Baseline nucleotide polymorphisms within HBV target site in chronic hepatitis B subjects do not impact HBsAg reductions mediated by RNA interference therapeutic Imdusiran (AB-729)

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AASLD The Liver Meeting® Nov. 10-14, 2023 1459-C

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BACKGROUND

Imdusiran (AB-729) is a subcutaneously administered *N*-Acetylgalactosamine (GalNAc)-conjugated single trigger, pan-genotypic RNAi therapeutic that blocks all HBV RNA transcripts, resulting in suppression of viral replication and production of all viral antigens. In clinical study AB-729-001, multiple dose cohorts show mean HBsAg declines from baseline ranging from 1.8 to 2.6 log_{10} by week 48, end of treatment (Table 1)[1]. Imdusiran is safe, well-tolerated, and is currently in Phase 2 clinical development.

Table 1: Mean (SE) HBsAg log_{10} Change from Baseline for Subjects enrolled in AB-729-001[ab]

Visit Mean (SE)	Cohort E N=7	Cohort F N=7	Cohort I N=6	Cohort J N=7	Cohort K N=7	Cohort G N=7
Baseline	3.51 (0.20)	3.53 (0.17)	3.36 (0.23)	3.37 (0.28)	3.23 (0.14)	3.14 (0.14)
Treatment Week 12	-1.10 (0.15)	-1.02 (0.11)	-1.30 (0.19)	-1.06 (0.31)	-1.63 (0.39)	-1.56 (0.32)
Treatment Week 24	-1.84 (0.16)	-1.57 (0.09)	-1.79 (0.22)	-1.56 (0.25)	-1.99 (0.35)	-1.82 (0.29)
Treatment Week 48	-1.89 (0.18)	-1.90 (0.14)	-1.91 (0.32)	-1.80 (0.41)	-2.57 (0.61)	-2.05 (0.31)

[a] Data shown are for a minimum of 5 subjects/timepoint. Last dose of AB-729: Cohort E: Week 44; Cohorts F, I, G, K: Week 40; Cohort J: Week 36

[b] See Figure 1 for AB-729-001 clinical schematic, multiple dose cohorts (Part 3)

OBJECTIVES

- Characterize and report target site variants in subjects enrolled in AB-729-001 at baseline and variants identified in sequences obtained from the HBVdb (INSERM)
- Identify and assess the prevalence of target site variants across genotypes
- Assess imdusiran activity against target site variants and evaluate viral fitness using an *in vitro* cell model

MATERIALS AND METHODS

- HBV RNA or total nucleic acid extracted from plasma of CHB subjects enrolled in Parts 2 and 3 of clinical study AB-729-001 (Figure 1) was subjected to HBV-specific PCR amplification followed by Illumina MiSeq next generation sequencing (NGS)
- Prevalence and frequency of Single Nucleotide Polymorphisms (SNPs) within the HBV sequence targeted by imdusiran were determined from baseline sequences of subjects enrolled in AB-729-001 or from 8,895 sequences obtained from the HBVdb (INSERM)
- For genotype and 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)
- Sensitivity to imdusiran and variant fitness were determined using a cell-based *in vitro* assay. Single nucleotide changes were introduced by site-directed-mutagenesis into a genotype D HBV replicating plasmid and transfected into HepG2 cells

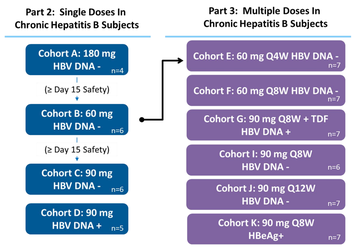


Figure 1: AB-729-001 clinical study design

Schematic of clinical study AB-729-001. NGS analysis was conducted on baseline plasma from subjects enrolled in Parts 2 and 3

RESULTS

Table 2: Conservation of Imdusiran Target Sequence Across Cohorts at Baseline

Cohort	HBV DNA (HBeAg) Status at Baseline	# Baseline Samples with NGS Data	# Subjects with Conserved Target Site Sequence (% Conserved)
A	Negative (HBeAg +/-)[b]	3 of 4	3 (100%)
B	Negative (HBeAg-)	3 of 6	3 (100%)
C	Negative (HBeAg-)	3 of 6	1 (33.3%)
D	Positive (HBeAg-)	5 of 5	5 (100%)
E	Negative (HBeAg-)	3 of 7	2 (66.7%)
F	Negative (HBeAg-)	4 of 7	4 (100%)
G[a]	Positive (HBeAg-)	7 of 7	6 (85.7%)
I	Negative (HBeAg +/-)[b]	4 of 7[c]	4 (100%)
J	Negative (HBeAg +/-)[b]	5 of 7	5 (100%)
K	Negative (HBeAg+)	7 of 7	7 (100%)
	Total	44 of 63 (70%)	40 (90.9%)

Target site variants with total read frequencies ≥15% identified in baseline sequences

[a] Subjects initiated TDF treatment with imdusiran

[b] 1 subject in Cohorts A and I was HBeAg+ and 3 subjects in Cohort J was HBeAg+

[c] NGS data available for 4 subjects, however, 1 subject was removed from study due to protocol deviation

- NGS data was available for 44 of 63 (70%) baseline samples
- Imdusiran target site sequence was conserved in 90.9% of baseline samples analyzed

Table 3: Frequency and Prevalence of Baseline SNPs within the Imdusiran Target Sequence

Target Site Variant	Subject	Cohort	HBV Genotype	Frequency (%)
C1587T	29	C	C	15
T1590A / G	44	E	D	31.2 / 65.8
A1593C	33	C	B	36.1
A1593G	57	G	C	37.0

Variants with total read frequencies ≥15% are shown. Frequency (%) = number of variant reads / total NGS reads.

- C1587T, T1590A/G, and A1593C/G variants were observed at frequencies ≥15% with a prevalence of 2.3%
- Subject 44 had both T1590A and T1590G at frequencies of 31.2% and 65.8%, respectively
- HBV genotype of subjects enrolled in AB-729-001 were: A: 1.6%, B: 13.1%, C: 34.4%, D: 26.2%, E: 1.6%, Not Available: 23%

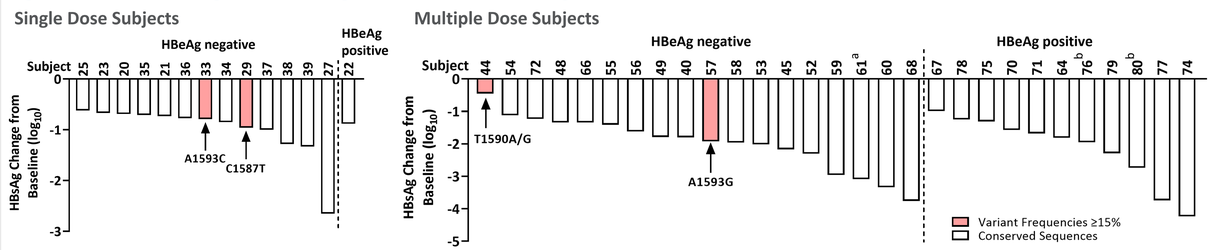
Table 4: Imdusiran HBsAg Activity against Target Site Variants

Nucleotide Position	Target Site Variant	EC_{50} fold change over WT	Nucleotide Position	Target Site Variant	EC_{50} fold change over WT
WT		1.00	1589	C1589A	1.31 ± 0.40
1580	T1580A	0.71 ± 0.29		C1589G	1.93 ± 1.40
1581	G1581A	0.99 ± 0.57	1590	T1590A	1.40 ± 0.31
1582	C1582G	1.06 ± 0.58		T1590G	1.63 ± 1.16
1587	C1587T	0.70 ± 0.08	1591	T1591C	1.84 ± 1.42
1588	G1588A	0.92 ± 0.25	1593	A1593C	2.42 ± 1.38
	G1588C	1.18 ± 0.26		A1593G	1.59 ± 0.94
	G1588T	0.97 ± 0.62			

Fold change of imdusiran HBsAg activity EC_{50} against variant over wildtype was calculated for each independent experiment and average data and standard deviation from N=3 independent experiments is shown

- Wild type and target site variants have similar imdusiran activity with variant HBsAg EC_{50} fold change over wildtype < 3-fold (range 0.71 to 2.42-fold)
- Imdusiran retains *in vitro* activity against T1590A or T1590G variants identified in Subject 44

Figure 2: Individual HBsAg Decline, HBeAg Status, and Imdusiran Target Site SNP Profile

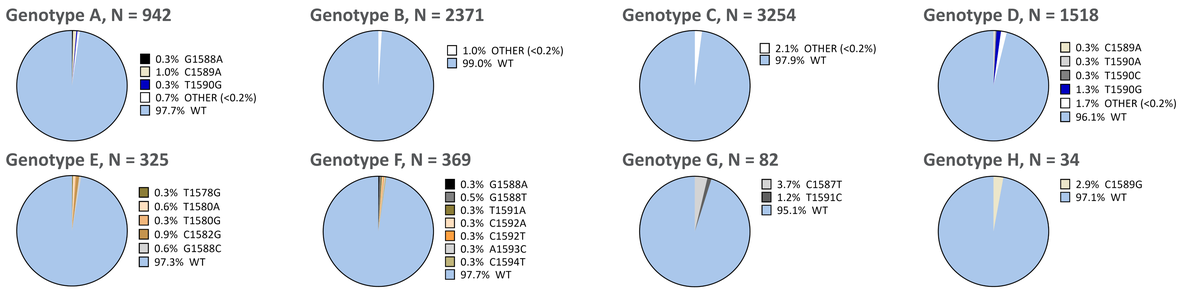


HBsAg decline is shown only for subjects with NGS data enrolled in single or multiple dose cohorts at week 12 or week 48, respectively

[a] log_{10} HBsAg change from baseline measured at week 4 [b] log_{10} HBsAg change from baseline measured at week 40

- For single dose subjects, HBsAg change from baseline at week 12 ranged from 0.62-2.65 log_{10} (N = 12) for subjects with conserved sequence and 0.79-0.96 log_{10} (N = 2) for subjects containing variants
- For multiple dose subjects, HBsAg change from baseline ranged from 0.99-4.24 log_{10} (N = 27) for subjects with conserved sequence and 0.45-1.92 log_{10} (N = 2) for subjects containing variants
- Both T1590A and T1590G variants were observed in Subject 44 where HBsAg reduction from baseline at Week 48 was 0.45 log_{10}. Subject 44 had a max HBsAg reduction of 1.0 log_{10} at Week 28 on treatment
- Subjects with variants C1587T, A1593C, and A1593G had similar HBsAg response to imdusiran as subjects with conserved target site sequence
- Imdusiran mediates similar HBsAg reductions in HBeAg negative and positive subjects

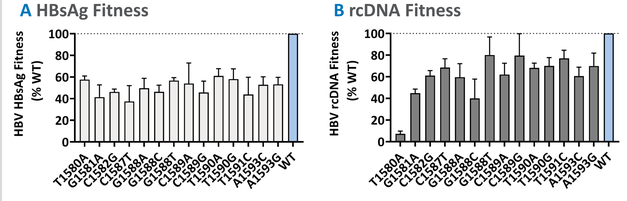
Figure 3: Prevalence and Distribution of Imdusiran Target Site Variants Across Genotypes in the HBVdb



8,895 HBVdb sequences (Genotypes A-H) were analyzed for SNPs in the imdusiran target sequence. Shown are variants with prevalence ≥0.3%. N = total number of sequences analyzed per genotype

- In HBVdb genotype sequences, imdusiran target site sequence is highly conserved (>95%); Genotypes B and C showed highest level of conservation (>98%)
- 9 variants with prevalence ≥0.5% in at least one genotype were identified: T1580A, C1582G, C1587T, G1588C, G1588T, C1589A, C1589G, T1590G, T1591C
- G1588A, C1589A, and T1590G variants were observed in two genotypes with prevalence ≥0.3%
- T1590A/G variants were both observed in HBVdb Genotype D and Subject 44 (Genotype D)
- Imdusiran target site conserved at ≥97.5% in all 9,835 sequences in HBVdb database

Figure 4: Fitness of Target Site Variants



Variant viral fitness was calculated as a percentage of wildtype extracellular HBsAg (CLIA assay) (A) or intracellular rcDNA (bDNA assay) (B)

- Imdusiran target site variants (including T1590A/G observed in subject 44) have reduced fitness relative to wildtype as determined by HBsAg expression (37.5-61%) and rcDNA formation (7.3-80%)
- T1580A mutation results in a premature stop codon in the X gene

CONCLUSIONS

- NGS analysis of AB-729-001 baseline sequences show conservation of the imdusiran target site sequence to be 90.9%
- C1587T, T1590A/G, and A1593C/G variants were identified in baseline samples with frequencies ≥15% in 4 subjects enrolled in AB-729-001. Except for A1593G, these variants were observed in the HBVdb at frequencies >0.3%
- Imdusiran target site is highly conserved between 95.1-99% across genotype sequences present in the HBVdb database
- Imdusiran retains activity against target site variants identified at baseline from clinical subjects and in HBVdb as determined in an HBV cell model

REFERENCES / ACKNOWLEDGEMENTS

1. MF Yuen, *et al.*, Global Hepatitis Summit, Paris, April 25-28, 2023

We would like to thank Elina Medvedeva for statistical support.

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AASLD 2023
10-14 November 2023