
Safety Results	**Summary of Safety – On-Treatment Phase** During the On-Treatment Phase, wherein subjects received VBR+NrtI for a mean (min, max) duration of 63.5 (1.1, 103.9) weeks, 59% of subjects (54 of the total 92 enrolled) reported at least 1 TEAE. Most subjects reported Grade 1 or 2 TEAEs (50/92; 54%). Grade 3 TEAEs were reported by 4 of 92 subjects (4%), and included ALT increased (3 subjects) and AST increased (1 subject). No Grade 4 TEAEs or deaths were reported. One subject reported a TE SAE of suicidal ideation, considered not related to study treatment. Two (2/92; 2%) subjects reported a TEAE leading to study drug discontinuation; these TEAEs were Grade 3 ALT increased (1 subject) and Grade 3 SAE of suicidal ideation (1 subject). Treatment-emergent AEs reported by ≥ 5% of subjects were upper respiratory tract infection (10/92; 11%), nasopharyngitis (6/92; 7%), and fatigue (5/92; 5%). Treatment-emergent AEs of rash (including rash, rash erythematous, rash macular, rash papular, and rash pruritic) were reported by 9/92 subjects (10%); all events were Grade 1. Treatment-emergent Grade 3 laboratory abnormalities were observed among 8% (7/92) of subjects, and included ALT increased (3 subjects), AST increased (2 subjects), amylase increased (1 subject), prothrombin INR increased (1 subject), and prothrombin time increased (2 subjects). No Grade 4 TE laboratory abnormalities were observed. **Summary of Safety – Following Implementation of Treatment Action** **Subjects Who Discontinued Both VBR and NrtI (Off-Treatment Phase):** During the Off-Treatment Phase, 41% of subjects (17/41 who met criteria to discontinue treatment with both VBR and NrtI) reported TEAEs. Twelve percent (5/41) of subjects reported Grade 1 AEs, 20% (8/41) reported Grade 2 AEs, and 10% (4/41) reported Grade 3 AEs. The Grade 3 AEs were ALT increased (2/41; 5%), AST increased (1/41; 2%), procedural hemorrhage (1/41; 2%), procedural pain (1/41; 2%), and seizure (1/41; 2%). No Grade 4 AEs or deaths were reported. Two (2/41; 5%) subjects reported an SAE. The SAEs were procedural pain and seizure. These SAEs were not considered related to study treatment and resolved during the study. Common AEs reported by ≥ 5% of subjects were ALT increased (11/41; 27%), AST increased (2/41; 5%), back pain (2/41; 5%), headache (2/41; 5%), and nausea (2/41; 5%). Most subjects had a Grade 1 or 2 laboratory abnormality of (25/40; 63%). Two subjects (2/40; 5%) had a Grade 3 laboratory abnormality and 1 subject (1/40; 3%) had a Grade 4 laboratory abnormality. The Grade 3 laboratory abnormalities were ALT increased (2/40; 5%) and AST increased (1/40; 3%). The Grade 4 laboratory abnormality was ALT increased (1/40; 3%). **Subjects Who Discontinued Both VBR and NrtI, then Restarted NrtI (NrtI-Restart Phase):** Upon restarting NrtI, 30% (9/30) of subjects reported an AE, with 10% (3/30) reporting a Grade 1 AE, 10% (3/30) reporting a Grade 2 AE, and 10% (3/30) reported a Grade 4 AE. The Grade 4 AE was ALT increased (3/30; 10%), which resolved during the study. No AE of Grade 3, death, or SAEs were reported. The only AE reported by ≥ 5% of subjects overall was ALT increased (5/30; 17%). Most subjects (19/30; 63%) reported laboratory abnormalities of Grade 1 or 2. Four subjects (4/30; 13%) had Grade 3 laboratory abnormalities and 4 subjects (4/30; 13%) had Grade 4 laboratory abnormalities. Grade 3 laboratory abnormalities were AST increased (4/30; 13%), ALT increased (3/30; 10%), and glucose increased (1/30; 3%). Grade 4 laboratory abnormalities were ALT increased (4/30; 13%) and AST increased (1/30; 3%).

	Subjects Who Continued NrtI/ETV (Off-Treatment Phase): Among subjects who continued treatment with NrtI/ETV, only a single subject (1/24; 4%), reported an AE of Grade 1. No AEs of Grade 2, 3, or 4, SAEs or death were reported by these subjects. All laboratory abnormalities were of Grade 1.
Conclusions	**The efficacy conclusions of this study are:** • In this long-term extension study, VBR treatment in addition to NrtI therapy led to deep viral suppression as evidenced by reductions in HBV DNA and HBV pgRNA, and to a lesser degree, reductions in viral antigens. o The observed on-treatment change in viral parameters varied by the patient population and Study 211 Baseline values. o There was no evidence of treatment-emergent virologic resistance in subjects who were compliant to study drug. • Among the subset of subjects who met criteria to discontinue all antiviral treatment, none achieved SVR. The primary endpoint was not met. **The safety conclusions of this study are:** • VBR administered in combination with NrtI was generally safe and well-tolerated with few discontinuations due to AEs and SAEs and no deaths. Most Grade 3 and 4 AEs and laboratory abnormalities were related to elevations in ALT and occurred in the Off-Treatment or NrtI-Restart Phases. • No trends in safety in terms of vital signs, ECG, or physical examination were noted.
Study Report Version – Date	Version 1.0 – 11 Feb 2022