Hepatitis B virus core protein variant profiles observed in chronic hepatitis B patients treated with capsid inhibitor AB-836

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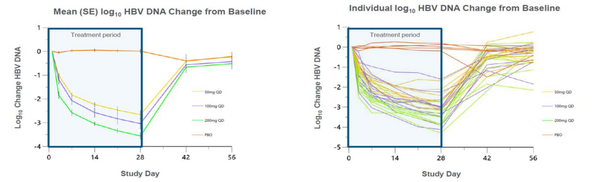
BACKGROUND

Current treatments for chronic hepatitis B virus (HBV) infection are limited to nucleos(t)ide analogues (NA) or pegIFN alfa which have low cure rates and often necessitate life-long treatment. AB-836 is an oral, pan-genotypic, CAM-E (empty) capsid assembly modulator that obstructs HBV pre-genomic RNA (pgRNA) encapsidation by binding to HBV core protein and accelerating capsid assembly. Previous *in vitro* analysis revealed core protein variants T33N and I105T show decreased sensitivity to AB-836 with EC_{50} fold changes of 64.8 and 8.3, respectively[1].

Data from a first-in-human clinical study (AB-836-001) show AB-836 resulted in robust mean HBV DNA declines from baseline of 2.66 log_{10} (Cohort F, 50 mg), 3.04 log_{10} (Cohort G, 100 mg), and 3.55 log_{10} (Cohort H, 200 mg), respectively, with no on-treatment rebound of viral titers (Figure 1)[2]. Clinical Development of AB-836 has been discontinued due to safety findings.

Figure 1 HBV DNA Mean and Individual Log_{10} Change from Baseline for Subjects Enrolled in AB-836-001[2]



OBJECTIVES

- Report *in vitro* HBV fitness and AB-836 activity against an expanded panel of core protein variants
- Report on prevalence of core protein variants from placebo and on-treatment subjects
- Explore regional and genotypic prevalence of observed HBV core variants

MATERIALS AND METHODS

- Viral fitness and AB-836 activity were determined using a cell-based *in vitro* assay. Mutations were introduced by site-directed-mutagenesis into a genotype D HBV replicating plasmid and transfected into HepG2 cells
- DNA extracted from plasma of CHB subjects enrolled in Part 3 of clinical study AB-836-001 (Figure 2) was subjected to HBV-specific PCR amplification followed by Illumina MiSeq next generation sequencing (NGS)
- Prevalence and frequency of HBV core variants at 31 amino acid sites located in and proximal to the AB-836 binding site were evaluated
- For variant calling, NGS data were compared against genotype-specific references: Genbank accession numbers: X02763 (GtA), AB219428 (GtB), GQ924620 (GtC), AF121240 (GtD), AB106564 (GtE), AY090458 (GtF), AF160501 (GtG), FJ356716 (GtH), and EU833891 (GtI)

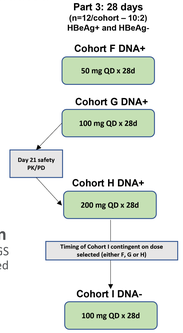


Figure 2 AB-836-001 clinical study design
Schematic of Part 3 of clinical study AB-836-001. NGS analysis was conducted on plasma from subjects enrolled in cohorts F, G, H, and I.

RESULTS

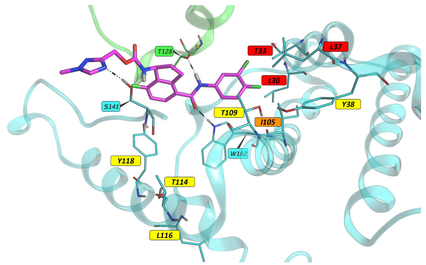
Table 1 *In vitro* Potency of 2nd and 3rd Generation CAM-E Inhibitors Against Wildtype and Core Protein Variants

Core Protein Variant	Fitness (%)	3rd Generation AB-836		2nd Generation AB-506
		EC_{50} µM	Fold Change	Fold Change
Wildtype	100	0.012-0.019 ±0.003-0.002	-	-
D29G	44	0.047 ±0.009	2.5	ND
L30F	27	0.056 ±0.006	**4.7**	**8.0**
T33N	79	0.777 ±0.091	**64.8**	**369**
T33S	115	0.028 ±0.007	1.4	2.6
T33Q	144	0.509 ±0.094	**42.4**	**223**
L37Q	66	0.220 +0119	**20.8**	**16.7**
Y38F	110	0.013 ±0.004	1.1	1.7
Y38H	103	0.009 ±0.002	0.5	0.5
I105T	56	0.099 ±0.044	**8.3**	**19.9**
I105V	98	0.015 ±0.006	1.3	1.4
T109M	46	0.024 ±0.012	2	1.9
T109I	ND	0.007 ±0.003	0.4	ND
T109S	43	0.027 ±0.006	1.4	2.8
T114I	74	0.023 ±0.002	1.2	2.4
Y118F	16	0.009 ±0.001	0.8	ND
Y132F	71	0.004 ±0.002	0.2	0.5
Y38F_T109S	ND	0.020 ±0.006	1	ND

AB-836 mean EC_{50} values for intracellular rcDNA inhibition (bDNA assay, n≥3 ±SD). HBV core variant fitness and EC_{50} fold change relative to wildtype. ND = Not Determined

- AB-836 is more potent against L30F, T33N/Q, L37Q, and I105T core variants than 2nd generation CAM-E inhibitor AB-506
- Amino acid changes at sites D29, Y38, T109, T114, Y118, and Y132 remain sensitive to AB-836

Figure 3 Crystal Structure of HBV Core Protein in Complex with CAM-E Inhibitor



Interaction of HBV core positions S141 (blue), W102 (blue) and T128 (green, adjacent core protein) with AB-506 (pink). Positions resistant to AB-836 and AB-506 are in red. Positions of high variant plasticity from clinical sequencing are in yellow. I105 is characterized by both red and yellow (orange).

Table 2 Available NGS Data from On-treatment or Placebo Subjects Enrolled in Cohorts F, G, and H

Cohort	# Subjects Sequenced	Enrollment Status	Baseline	Day 28	Day 56
F	13	On-Treatment	11[a]	4	9
		Placebo	2	2	2
G	13	On-Treatment	11	6	10
		Placebo	2	2	2
H	11	On-Treatment	10	4	8
		Placebo	1	1	0
# Samples Sequenced			37 / 37	19 / 36	31 / 36

Summary of available NGS data for subjects at Baseline, Day 28, and Day 56. Unable to amplify HBV DNA amplicon from Cohort I, NA-treated subjects.
[a] NGS data was included for one subject at screening where Baseline, Day 28, and Day 56 samples were not available

- Inability to obtain amplicon for sequencing from Day 28 samples coincides with AB-836 treatment and viral titer decline

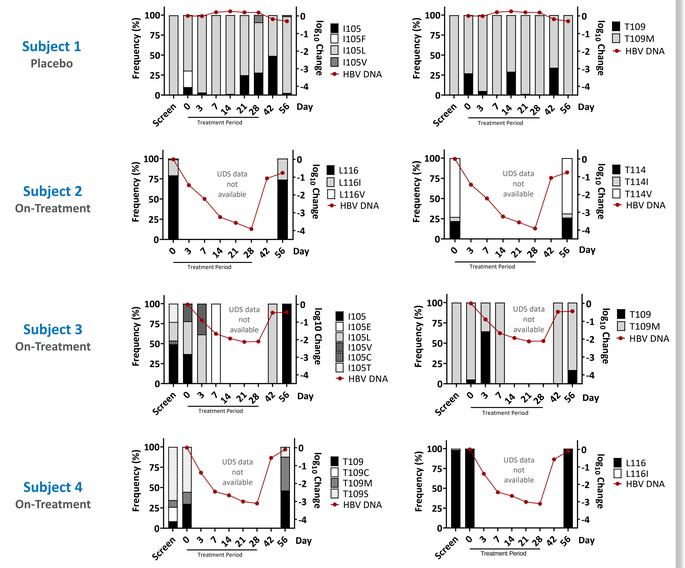
Table 3 Prevalence of HBV Core Variants in AB-836-001 Subjects at Baseline, Day 28 (End of Treatment), and Day 56 (Follow-up)

AA location	Amino Acid Change	Baseline, N=37		Day 28 (End of Treatment), N=18			Day 56 (Follow-up), N=31			HBVdb Prevalence (%)
		# subjects at Baseline	Prevalence (%)	Placebo (N=5)	Active (N=13)	Prevalence (%)[a]	Placebo (N=5)	Active (N=26)	Prevalence (%)[a]	
Y38	Y38F	7	**18.9**	1		**5.6**	1	2	**9.7**	2.5
	Y38H	4[b]	**10.8**			**0.0**	1	1	**6.5**	0.9
I105	I105F	1	**2.7**			**0.0**			**0.0**	0.1
	I105N		**0.0**		1	**5.6**			**0.0**	0.0
	I105L	2	**5.4**	1		**5.6**	1	2	**9.7**	0.7
	I105V	2	**5.4**	2		**11.1**	1		**3.2**	1.2
	I105T		**0.0**			**0.0**			**0.0**	0.7
T109	T109M	4	**10.8**	1	1	**11.1**	1	3	**12.9**	0.7
	T109S	1	**2.7**			**0.0**		1	**3.2**	0.2
T114	T114A		**0.0**		1	**5.6**		1	**3.2**	0.1
	T114I	3	**8.1**	1		**5.6**	1	3	**12.9**	0.5
	T114V	5	**13.5**			**0.0**		3	**9.7**	1.4
I116[c]	I116L	3	**8.1**		2	**11.1**		3	**9.7**	75.7
	I116V	1	**2.7**		1	**5.6**		1	**3.2**	1.5
	L116I	5	**13.5**	1		**5.6**		5	**16.1**	19.2
	L116V	2	**5.4**			**0.0**			**0.0**	1.5
Y118	Y118F	1	**2.7**	1		**5.6**	1		**3.2**	0.4

Range of prevalence (%) is shown as a heat map for core variants where frequencies were >15% for at least one subject. % Prevalence = (# subjects with core variant / total # subjects with available sequence, N). % Frequency = (number of variant reads / total NGS reads). Prevalence of core variants present in the HBVdb database are shown.
[a] Some variants observed in subjects at Baseline were not detected at Day 28 or Day 56 due to HBV DNA amplification failure
[b] NGS data included from 1 "screening" sample where Baseline, Day 28, and Day 56 was not available
[c] I116 is wildtype in genotypes D and E. L116 is wildtype in genotypes A, B, and C

- Multiple variants with frequencies >15% were observed at amino acid sites Y38, I105, T109, T114, I116, and Y118
- Variants with known resistant profiles to AB-836 (L30F, T33N, T33Q, L37Q, and I105T) were not observed at frequencies >2% at Baseline, Day 28, or Day 56

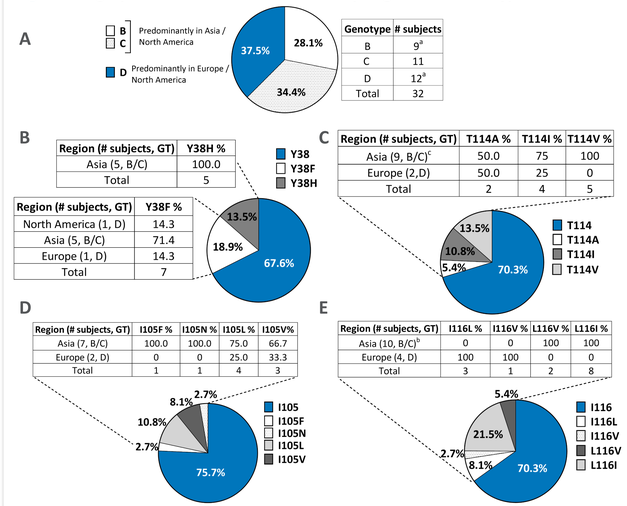
Figure 4 Multiple Core Variants Observed in Placebo or AB-836 Treated Subjects



- Subjects with multiple HBV core variants at positions T109, T114, and L116 at baseline showed HBV DNA declines of >3 log_{10} by Day 28 (end of treatment)
- In Subject 3 where HBV DNA declines reached 2.1 log_{10} by Day 28, fluctuations of I105 variants were observed between screening and Day 56
- Fluctuations of variant prevalence at sites Y38*, I105, T109, T114*, and Y118* were observed in placebo Subject 1

*Data not shown

Figure 5 Highly Variable HBV Core Variants by Region and Genotype



Percentage of HBV genotypes per region (A). Prevalence of core variants at positions Y38 (B), T114 (C), I105 (D), and I116 (E) are shown in pie charts. Distribution of variants across genotypes is reflected in tables.
[a] Genotype data not available for 5 subjects from which NGS data was available
[b] Includes 1 subject from North America
[c] Genotype data not available for 2 subjects

- Subjects enrolled in this study were predominantly from Asia and Europe with HBV Gts B, C, or D
- Y38F/H, T114I/V, and I105 variants appear to be more prevalent in Gt B/C subjects
- I116L/V variants are associated with Gt D subjects, whereas L116I/V variants associate more closely with Gts B/C

CONCLUSIONS

- *In vitro* analysis of HBV core variants L30F, T33N, T33Q, L37Q, and I105T result in AB-836 EC_{50} fold changes of 4.7, 64.8, 42.4, 20.8, and 8.3, respectively. These sites are proximal to the AB-836 binding pocket within the core protein.
- Multiple variants with frequencies >15% were observed in both placebo and on-treatment subjects. For on-treatment subjects, response to AB-836 treatment was robust and none experienced on-treatment viral rebound.
- HBV core amino acids Y38, I105, T109, T114, I116, and Y118 are sites of high plasticity and appear to be under low conservation pressure.
- Obtaining a better understanding of natural fluctuations of core variants over time in CHB subjects may help inform interpretation of on-treatment effects.

REFERENCES / ACKNOWLEDGEMENTS

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2. E. Gane, et al., Poster SAT-392 at EASL Congress, London, England, 22-26 June 2022
3. N. Mani, et al., Antiviral Res. 2022. DOI: 10.1016/j.antiviral.2021.105211

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