Development of Second Generation RNA Interference Therapy for Hepatitis B Virus Infection

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AIMS

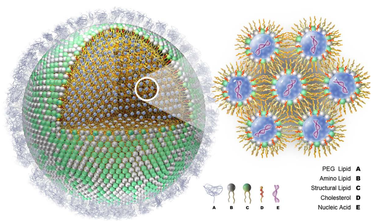
Here we describe ARB-1740, an improved second-generation RNA interference therapeutic for chronic Hepatitis B infection (CHB).

- CHB is a global health challenge with 240 million individuals at risk of serious complications such as liver cirrhosis and cancer
- HBV proteins such as surface antigen (HBsAg) are associated with immune impairment, leading to persistent infection and posing a challenge for development of a functional cure
- ARB-1740 is 3 siRNAs encapsulated in lipid nanoparticles (LNP); the drug utilizes a gene silencing mechanism to destroy HBV RNAs enabling suppression of all HBV antigens and promoting host immune recognition and viral control

METHODS

- **In Vivo Model 1:** Immunodeficient SCID mice produce HBV from genotype D 1.3× overlength plasmid copy that had been administered to the liver via hydrodynamic injection (HDI) (Guidotti 1995, Yang 2002)
- **In Vivo Model 2:** PXB uPA-SCID mice are chronically infected with genotype C virus in humanized livers, with baseline serum HBV DNA ~2-3×10e8 copies/mL and serum HBsAg ~3-4×10e3 IU/mL (PhoenixBio Co., Ltd.)
- RNASeq: Next generation sequencing was conducted on low-dose treated HDI mouse liver RNA (0.03 mg/kg, n=6, pooled) using an Illumina HiSeq platform
- Antiviral activity against different genotypes and NUC-resistant variants was determined using a transient transfection cell culture model
- Drug-drug interaction study was conducted in a HepDE19 cell culture model in checkerboard format and analyzed as per Prichard and Shipman 1990

LNP DELIVERY TECHNOLOGY



Lipid Nanoparticles

protect the siRNA drug against degradation in the bloodstream,

enabling delivery to hepatocytes, the site of HBV replication.

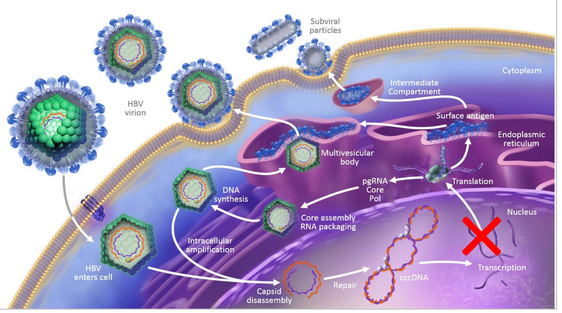
Drug products utilizing Arbutus LNP technology have advanced to Phase 3 trials. With >400 patients treated across multiple disease areas, LNP-enabled siRNA drugs have strong clinical validation, benefiting from proprietary leading-edge nucleic acid drug (siRNA and mRNA) formulation development.

THERAPEUTIC APPROACH

ARB-1740 interferes with HBV life cycle by destroying viral RNAs

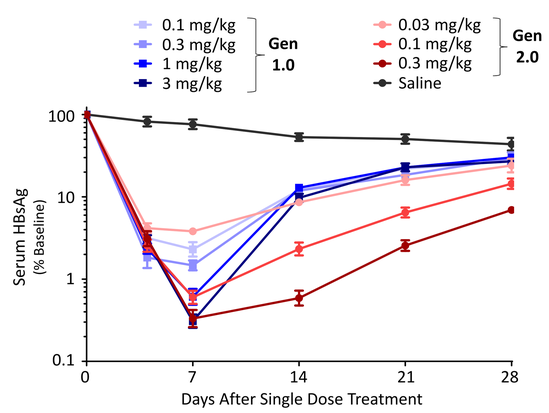
The primary aim of ARB-1740 is to facilitate a functional cure for chronic HBV infection by **reducing the levels of HBsAg in the body**

- HBsAg promotes host immune tolerance of virus
- Removal should promote immune recognition & viral clearance



RESULTS

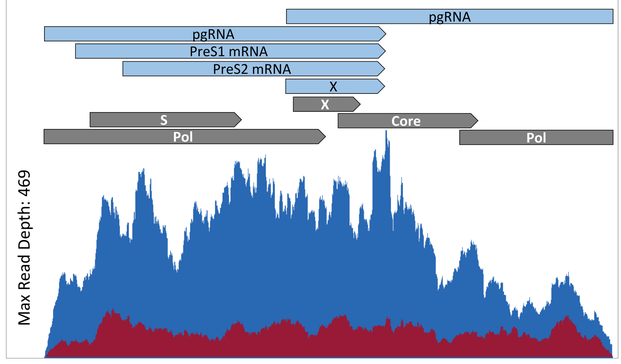
1. Improved Drug Potency and Duration of Activity In Vivo



ARB-1740 is up to 10-fold more potent compared to first generation ARB-1467, with a slower rate of resolution at all dose levels tested (HDI mice, n=5 ± SEM)

2. ARB-1740 Inhibits All HBV RNAs Including HBx Transcript

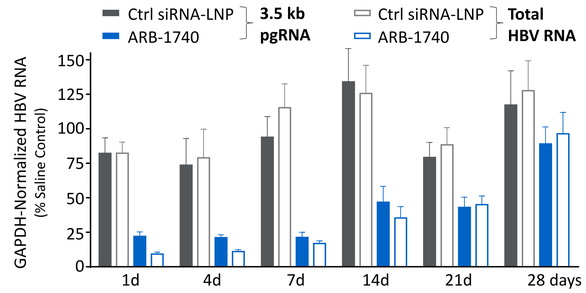
	Total HBV Reads	Reads Normalized to RNA Length		
		3.5 kb RNA	2.4/2.1 kb RNA	0.7 kb RNA
Saline	7372	2280	761	885
ARB-1740	1676	517	124	110



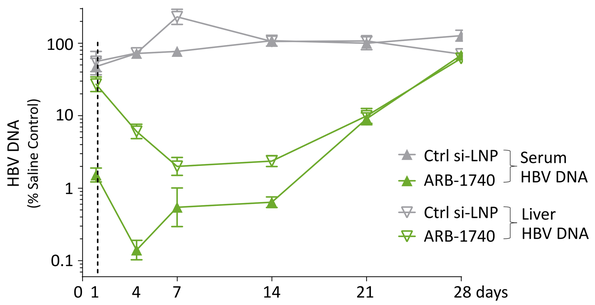
3. ARB-1740 Rapidly Suppresses Multiple Elements of Hepatitis B Throughout the Body

Single dose in HDI mice (n=5 ± SEM) on Day 0

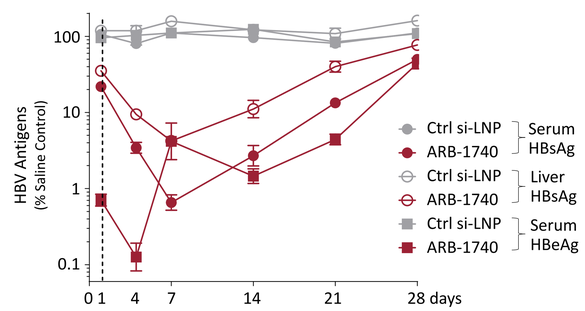
A. ARB-1740 reduces viral RNAs in the liver



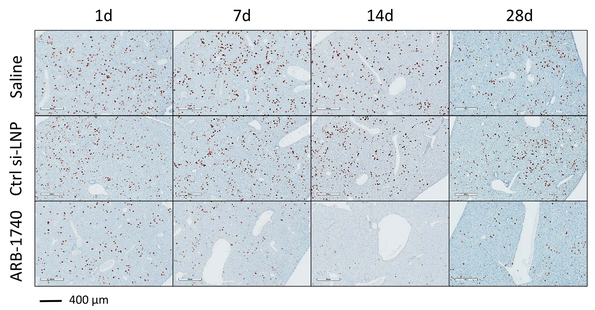
B. ARB-1740 reduces HBV DNA in serum and the liver



C. ARB-1740 reduces HBsAg and HBeAg in serum and the liver

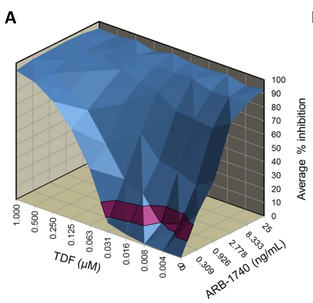


D. ARB-1740 reduces HBV core antigen in the liver



4. ARB-1740 Complements Standard-of-Care NUC Treatment and Inhibits NUC-Resistant Virus Variants

A

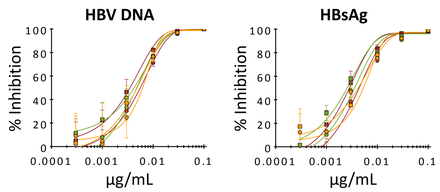


No antagonism observed in ARB-1740 + tenofovir disoproxil fumarate (TDF) drug combination

Intracellular rcDNA measured by bDNA assay (n=3 ± SD)

B

HBV Variant	rcDNA (EC_{50}, ng/mL)			HBsAg (EC_{50}, nM)		
	ARB-1740	ETV	LAM	ARB-1740	ETV	LAM
rtM204I	5.88	--	>100,000	4.18	--	>100,000
rtM204I + V173L	4.38	--	>100,000	4.88	--	>100,000
rtM204I + S202G	3.55	10,700	--	2.62	10,373	--
rtM204I + L180M	4.73	--	>100,000	1.49	--	>100,000
rtM204I + S202G + M250V	5.08	9,042	--	4.44	6,063	--
Wildtype	3.34	1.89	30.63	2.72	>100	>1000



ARB-1740 is equally effective against NUC-resistant variants as it is against wildtype virus.

Unlike approved nucleos(t)ide analogs entecavir (ETV) and lamivudine (LAM), ARB-1740 inhibits viral replication as well as production of viral antigen.

Intracellular encapsidated HBV DNA measured by qPCR, HBsAg by ELISA (n=3 ± SD).

5. Pan-Genotypic Activity of ARB-1740

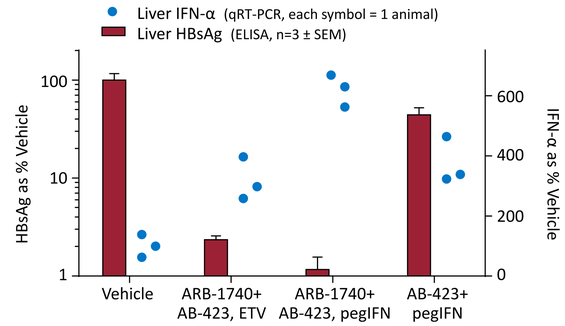
	% Viral Genomes Matched in Each Hepatitis B Genotype								
	A	B	C	D	E	F	G	H	A-H
siRNA 1	99	95	90	97	97	97	99	97	94
siRNA 2	98	99	98	96	97	100	96	65	97
siRNA 3	83	87	95	91	97	95	95	92	92

High match frequencies predicting conservation of activity against all genotypes with >99.9% probability of ≥1 of the 3 siRNAs in ARB-1740 having a complete match to any HBV strain. Core siRNA regions (antisense positions 2-18) were compared against >6000 HBV genomes in public databases.

	HBV DNA (EC_{50}, ng/mL)		HBsAg (EC_{50}, ng/mL)	
	ARB-1740	ETV	ARB-1740	ETV
Genotype A	5.19	0.32	3.90	>100
Genotype A2	4.16	1.27	3.62	>100
Genotype B	4.26	0.50	5.49	>100
Genotype C	3.91	0.78	4.18	>100
Genotype D	5.98	2.62	5.96	>100

ARB-1740 is equally potent against genotypes A-D with sub-nM EC50s. Unlike ETV, it directly inhibits both HBV DNA and HBV surface antigen to equivalent degree.

6. HBsAg Removal Correlates with ↑ Innate Immune Response



Removal of HBsAg by ARB-1740 correlated with gain in human IFN-α expression. This innate immune response in engrafted human hepatocytes of PXB mice was further potentiated by combining ARB-1740 and pegylated interferon treatments. Neither ETV nor capsid inhibitor AB-423 affect HBV antigens (abstracts 232, 233).

CONCLUSIONS

ARB-1740, a second-generation siRNA therapeutic for HBV, has:

- ≥10-fold greater in vivo potency & extended activity duration
- Efficacy against HBsAg derived from cccDNA and integrated DNA
- Pan-genotypic activity; inhibits eAg, core protein & replication
- Complementary function to standard-of-care NUCs which only suppress replication; & inhibits NUC-resistant variant replication
- Provided in vivo support for immune de-repression approach, showing elevations in host immune response markers 2 weeks after end of ARB-1740 dosing, and in combination with pegIFN

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