Hepatitis B Virus Core Protein Variants Observed In a First-In-Human Placebo-Controlled Study of a Capsid Inhibitor

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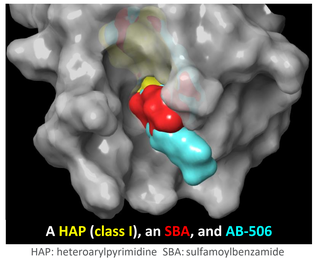
Curing Chronic Hepatitis B

BACKGROUND & AIMS

- Hepatitis B virus (HBV) capsid inhibitors (CI) are being intensively studied as potential components of new combination regimens for the treatment of chronic hepatitis B (CHB) infection
- Current standard-of-care nucleos(t)ide analogs (NAs) do not completely inhibit HBV replication and have limited ability to reduce cccDNA pools in the liver.[1] Additional agents such as CIs will be needed to more effectively shut down the virus.
- AB-506 is an oral, class II, selective HBV CI with pan-genotypic activity and combines effectively with NAs *in vitro*[2]
- Here we describe the prevalence and impact of HBV core protein variants observed in CHB subjects during the AB-506-001 first-in-human trial[3] which has since been halted
- These prevalence findings may also be relevant for other molecules accessing the same binding pocket



HAP: heteroarylpyrimidine SBA: sulfamoylbenzamide

Fig 1: X-ray Crystal Structure of CI Bound to Core Dimer:Dimer Interface

METHODS

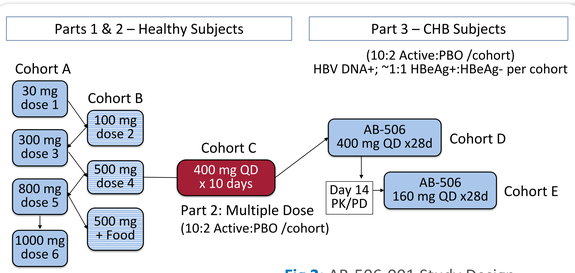


Fig 2: AB-506-001 Study Design

Key Eligibility Criteria for CHB Subjects:

- Healthy males or females aged 18 to 65 years
- Documented chronic HBV infection (HBsAg+ >6mon + negative HBcAb-IgM)
- HBV-DNA ≥2,000 IU/mL (HBeAg-neg) or ≥20,000 IU/mL (HBeAg-pos)
- HBsAg ≥250 IU/mL
- HBV genotype A, B, C, or D
- No evidence of cirrhosis, advanced fibrosis or HCC (Fibroscan, ultrasound)
- ALT or AST ≤5 × upper limit of normal (2016 AASLD criteria for ALT)

HBV DNA Sequencing:

- DNA was extracted from pre-treatment plasma collected from the 24 non-cirrhotic, HBeAg+/-, HBV DNA-positive subjects enrolled in AB-506-001 and an additional 28 subjects that were screened but not enrolled in the study
- Extracted DNA samples were subjected to HBV-specific PCR amplification followed by Illumina MiSeq next generation sequencing (NGS)
- NGS data were compared against genotype specific references (Genbank accession nos. X02763 (gtA), AB219428 (gtB), GQ924620 (gtC), AF121240 (gtD)); only select core protein variants of interest are reported here
- Variants were considered present if frequency was > 5%

RESULTS

Table 1: CHB Subject Baseline Characteristics

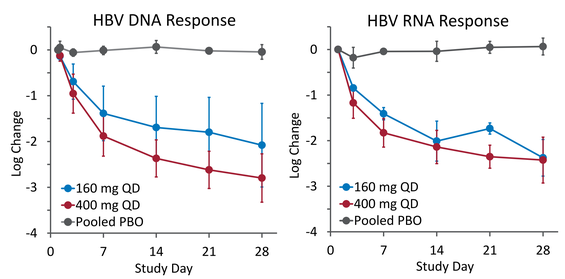
Baseline Measure		Cohort D 400 mg QD (N=10)	Cohort E 160 mg QD (N=10)	Pooled Placebo (N=4)
Age (years) [Mean (SD)]		41.7 (9.5)	41.3 (12.4)	40.8 (9.3)
Male Gender [n (%)]		5 (50)	5 (50)	0
Race [n, (%)]	Asian	8	5	2
	White	1	5	2
	Pacific Islander	1	0	0
Genotype [n, (%)]	A	0	0	0
	B	2	0	0
	C	7	5	2
	D	1	5	2
HBV eAg Positive [n, %]		3	7	2
ALT (U/L) Mean (SD)]		37.1 (20.3)	27.9 (17.2)	28.1 (11.6)
HBV DNA (Log_{10} IU/mL) [Mean (SD)]		6.99 (2.11)	5.21 (1.43)	5.40 (2.18)
HBV RNA (Log_{10} IU/mL) [Mean (SD)]		5.90 (2.12)	4.68 (1.29)[a]	5.37 (1.99)[b]
HBsAg (Log_{10} IU/mL) [Mean (SD)]		4.23 (0.66)	3.62 (0.56)	3.52 (0.60)

[a] 3 subjects Target Not Detected (TND); [b] 2 subjects TND

Table 2: Log_{10} Change from Baseline at Day 28/End of Treatment (EOT)

HBV Parameter (Log_{10} IU/mL) [Mean (SD)]	Cohort D 400 mg QD[a] HBeAg+ [N=7]	HBeAg- [N=3]	ALL [N=10]	Cohort E 160 mg QD HBeAg+ [N=3]	HBeAg- [N=7]	ALL [N=10]	Pooled Placebo ALL [N=4]
HBV DNA	-2.9 (0.58)	-2.5[b] (0.23)	-2.8 (0.57)	-2.2 (0.39)	-2.0 (1.1)	-2.1 (0.91)	-0.045 (0.16)
HBV RNA	-2.4 (0.50)	All[c] <LLOQ	-2.4 (0.50)	-2.5[d] (0.54)	-2.22[e]	-2.37 (0.40)	0.066 (0.19)
HBsAg	0.116 (0.208)	0.107 (0.001)	0.113 (0.176)	-0.0213 (0.029)	-0.0214 (0.082)	-0.0213 (0.069)	0.006 (0.07)

[a] 2 subjects discontinued (DC) for ALT excluded; [b] 1 subject <LLOQ; [c] 1 <LLOQ at baseline; [d] N=2 (1 <LLOQ by Day 28); [e] N=1 (5 <LLOQ at baseline, 1 <LLOQ by Day 28)



NOTE: Grade 4 ALT Subjects in Cohort D (400 mg QD) excluded post-discontinuation at Days 23 and 24

Fig 3: Virological Responses to AB-506 Treatment

AB-506 demonstrated potent inhibition of HBV replication with mean declines in HBV DNA and HBV RNA of 2.8 and 2.4 log_{10}, respectively.

Further development of AB-506 has been discontinued due to observation of reversible ALT increases on treatment in a subset of Asian CHB as well as healthy subjects ≥ Day 14.[4] An immune component of these flares cannot be ruled out.

One CHB subject with ALT flare experienced persistent HBeAg (>2.6 log_{10}) and HBsAg (>2.2 log_{10}) declines from baseline 9-10 months post-flare.[4]

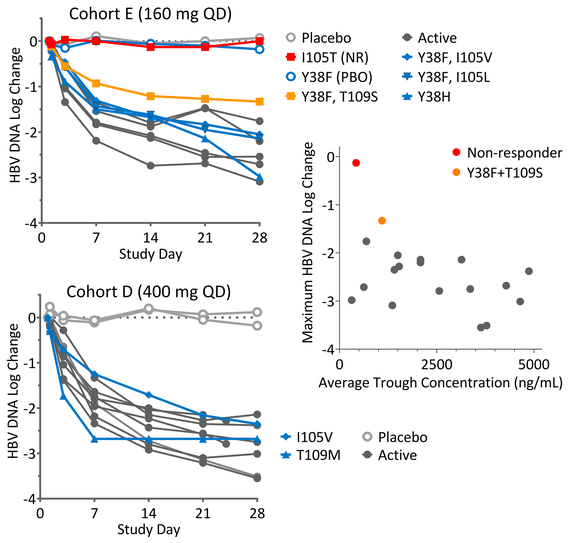


Fig 4: Effect of Pre-existing Core Variants on Individual Responses

- No viral breakthroughs occurred during 28-day monotherapy
- 1 of 20 subjects did not respond to treatment (NR); correlated with pre-existing I105T variant which reduced *in vitro* potency 19.9-fold (Table 4)
- Baseline substitutions at Y38, T109 were also noted in active subjects
- The weakest response (-1.33 log_{10} HBV DNA) aside from the NR was observed in the only active subject carrying Y38F+T109S
- Potency *vs.* variant appears more important than PK in this dose range

Table 3: Frequency of Pre-existing HBV Core Variants in CHB Subjects Recruited to AB-506-001

Core Protein Variant	Screened Subjects (N=52) Placebo (N=4)	Active (N=20)	Not Enrolled (N=28)	Prevalence Observed (%)	HBVdb (%)[a]
T33N	-	-	1	1.9	0.02
T33S	-	-	1	1.9	0.04
Y38F	1	3	9	25	3.1
Y38H	-	1	1	3.8	1.2
I105T	-	1	3	7.7	0.6
I105V	-	2	5	13	1.1
T109M	-	1	2	5.8	0.7
T109S	-	1	1	3.8	0.1
Y118F	-	-	1	1.9	0.4

[a] within 10,975 HBV genome sequences archived as of Sep 12, 2019 at https://hbvdb.ibcp.fr

Additional baseline HBV sequencing of 28 screened subjects, with no known history of receiving any CI, was conducted to better understand the prevalence of I105T and other relevant variants in the clinical CHB population. Some subjects were found to carry multiple of these variants.

NEXT-GENERATION MOLECULES

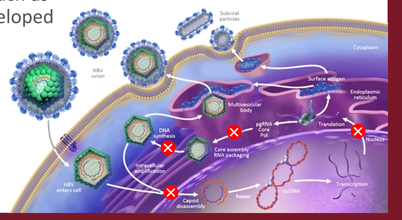
Table 4: *In Vitro* Potency of AB-506 and Next-Generation Molecules Against Wildtype and Core Protein Variants

Core Protein Variant	2nd Generation AB-506 EC_{50} µM	Fold Change	3rd Generation AB-836 EC_{50} µM	Fold Change	4th Generation Representative EC_{50} µM	Fold Change
Wildtype	0.063 ±0.009	-	0.012 ±0.003	-	0.004 ±0.001	-
T33N	23.23 ±1.59	369	0.777 ±0.091	64.8	0.059 ±0.029	14.8
T33S	0.162 ±0.044	2.6			0.003 ±0.001	0.8
Y38F	0.106 ±0.020	1.7	0.013 ±0.004	1.1	0.004 ±0.001	1.0
Y38H	0.032 ±0.005	0.5			0.004 ±0.000	1.0
I105T	1.255 ±0.559	19.9	0.099 ±0.044	8.3	0.007 ±0.004	1.8
I105V	0.087 ±0.027	1.4	0.015 ±0.006	1.3	0.004 ±0.002	1.0
T109M	0.119 ±0.109	1.9	0.024 ±0.012	2.0	0.002 ±0.001	0.5
T109S	0.174 ±0.061	2.8			0.004 ±0.001	1.0

Mean EC_{50} values for intracellular rcDNA inhibition (bDNA assay, n≥3 ±SD) against point mutation in a genotype D transient transfection model.

CONCLUSIONS

- Molecular epidemiology studies are critical to better assess the prevalence of circulating CI-resistant variants
- Next-generation CIs, such as AB-836, are being developed with improved variant coverage
- Multiple intervention modalities are needed to address the complexities of CHB



ACKNOWLEDGEMENTS & REFERENCES

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DISCLOSURES & CONTACT INFORMATION

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