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Curing Chronic Hepatitis B

Preclinical antiviral profile of AB-836, a potent, highly selective hepatitis B virus capsid inhibitor

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Abstract # OS-595

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DISCLOSURE STATEMENT

The authors affiliated with Arbutus Biopharma are current and former employees and may hold company stock.

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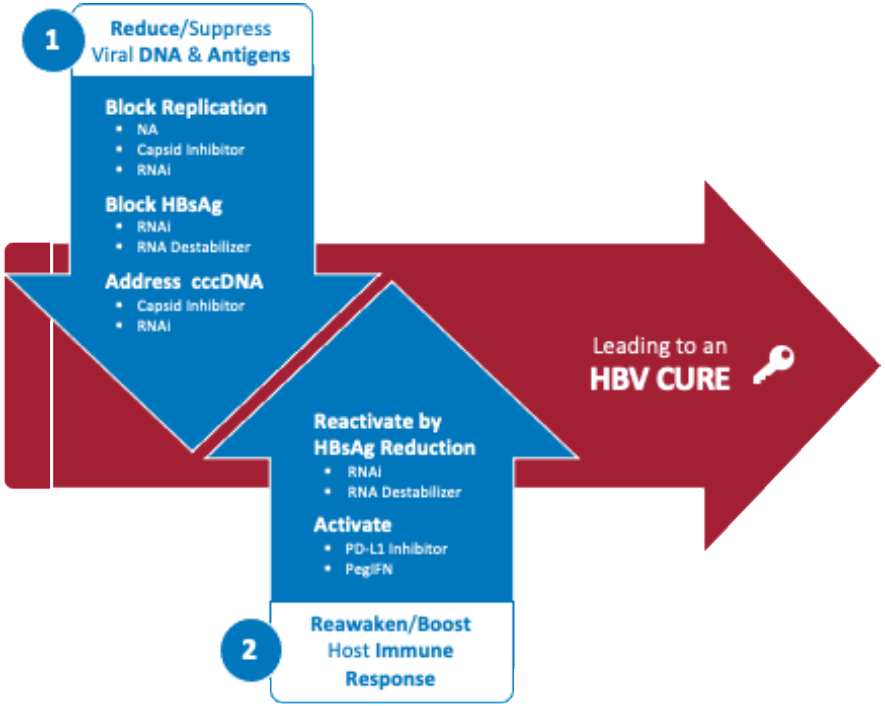
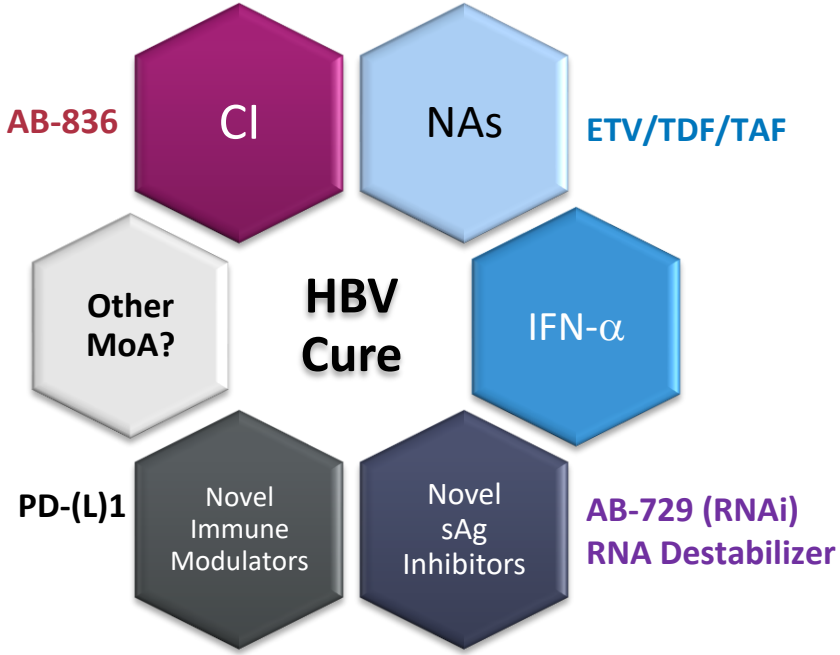
Therapeutic success in CHB: combination is key

Reduce viral DNA and antigens + activate/reactivate immune response





AB-729 EASL presentations: LBO-2764, Posters 2822, 2823, 2829

- Goal: Identify combinations that lead to improved functional cure in CHB patients

CI = capsid inhibitors; NAs = nucleos(t)ide analogs

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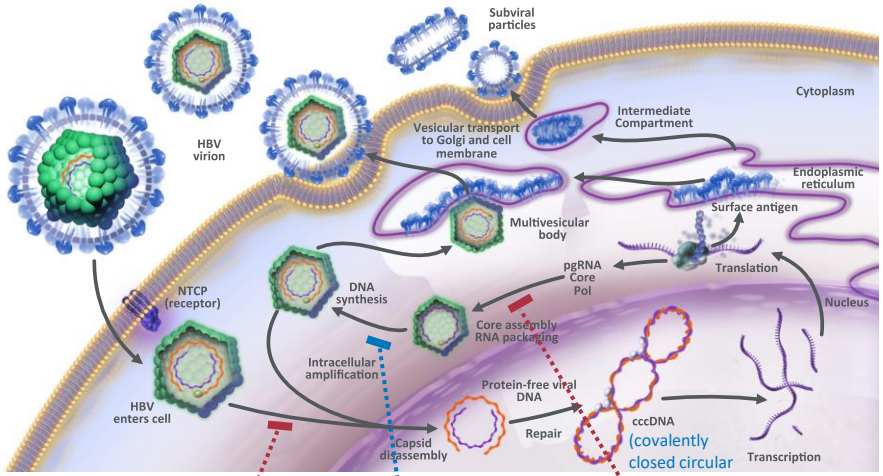
HBV capsid assembly

An attractive target for antiviral drug development

- Interfering with HBV capsid assembly with small molecule inhibitors has been shown to translate into antiviral activity in CHB patients
- Constitutes a novel mechanism that is distinct from the NAs:
  - CIs impact pgRNA encapsidation (primary MoA) but also interfere with proper uncoating of the incoming virion and cccDNA amplification (secondary MoA)
- NAs are “leaky”. Capsid inhibitors can shut down the “leakiness” of NAs in a combination regimen
- Capsid inhibitors may have the potential to play an important role in a curative regimen



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AB-836: a differentiated chemotype

Designed and optimized using structure-based drug design, medicinal chemistry, and SAR

Capsid Inhibitors Evolving Clinical Landscape

Attributes

2015

- Proof of Concept
- Suboptimal Efficacy
- BID; High Pill Burden

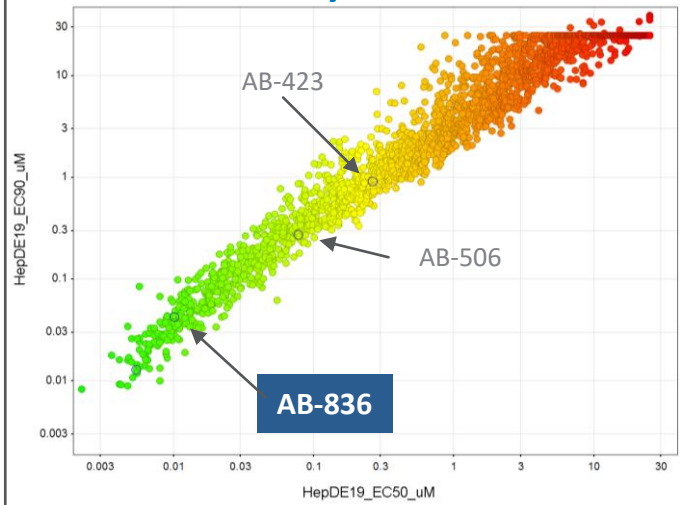
- Improved Efficacy
- QD/ Lower Pill Burden
- Combinations
- Suboptimal Resistance
- Lower potency vs 2nd MoA

2021

- Improved Potency
- QD; Lower Pill Burden
- Improved resistance coverage
- Improved potency vs 2nd MoA
- New Combinations

- Program aimed at discovering potent, chemically diverse capsid inhibitors
- Applies learnings from previous programs to build research target profile for candidate selection
- Novel chemotype designed based on a pharmacophore model derived from X-ray crystallographic data
  - Multiple chemotypes explored
  - Stringent preclinical selection criteria
- AB-836 represents a differentiating profile among internal and external capsid compounds

Discovery of AB-836



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AB-836 is a potent inhibitor of HBV replication *in vitro*

Inhibits rcDNA synthesis and cccDNA establishment in infection systems

- AB-836 originated from a novel chemical series differentiated from other chemotypes

HepDE19 rcDNA EC_{50} ± SD/CC_{50} (µM)	HBV infected Primary Human Hepatocytes		HBV infected HepG2-NTCP-C4		
	rcDNA EC_{50} ± SD/CC_{50} (µM)	sAg EC_{50} ± SD (µM)	rcDNA EC_{50} ± SD/CC_{50} (µM)	sAg EC_{50} ± SD (µM)	cccDNA EC_{50} ± SD (µM)
0.010 ± 0.003/ >25*	0.002 ± 0.0004/ >10**	0.050 ± 0.013	0.012 ± 0.005/ >10**	0.197 ± 0.015	0.175 ± 0.040

* Cell Titer Glo assay for cell viability **GAPDH RNA inhibition

- Demonstrates potent *in vitro* antiviral potency against primary and secondary mechanism
- Modest 2.3x decrease in potency in presence of 40% human serum
- AB-836 showed high degree of selectivity for inhibition of HBV when compared to a panel of RNA and DNA viruses with EC_{50} and CC_{50} of >30 µM involving multiple cell line backgrounds

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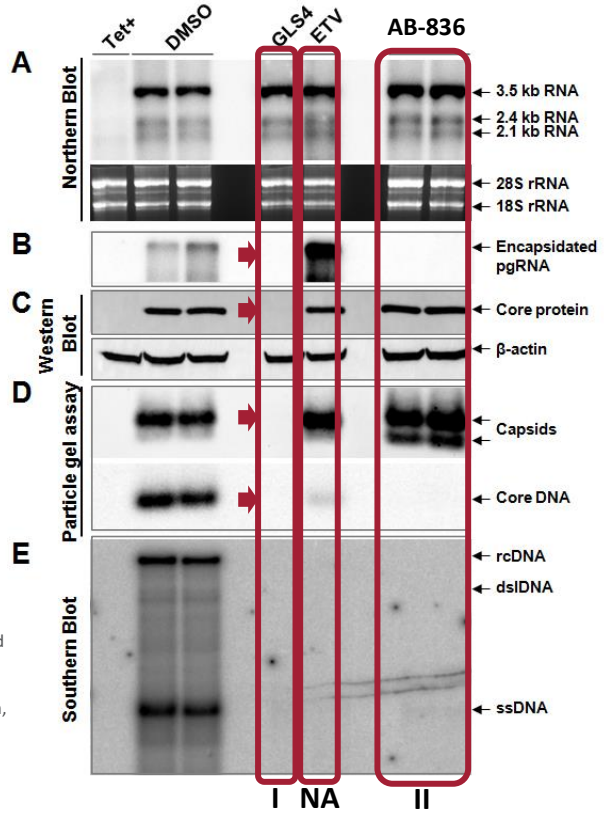
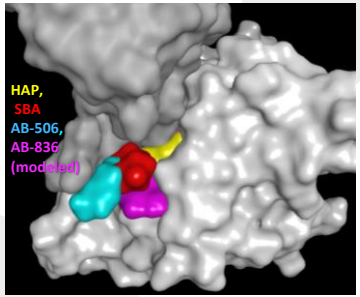
AB-836 inhibits pgRNA encapsidation *in vitro*

AB-836 treatment results in the formation of empty capsids in cells



HepDE19 cells were treated with GLS-4, ETV or AB-836 at 1 μM or DMSO for 6 days. HBV expression products and cellular housekeeping gene products were analyzed using southern, northern, western, and particle gel assays

- Two main classes of capsid inhibitors:
  - Aberrant capsids: e.g., HAPs such as GLS-4
  - Normal but empty capsids: e.g., JNJ-6379
- AB-836 inhibits HBV replication *via* formation of empty capsids
- Differentiated from NAs
- X-ray crystallography data from closely-related compounds confirms that AB-836 binds to the same site as HAPs and SBAs; at the dimer:dimer interface
- Improved potency *via* a unique binding mode
- Binding site highly conserved amongst genotypes of HBV



AB-836 shows pan-genotypic activity

Potent inhibition of HBV DNA in isolates representing HBV genotypes A - H

- Hepatitis B is classified into 10 genotypes (A – J), of which genotypes A to D are the most prevalent, while I and J are very uncommon
- It is important that any new therapy for CHB demonstrates broad genotype coverage
- Using a HepG2 transient transfection system, the antiviral activity of AB-836 was evaluated against cloned sequences representing genotypes A to H
- As shown in the table, AB-836 demonstrated potent inhibition of all tested HBV isolates

HBV GENOTYPE	HBV DNA AVG. EC_{50} ± SD (μM)
A	0.017 ± 0.004
A	0.007 ± 0.001
B	0.004 ± 0.001
C	0.004 ± 0.002
D	0.012 ± 0.003
E	0.066 ± 0.035
E	0.014 ± 0.000
F2	0.006 ± 0.001
G	0.006 ± 0.001
H	0.007 ± 0.001

n ≥ 3 independent determinations

Plasmid DNA HepG2 transient transfection assay

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AB-836 shows potent inhibition of core variants

All variants tested showed sub-micromolar EC_{50} values for replication inhibition (HBV DNA)

HBV Core Variant	AB-836 Avg. EC_{50} ± SD (µM)	AB-506 Avg. EC_{50} ± SD (µM)
WT (GT-D)*	0.012 ± 0.003	0.063 ± 0.018
L30F	0.056 ± 0.006	0.504 ± 0.102
T33N	0.777 ± 0.091	23.230 ± 1.588
T33Q	0.509 ± 0.094	14.061 ± 5.882
L37Q	0.250 ± 0.119	1.050 ± 0.308
Y38F	0.013 ± 0.004	0.106 ± 0.020
I105T	0.099 ± 0.044	1.255 ± 0.559
I105V	0.015 ± 0.006	0.087 ± 0.027
T109M	0.024 ± 0.012	0.087 ± 0.027

*HBV genotype D background for all variants; HBV DNA measured with bDNA assay

HBV Core Variant	AB-836 Avg. EC_{50} ± SD (µM)	AB-506 Avg. EC_{50} ± SD (µM)
WT (GT-D)*	0.019 ± 0.002	0.063 ± 0.018
D29G	0.047 ± 0.009	-
T33S	0.028 ± 0.007	0.162 ± 0.044
Y38H	0.009 ± 0.002	0.032 ± 0.005
T109I	0.007 ± 0.003	-
T109S	0.027 ± 0.006	0.174 ± 0.061
T114I	0.023 ± 0.002	0.148 ± 0.044
Y118F	0.009 ± 0.001	-
Y132F	0.004 ± 0.002	0.032 ± 0.009
Y38F + T109S	0.020 ± 0.006	-

n ≥ 3 independent determinations

- Core variants maintain susceptibility to SOC NAs;
- Conversely AB-836 showed comparable activity against a panel of NA-resistant variants

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AB-836 shows a favorable preclinical PK profile

QD dosing potential in humans: high multiples over the EC_{90} in the liver (24 h post dose)

- Pharmacokinetic properties of AB-836 were assessed in rodent and non-rodent species at doses of 2 mg/kg IV and 10 mg/kg oral
- AB-836 demonstrated low systemic clearance
  - IV clearance decreased with a concomitant increase in half-life from mouse, rat, and monkey
- Oral bioavailability ranged from 30 – 100% with high liver:plasma ratio in rodents
- PK profile projects QD dosing potential in humans

Mouse

Test Cmpd	PO AUC_{inf} (ng/mL*h)	IV CL (mL/min/kg)	IV $T_{1/2}$ (h)	[24 h liver] (ng/mL)	[24 h] liver fold over EC_{90}
AB-836	13,040	**13**	3.1	395	25x

Rat

Test Cmpd	PO AUC_{inf} (ng/mL*h)	IV CL (mL/min/kg)	IV $T_{1/2}$ (h)	[24 h liver] (ng/mL)	[24h] liver fold over EC_{90}
AB-836	5,740	**11**	4.4	334	20x

Monkey

Test Cmpd	PO AUC_{inf} (ng/mL*h)	IV CL (mL/min/kg)	IV $T_{1/2}$ (h)	[24 h liver] (ng/mL)	[24 h] liver fold over EC_{90}
AB-836	6,740	**9**	5.2	ND	ND

ND – Not determined

IV PK was done at 2 mg/kg and PO PK data was done at 10 mg/kg`

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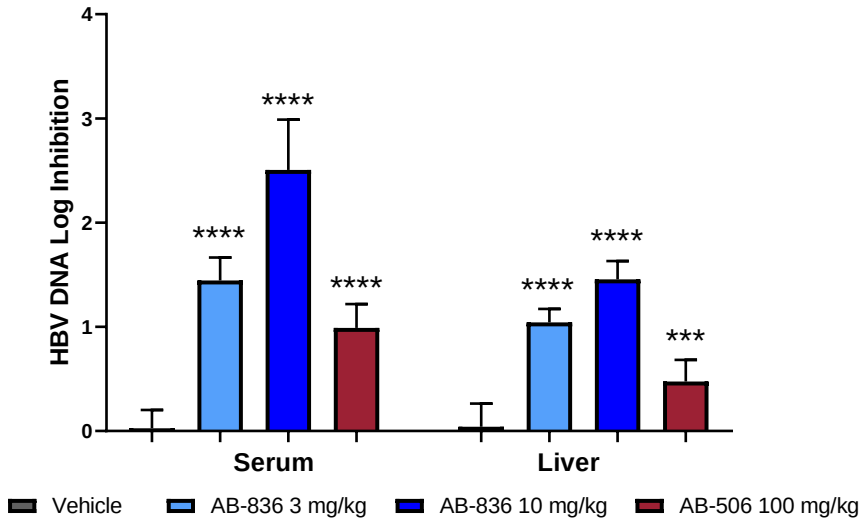
AB-836 shows robust multi-log HBV inhibition *in vivo*

AB-836 reduced HBV DNA in serum and liver of HBV HDI mice (QD, 7 days)

- A hydrodynamic injection (HDI) mouse model of HBV was used to evaluate AB-836
- AB-836 treatment resulted in dose responsive HBV DNA inhibition in serum as well as liver in HDI mice
- Up to 2.5 log_{10} reduction in serum HBV DNA observed when dosed orally at 10 mg/kg once daily for 7 days

  -AB-836 greater than 33× more active *vs.* our prior generation capsid inhibitor

NOD-Scid



Data: Mean ± SD for n=5-6; **** P<0.0001 *vs* Vehicle, One-Way ANOVA with Dunnett's multiple comparisons test

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Conclusions: AB-836 preclinical profile

Chemically differentiated *vs.* AB-506, potent inhibitor, QD dosing potential in humans

- It is a rationally designed chemotype differentiated from other capsid inhibitors
- Shows potent inhibition of both the primary and secondary MoA in cell culture HBV models
- Demonstrates empty capsid formation phenotype devoid of pgRNA and rcDNA: Class II capsid inhibitor
- Engages in a unique electrostatic interaction with core protein inferred from X-ray crystallography studies in comparison to other reported capsid inhibitors
- Has an improved activity profile against a core variant panel *in vitro*
- AB-836 possesses favorable PK properties across rodent and non-rodent species with high liver:plasma ratios and good oral bioavailability
  - PK profile projects QD dosing potential in humans
- Demonstrates robust multi-log reduction of HBV DNA in a HDI mouse model
- AB-836 is currently undergoing Phase 1 clinical trials

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