# HBV Target Site for the RNA Interference Therapeutic Imdusiran is Highly Conserved in Chronic Hepatitis B Subjects

**Christine L Espiritu, Andrzej Ardzinski, Holly Micolochick Steuer, Varun Sharma, Kevin Gray, Deana Antoniello, Timothy Eley, Karen D Sims, Andrea Cuconati, Angela M Lam, Michael J Sofia, and Emily P Thi**

Arbutus Biopharma, Warminster, PA, USA

AASLD The Liver Meeting® Nov. 15-19, 2024 1391

Arbutus BIOPHARMA *Curing Chronic Hepatitis B*

## BACKGROUND

Imdusiran (AB-729, IDR) is an *N*-Acetylgalactosamine-conjugated small interfering RNA (siRNA) currently being investigated for the treatment of chronic hepatitis B (CHB) in multiple Phase 2 combination studies with pegylated interferon-alfa 2a (AB-729-201, IM-PROVE I)[1] and the immunotherapeutic VTP-300 (AB-729-202, IM-PROVE II)[2]. In a previous study analyzing IDR sequences from subjects enrolled in AB-729-001 (Phase 1a/1b clinical study) and the HBVdb database[3], we showed the following:

- Baseline sequences from subjects enrolled in AB-729-001 had 90.9% IDR sequence conservation
- Target site variants C1587T, T1590A/G, and A1593C/G were identified in 4 subjects. These variants retained sensitivity to IDR *in vitro*
- The IDR target site is highly conserved (95.1-99%) across genotype sequences in the HBVdb database

Here we expand our next generation sequencing (NGS) analysis of the IDR target site to subjects enrolled in IM-PROVE I and IM-PROVE II, providing comprehensive data from over 100 subjects at baseline.

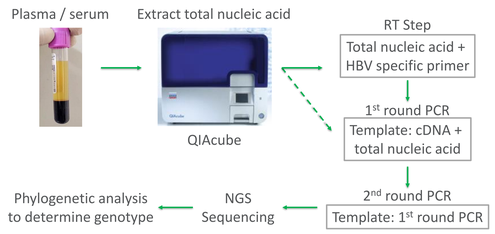
## OBJECTIVES

- Characterize and report target site variants at baseline in subjects enrolled in IM-PROVE I and IM-PROVE II
- Test IDR and nucleos(t)ide analog (NA) activity against target site variants and evaluate viral fitness using an *in vitro* cell model
- Evaluate pan-genotypic activity of IDR in subjects enrolled in clinical studies

## MATERIALS AND METHODS

- HBV RNA or total nucleic acid extracted from plasma of CHB subjects enrolled in clinical studies AB-729-001 (Parts 2 and 3), IM-PROVE I, and IM-PROVE II 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 IDR were determined from baseline sequences
- For genotype and variant identification, 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 1: HBV Genotype Workflow




- Variant fitness and sensitivity to IDR and nucleos(t)ide analogs 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. Extracellular HBsAg was measured by chemiluminescence immunoassay (CLIA) and intracellular rcDNA by branched DNA (bDNA) assay
- An update on the IM-PROVE I clinical study will be presented in Late Breaker Poster 5036[4]
- An update on the IM-PROVE II clinical study will be presented in Late Breaker Poster 5025[5]

## RESULTS

### Table 1: Frequency of Baseline SNPs within the IDR Target Sequence

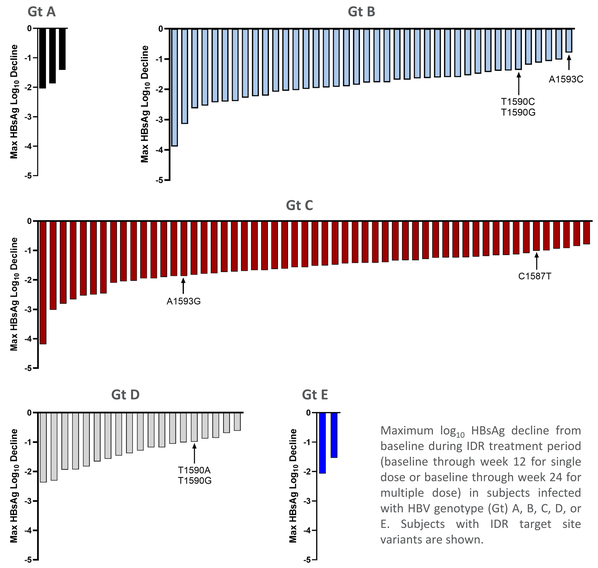
| Study | # Sequences Submitted | # Sequences with NGS data | # Conserved Sequences (% Conserved) | Subject ID | Genotype | Variant (% Frequency) |
|---|---|---|---|---|---|---|
| AB-729-001[a] | 63 | 44 | 40 (90.9) | 29 | C | C1587T (15) |
| | | | | 33 | B | A1593C (36) |
| | | | | 44 | D | T1590A (31) T1590G (66) |
| | | | | 57 | C | A1593G (37) |
| IM-PROVE I | 43 | 20 | 20 (100) | All Subjects conserved | | |
| IM-PROVE II | 62 | 40 | 39 (97.5) | 12 | B | T1590C (23) T1590G (24) |
| Total Subjects with Conserved IDR Sequence | | | 99 / 104 (95.2) | | | |

168 baseline samples were submitted for NGS analysis of the IDR target site; 62% of these samples were successfully sequenced. Target site variants with total read frequencies ≥15% were identified

[a] Data previously reported[3]

- 95.2% of enrolled subjects had conserved IDR target site sequence
- IDR target site variants C1587T, A1593C/G, and T1590A/C/G were observed across 5 subjects infected with HBV genotype B, C, or D
- Variant T1590C was observed together with T1590G in 1 subject enrolled in IM-PROVE II at frequencies of 23 and 24%, respectively
- Prevalence of C1587T, A1593C/G, and T1590A/C variants were each observed to be 0.96%, whereas prevalence of T1590G was 1.9%

### Figure 2: Individual On-Treatment HBsAg Decline in Subjects Across Genotypes A-E




Maximum $log_{10}$ HBsAg decline from baseline during IDR treatment period (baseline through week 12 for single dose or baseline through week 24 for multiple dose) in subjects infected with HBV genotype (Gt) A, B, C, D, or E. Subjects with IDR target site variants are shown.

- IDR has similar activity across genotypes A-E with mean HBsAg $log_{10}$ change from baseline of -1.75 (GtA), -1.78 (GtB), -1.55 (GtC), -1.29 (GtD), and -1.78 (GtE)
- IDR treatment is active against target site variants; HBsAg $log_{10}$ decline ranged from -0.79 to -1.87

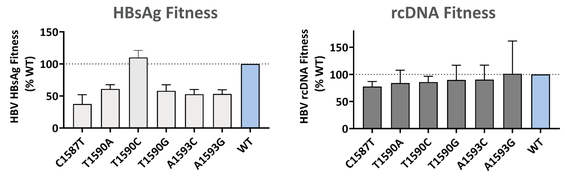
### Table 2: IDR and NA Activity Against Target Site Variants

| Nucleotide Position | Target Site Variant | IDR HBsAg $EC_{50}$ Fold Change over WT | ETV rcDNA $EC_{50}$ Fold Change over WT | TAF rcDNA $EC_{50}$ Fold Change over WT |
|---|---|---|---|---|
| | WT | 1.00 | 1.00 | 1.00 |
| 1587 | C1587T | **0.69** ± 0.08 | **1.84** ± 0.01 | **1.22** ± 0.01 |
| 1590 | T1590A | **1.37** ± 0.31 | **1.71** ± 0.02 | **1.55** ± 0.01 |
| | T1590C | **1.27** ± 0.29 | **0.95** ± 0.00 | **0.94** ± 0.00 |
| | T1590G | **1.37** ± 1.16 | **1.49** ± 0.03 | **1.56** ± 0.01 |
| 1593 | A1593C | **2.05** ± 1.38 | **0.39** ± 0.01 | **0.79** ± 0.01 |
| | A1593G | **1.43** ± 0.94 | **0.86** ± 0.01 | **0.79** ± 0.01 |

IDR HBsAg $EC_{50}$ activity was determined and fold change of variant over wildtype was calculated. Variant fold change of rcDNA $EC_{50}$ was similarly determined for entecavir (ETV) and tenofovir alafenamide (TAF). $EC_{50}$ activity was calculated for each experiment and mean data and standard deviation from N=3 independent experiments is shown.

- IDR activity is similar against wild type and target site variants with variant HBsAg $EC_{50}$ fold change over wildtype <3-fold (range 0.69 to 2.05-fold)
- IDR target site variants remain sensitive to NAs with rcDNA $EC_{50}$ fold change over wild type <3-fold against ETV and TAF

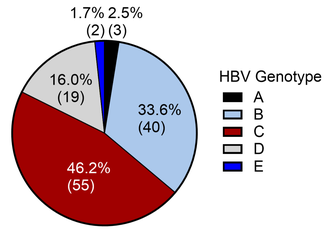
### Figure 3: Fitness of Target Site Variants




Variant fitness was calculated as a percentage of wildtype extracellular HBsAg (A) or intracellular rcDNA (B). Mean and standard deviation from N=3 independent experiments is shown.

- IDR target site variants C1587T, T1590A/G, and A1593C/G have reduced fitness relative to wildtype, whereas T1590C is similarly fit as determined by HBsAg expression
- Viral replication of rcDNA in target site variants range from 77.6% to 101% of wildtype

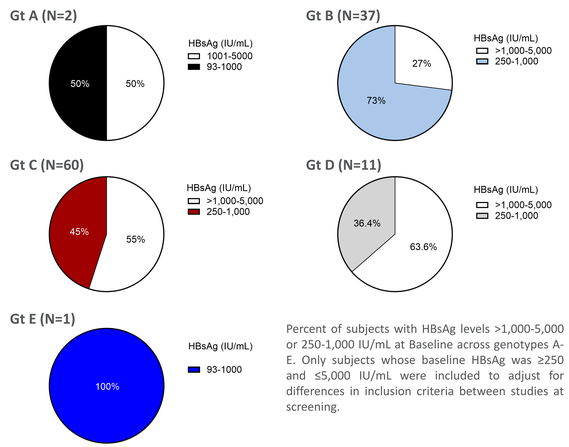
### Figure 4: HBV Genotype Distribution Across Subjects Enrolled in AB-729-001, IM-PROVE I, and IM-PROVE II




Percent of subjects infected with HBV Genotype A-E. Number of subjects per genotype are indicated in parentheses. 166 baseline samples were submitted for genotype analysis by NGS: AB-729-001 (N=61, data pending for 2 subjects), IM-PROVE I (N=43), IM-PROVE II (N=62). PCR amplification failed for 47 subjects; genotype will be assessed by IMMUNIS HBV Genotype EIA

- Majority of subjects enrolled across studies were infected with HBV genotypes B, C, or D

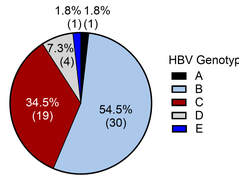
### Figure 5: Baseline HBsAg Levels Across Genotypes




Percent of subjects with HBsAg levels >1,000-5,000 or 250-1,000 IU/mL at Baseline across genotypes A-E. Only subjects whose baseline HBsAg was ≥250 and ≤5,000 IU/mL were included to adjust for differences in inclusion criteria between studies at screening.

- 73% of genotype B subjects had baseline HBsAg between 250-1,000 IU/mL, whereas only 36.4% of genotype D subjects had HBsAg levels in this range
- Distribution of HBsAg baseline levels across genotype C subjects was similar

### Figure 6: Subjects with HBsAg ≤ 1000 IU/mL at Baseline




Overall genotype distribution in subjects with HBsAg ≤1000 IU/mL at baseline. 46.2% (55 of 119) of all subjects for whom genotype information was available had baseline HBsAg ≤1000 IU/mL. Number of subjects with Gt A-E are indicated in parentheses.

- Most subjects with baseline HBsAg ≤1000 IU/mL had genotype B or C

## CONCLUSIONS

- The IDR target site sequence is highly conserved (95.2%) across subjects enrolled in AB-729-001, IM-PROVE I, and IM-PROVE II
- Target site variants C1587T, T1590A/C/G, and A1593C/G were identified in baseline sequences and remain sensitive to IDR treatment
- Nucleos(t)ide analogs maintain activity against IDR target site variants
- IDR variants are similarly or less fit compared to wild type HBV *in vitro*
- Most subjects with baseline HBsAg ≤1000 IU/mL had genotype B or C

## REFERENCES / ACKNOWLEDGEMENTS

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## CONTACT

Emily Thi, ethi@arbutusbio.com
Sr. Director
Immunology and Biomarkers Research

Authors affiliated with Arbutus Biopharma are current or former employees and may own company stock