Continued Therapy with ABI-H0731 + NrtI Results in Sequential Reduction/Loss of HBV DNA, HBV RNA, HBeAg, HBcrAg and HBsAg in HBeAg-Positive Patients

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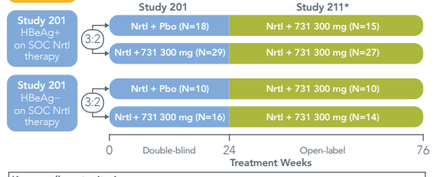
Introduction

- Chronic hepatitis B (CHB) is a prevalent infection of the liver affecting an estimated 257 million people worldwide[1]
- While currently approved nucleos(t)ide reverse transcriptase inhibitors (NrtI) provide long-term viral suppression in most patients, they fail to eliminate ongoing NrtI-refractory viral infection, with patients remaining at risk for hepatocellular carcinoma and end-stage liver disease[2,3]
- Elimination of NrtI-refractory viral infection and depletion of covalently closed circular DNA (cccDNA) pools will be required to achieve HBV cure
- HBV DNA and pregenomic (pg)RNA are the primary surrogate markers for active viral infection and presence of cccDNA, respectively, while other surrogate markers of cccDNA are HBcrAg and HBeAg
- The temporal correlation of HBsAg levels with elimination of cccDNA pools is complicated by potentially high proportions of HBsAg being generated from HBV integrants[4,5]
- Core protein inhibitors (CI) interfere with multiple aspects of the HBV lifecycle, including formation of viral nucleocapsids containing pgRNA, and trafficking of incoming nucleocapsids containing relaxed circular DNA to the nucleus to generate new cccDNA molecules
- ABI-H0731 is an orally administered, potent and selective small molecule inhibitor of the HBV core protein currently in Phase 2 development; Favorable safety and tolerability have been demonstrated with 127 CHB patients treated for at least 4 weeks and over 50 CHB patients treated for ≥40 weeks with ABI-H0731-containing regimens
- Here we report final Week 24 data from two clinical studies in NrtI-suppressed (201) and NrtI-naïve (202) patients with CHB, along with interim data from a long-term extension study (211) where all patients receive combination therapy

Study Design

Figure 1. Overview of ABI-H0731 Phase 2a Studies



Key enrollment criteria
- Chronic HBV infection in good general health
- Metavir F0-F2 or equivalent (no history of hepatic decompensation)
- Study 202: HBV DNA >10^5 IU/mL; ALT <10x ULN
- Study 201: HBsAg >400 IU/mL (HBeAg+) or >100 IU/mL (HBeAg−); ALT <5x ULN

*n values represent the 87 patients who transitioned to 211, remain on treatment to date, and were included in this analysis
ETV, entecavir; Pbo, placebo; SOC, standard of care

- Of the 97 subjects completing Study 201 or 202, 87 are currently receiving ABI-H0731 + NrtI and have been treated for at least 16 weeks in Study 211 (ie, the minimum cumulative treatment time with ABI-H0731 + NrtI is ≥40 weeks)

HBV DNA and pgRNA Assays

- Four viral nucleic acid assays (including 3 assays developed at Assembly Biosciences [ASMB]) were utilized in the clinical studies to evaluate the antiviral response in each cohort population to assess specific virologic endpoints (ie, changes from baseline vs. categorical assessment of target detection)

202 (Rx-naïve)	**HBV DNA Assay (lower limit of quantification [LLOQ] = 20 IU/mL)** – Cobas TaqMan Ver 2.0, used for measuring change from baseline across a broad dynamic range **HBV pgRNA Assay (LLOQ = 135 U/mL)** – ASMB real-time quantitative PCR (RT-qPCR), used for measuring change from baseline across a broad dynamic range after digestion of high levels of interfering HBV DNA
201 (NrtI-suppressed)	**HBV DNA Assay (LLOQ = 5 IU/mL)** – ASMB semi-quantitative PCR, used for measuring "Target Not Detected" with high sensitivity **HBV pgRNA Assay (LLOQ =35 U/mL)** – ASMB RT-qPCR, used for measuring change from baseline over a broad dynamic range in the absence of high levels of interfering HBV DNA
211 (201 & 202)	**HBV DNA and pgRNA Assays** – Study 211 utilized all four assays described above as determined by study origin (201 or 202) and the individual patient nucleic acid levels

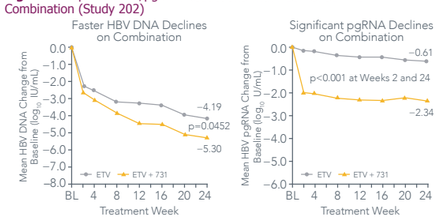
Results – Antiviral Activity

Table 1. Demographics and Baseline Characteristics

	Study 202	Study 201	
	HBeAg+ (N=25)	HBeAg+ (N=47)	HBeAg− (N=26)
Demographics			
Age, years, mean (range)	35 (20–66)	44 (20–66)	48 (34–64)
Female, n (%)	17 (68)	16 (34)	10 (38)
Asian, n (%)	24 (96)	42 (89)	21 (81)
Genotype B, C[a] (%)	11, 11 (88)	12, 19 (66)	4, 2 (23)
Baseline characteristics, mean (range)			
ALT U/L	56.7 (13–295)	26.8 (13–97)	24.7 (9–67)
HBV DNA log_{10} IU/mL[b]	8.0 (5.5–9.1)	45 (96% <LLOQ)	26 (100% <LLOQ)
HBV pgRNA log_{10} U/mL	7.2 (4.6–8.6)	3.5 (1.5–6.3)[c]	1.6 (1.5–2.6)[c]
HBsAg log_{10} IU/mL	4.6 (3.3–5.2)	3.5 (2.9–4.5)	3.1 (2.2–4.2)
HBeAg log_{10} IU/mL[d]	2.5 (−0.7–3.1)	0.5 (−0.9–2.6)	25 (96% <LLOQ)
HBcrAg log_{10} kU/mL	5.4 (2.8–6.2)	3.0 (1.4–4.8)	0.4 (−1.0–1.9)

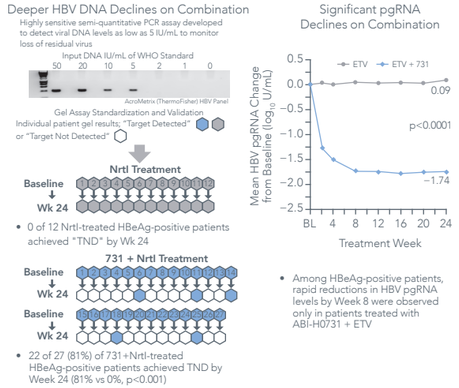
LLOQ, lower limit of quantification; [a]Genotypes in Study 201 were determined by sequence alignment; genotypes in Study 202 were determined by InnoLipa HBV at a central lab; [b]As measured by Roche Cobas qPCR, LLOQ = 20 IU/mL; Reported mean (range) for Study 202 and n (% <LLOQ) for Study 201; [c]Nine of 47 HBeAg-positive patients with baseline pgRNA <35 U/mL; 22 of 26 HBeAg-negative patients with baseline pgRNA <35 U/mL. HBV pgRNA values <35 U/mL were imputed at 34 U/mL; [d]Reported mean (range) for Study 202 and Study 201 HBeAg-positive patients, and n (% <LLOQ) for Study 201 HBeAg-negative patients

Figure 2. Superior DNA/pgRNA Reductions with ABI-H0731 + ETV Combination (Study 202)



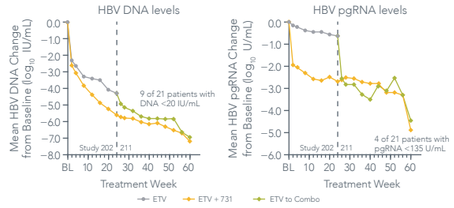
- Faster HBV DNA declines were observed in the ABI-H0731 + entecavir (ETV) arm than with ETV alone, with statistically significant declines in HBV DNA in the ABI-H0731 + ETV arm at Week 24 (p=0.0452)
- Rapid 2-log reductions in HBV pgRNA levels by Week 2 were observed only in patients receiving ABI-H0731 + ETV (p<0.001)
- The initial rapid phase decline of pgRNA is thought to be mechanism-based inhibition (ie, pgRNA not packaged and secreted into plasma), while the second slower phase decline is believed to reflect reduction in cccDNA pools

Figure 3. DNA/pgRNA Declines in NrtI-Suppressed, HBeAg-Positive Patients (Study 201)



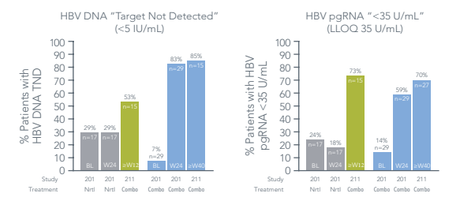
- 0 of 12 NrtI-treated HBeAg-positive patients achieved "TND" by Wk 24
- 22 of 27 (81%) of 731+NrtI-treated HBeAg-positive patients achieved TND by Week 24 (81% vs 0%, p<0.001)
- Among HBeAg-positive patients, rapid reductions in HBV pgRNA levels by Week 8 were observed only in patients treated with ABI-H0731 + ETV
- Baseline and Week 24 serum samples were assayed from all subjects for detectable HBV using ASMB PCR gel assay with LLOQ of 5 IU/mL
- As previously reported,[2,3] the vast majority of long-term NrtI-treated patients continue to harbor low level virus at the time of study entry
- Results show that the addition of ABI-H0731 can readily reduce viral load to levels not achieved by NrtI therapy alone in HBeAg-positive patients

Figure 4. Further DNA/pgRNA Declines with Extended Treatment (Study 202/211)



- Switch from ETV to ABI-H0731 + ETV resulted in immediate and enhanced declines in both HBV DNA and pgRNA levels, confirming the contribution of ABI-H0731 to the combination
- The mean HBV DNA and pgRNA declines from baseline at Week 48 were 6.3 logs and 3.0 logs, respectively, for patients treated with ABI-H0731 + ETV
- Continued HBV DNA declines are observed on combination therapy
- The observed acceleration in second phase decline of HBV pgRNA levels likely reflects reductions of cccDNA pools

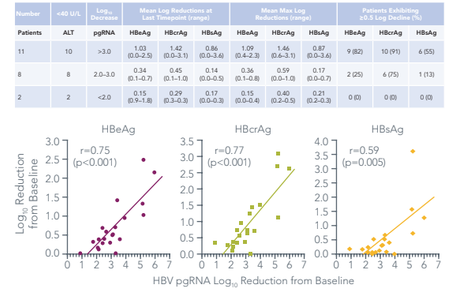
Figure 5. DNA/pgRNA Declines to Highly Suppressed Levels in NrtI-Suppressed Patients (Study 201/211)



- Only patients receiving ABI-H0731 + ETV therapy reduced HBV DNA levels to TND and pgRNA levels to <35 U/mL

Figure 6. Correlation Between HBV pgRNA Reductions and Viral Antigen Declines (Patients Treated 16–60 Weeks with ABI-H0731 + ETV in Study 202/211)

Number	<40 U/L	Log_{10} Decrease	Mean Log Reductions at Last Timepoint (range)			Mean Max Log Reductions (range)			Patients Exhibiting ≥0.5 Log Decline (%)		
Patients	ALT	pgRNA	HBeAg	HBcrAg	HBsAg	HBeAg	HBcrAg	HBsAg	HBeAg	HBcrAg	HBsAg
11	10	>3.0	1.03 (0.0–2.5)	1.42 (0.0–3.1)	0.86 (0.0–3.4)	1.09 (0.4–2.3)	1.46 (0.6–3.1)	0.87 (0.0–3.6)	9 (82)	10 (91)	6 (55)
8	8	2.0–3.0	0.34 (0.1–0.7)	0.45 (0.1–1.0)	0.14 (0.0–0.5)	0.36 (0.1–0.8)	0.59 (0.0–1.0)	0.17 (0.0–0.7)	2 (25)	6 (75)	1 (13)
2	2	<2.0	0.15 (0.9–1.8)	0.29 (0.3–0.3)	0.17 (0.0–0.3)	0.15 (0.0–0.4)	0.40 (0.2–0.5)	0.21 (0.2–0.3)	0 (0)	0 (0)	0 (0)



r is Spearman's correlation between reduction in pgRNA and HBV antigen. The straight line fit is calculated by choosing the line that minimizes the least square sum of the vertical distance d, of all the selected markers pictured by using the following equation: y = a + bx, where "a" is the intercept and "b" is the slope.

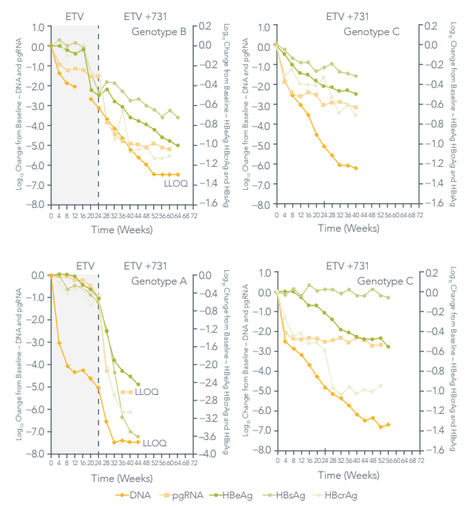
- cccDNA is the template (only known source) of HBV pgRNA
- The addition of ABI-H0731 resulted in multi-log reductions in pgRNA levels, while NrtI therapy fails to significantly reduce pgRNA levels
- The initial phase decline of pgRNA (≤2 logs) was not associated with HBV antigen declines
- The second phase decline of pgRNA appears to reflect decline in cccDNA pools, as pgRNA reductions greater than 3 logs are associated with the greatest level of declines in HBeAg and HBcrAg (surrogate markers of cccDNA)

Table 2. Progression of Viral Markers in HBV NrtI-Suppressed Patients (Patients Treated 16–60 Weeks with ABI-H0731 + NrtI in Study 201/211)

Parameter	Patients, n (%)
Combination Treatment ≥40 weeks	27 (100)
ALT ≤40 U/L	25 (93)
DNA TND (<5 IU/mL)	23 (85)
pgRNA <35 U/mL	19 (70)
DNA TND + pgRNA <35 U/mL	18 (67)
HBeAg <1 IU/mL and/or experienced a >0.5 log decline	14 (52)
HBcrAg <100 kU/mL and/or experienced a >0.5 log drop	9 (33)
HBsAg experienced a >0.5 log drop	1 (4)
DNA TND + pgRNA <35 U/mL + HBeAg <1 IU/mL or ≥0.5 log decline	10 (37)

- Viral markers in these patients receiving long-term NrtI treatment are significantly lower than in Rx-naïve patients, with several approaching the LLOQ
- Results are supportive of mixed source (cccDNA and integrants) HBsAg in long-term HBeAg-negative and NrtI-suppressed patients that appears different than other viral antigens, similar to prior reports[4,5]

Figure 7. Study 202/211 Individual Patients



- Individual patient profiles showing DNA and pgRNA declines on left y-axis and HBV antigen declines on right y-axis

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Results – Safety

Table 3. Final Summary of Safety Findings in Studies 201 and 202

Preferred Term, n (%)	24-Week Controlled Period			
	Rx-Naïve Patients (202)		Nrtl-Suppressed Patients (201)	
	ABI-H0731 + NrtI (N=13)	NrtI (N=12)	ABI-H0731 + NrtI (N=45)	NrtI (N=28)
Any Treatment-Emergent AE	7 (53.8)	5 (41.7)	25 (55.6)	9 (32.1)
Grade 1	6 (46)	4 (33)	17 (37.8)	6 (21.4)
Grade 2	1 (8)	1 (8)	8 (17.8)	2 (7.1)
Grade 3	0	0	0	1 (3.6)
Any Serious AE	0	0	0	0
Rash[a]	0	0	5 (11.1)	0
Upper Respiratory Tract Infection	1 (7.7)	1 (8.3)	5 (11.1)	1 (3.6)
Fatigue	0	0	1 (2.2)	1 (2.2)
Nausea	0	0	4 (8.9)	0
Pruritis	2 (15.4)	0	3 (6.7)	0
Headache	2 (15.4)	0	3 (6.7)	0

[a]5 patients receiving ABI-H0731 + NrtI reported a rash; 4 Grade 1 and 1 Grade 2; no systemic signs or laboratory abnormalities were observed and all patients continued treatment through Week 24

- ABI-H0731 was well-tolerated when administered with a NrtI for 24 weeks
- Overall, 26/58 subjects reported no AEs. Of the 32 subjects reporting ≥1 AE, 23 had Grade 1, and 9 had Grade 2 events. No serious AEs were reported
- With longer-term treatment in Study 211, the safety and tolerability profile is similar to the initial Week 24 placebo-controlled period

Table 4. Laboratory Abnormalities in Studies 201 and 202

Parameter, n (%)	24-Week Controlled Period					
	ABI-H0731 + NrtI (N=58)			NrtI (N=40)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
ALT (SGPT)	3 (5.2)	3 (5.2)	0	5 (12.5)	4 (10.0)	0
AST (SGOT)	4 (6.9)	2 (3.4)	0	6 (15.0)	3 (7.5)	0
Creatinine	0	0	0	2 (5.0)	0	0
Serum amylase	8 (13.8)	3 (5.2)	0	2 (5.0)	4 (10.0)	0
Serum glucose	8 (13.8)	1 (1.7)	0	10 (25.0)	2 (5.0)	0
Serum glucose decreased	1 (1.7)	1 (1.7)	0	3 (7.5)	0	0
Serum sodium	2 (3.4)	0	0	3 (7.5)	0	0
Serum uric acid	3 (5.2)	0	0	4 (10.0)	0	0
Urine blood	1 (1.7)	4 (6.9)	0	2 (5.0)	2 (5.0)	0

ALT, AST: Grade 1: 1.25 to <2.5 × ULN; Grade 2: 2.5 to <5.0 × ULN
Amylase Grade 1: 1.1 to <1.5 × ULN; Grade 2: 1.5 to <3.0 × ULN

- Overall, laboratory abnormalities were of Grade 1 or 2 severity and occurred in similar proportions of patients across the two treatment groups
- With longer-term treatment in Study 211, the profile of laboratory abnormalities are similar to those at Week 24
- Grade 3 elevations in ALT and/or AST have been observed in 3 patients treated with ABI-H0731 + NrtI beyond Week 24
  - In 2 patients, elevations were transient and normalized within a 4–8-week period while continuing on treatment
  - In 1 patient ALT and AST fluctuated during treatment and were asymptomatic and Grade 3 (217 U/L) and Grade 2 (145 U/L), respectively at Week 52 of combination therapy
  - All 3 patients remain on treatment

Conclusions

- The combination of ABI-H0731 + NrtI demonstrated faster and greater reductions in viral nucleic acid levels than NrtI therapy alone, with "DNA TND" and "pgRNA <35 U/mL" thresholds only being achieved in patients receiving ABI-H0731 + NrtI
- Long-term treatment with ABI-H0731 + NrtI results in continued deep reductions in HBV DNA and pgRNA as measured by high sensitivity PCR assays
- Second phase declines in pgRNA (>3 logs), a primary surrogate marker of cccDNA, were strongly associated with reductions in viral antigens, suggesting declining cccDNA pools
- ABI-H0731 is well-tolerated when chronically administered in combination with NrtI and no serious adverse events have been reported to date
- Treatment-emergent adverse events and laboratory abnormalities associated with ABI-H0731 + NrtI were generally Grade 1 or Grade 2 in severity and resolved without treatment interruption

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