Controlling HBsAg as a Key Component of a Combination Strategy to Affect an HBV Cure

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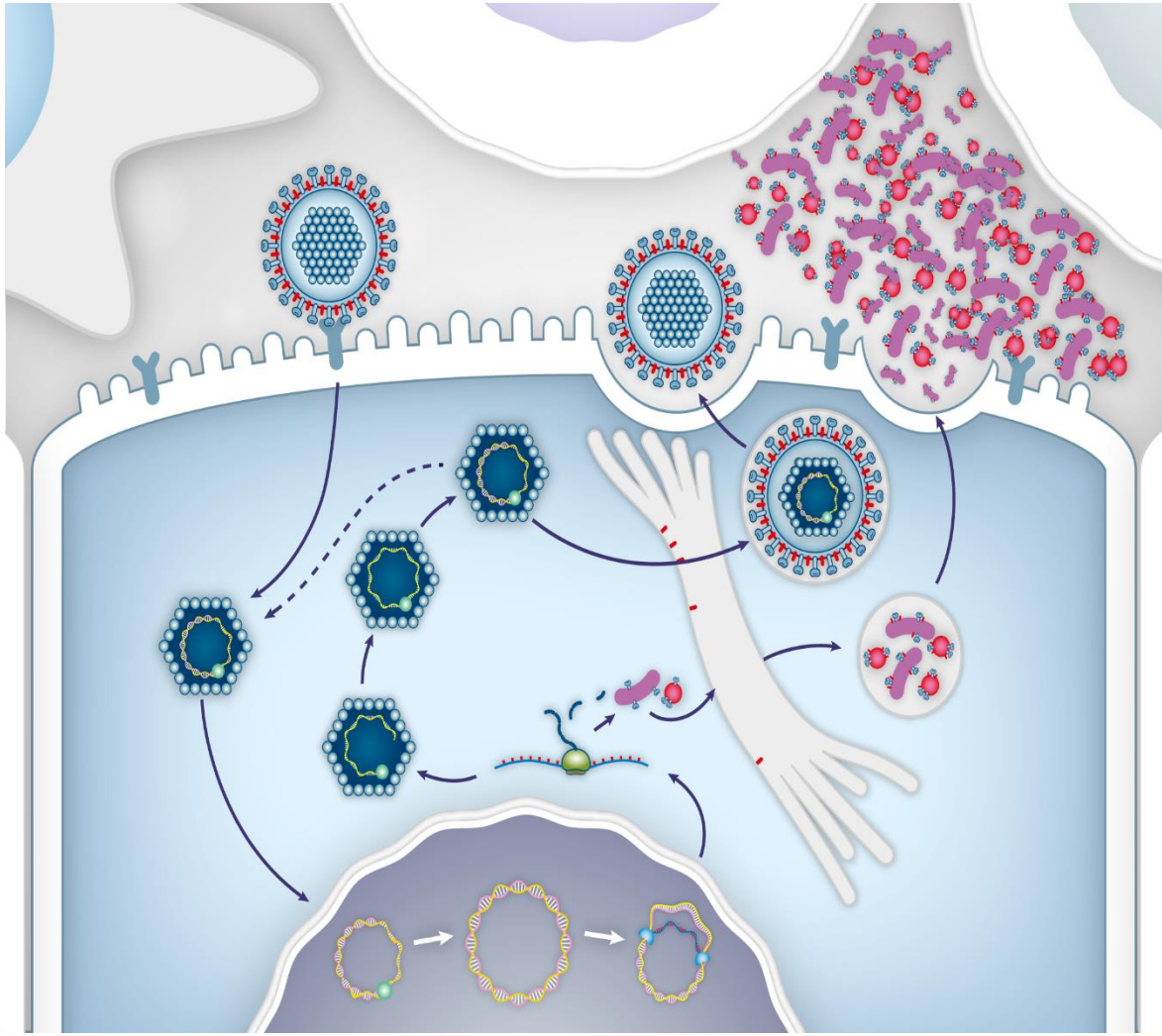
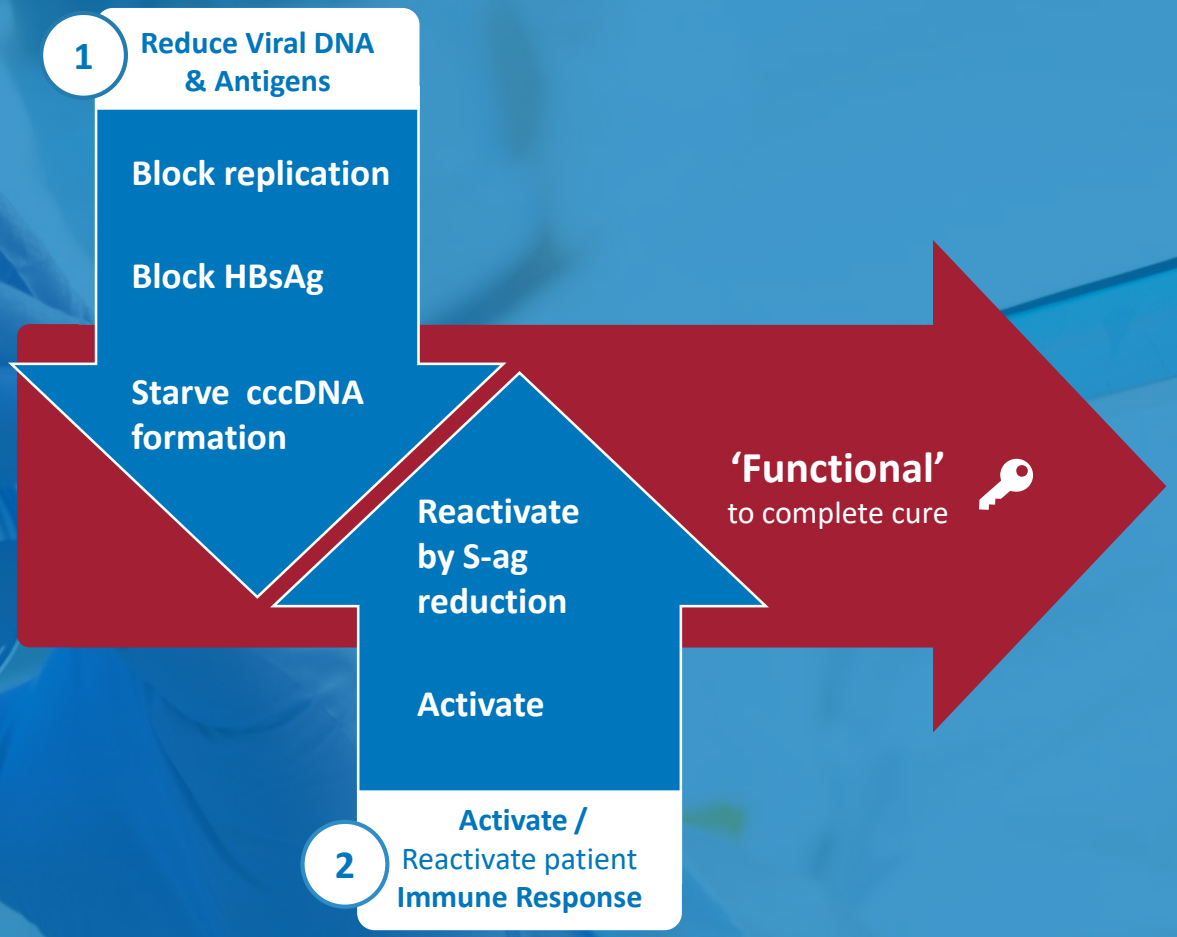
The Hepatitis B Virus

- Up to 10^{13} virons produced per day
- 10,000× more subviral particles produced comprised of surface antigen (HBsAg)
- Hypothesized that the host immune response is attenuated by viral antigens
- Functional cure is associated with loss of viral HBsAg



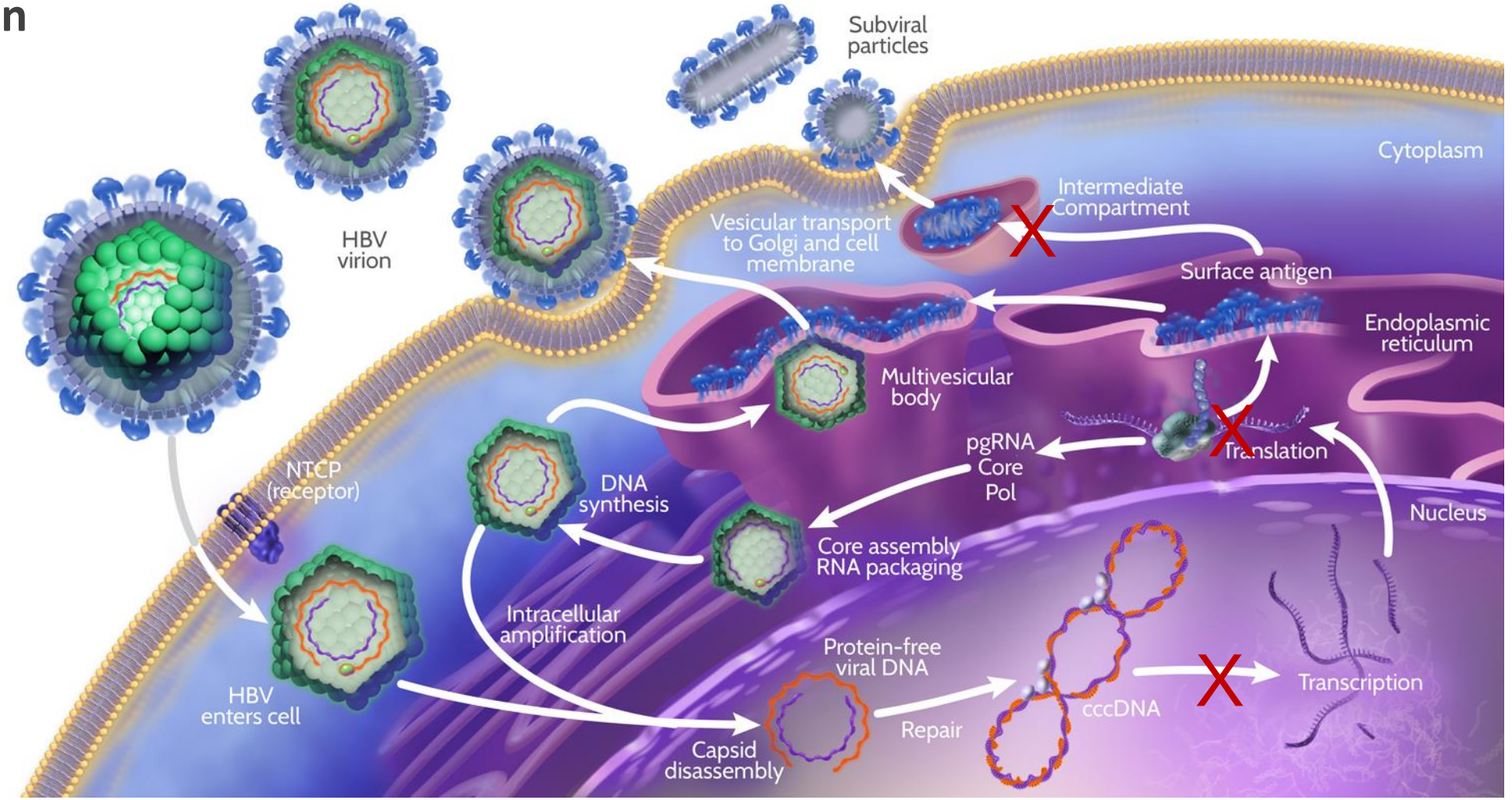
Keys to **Therapeutic Success** in HBV



Arbutus BIOPHARMA

Eliminating S-Antigen

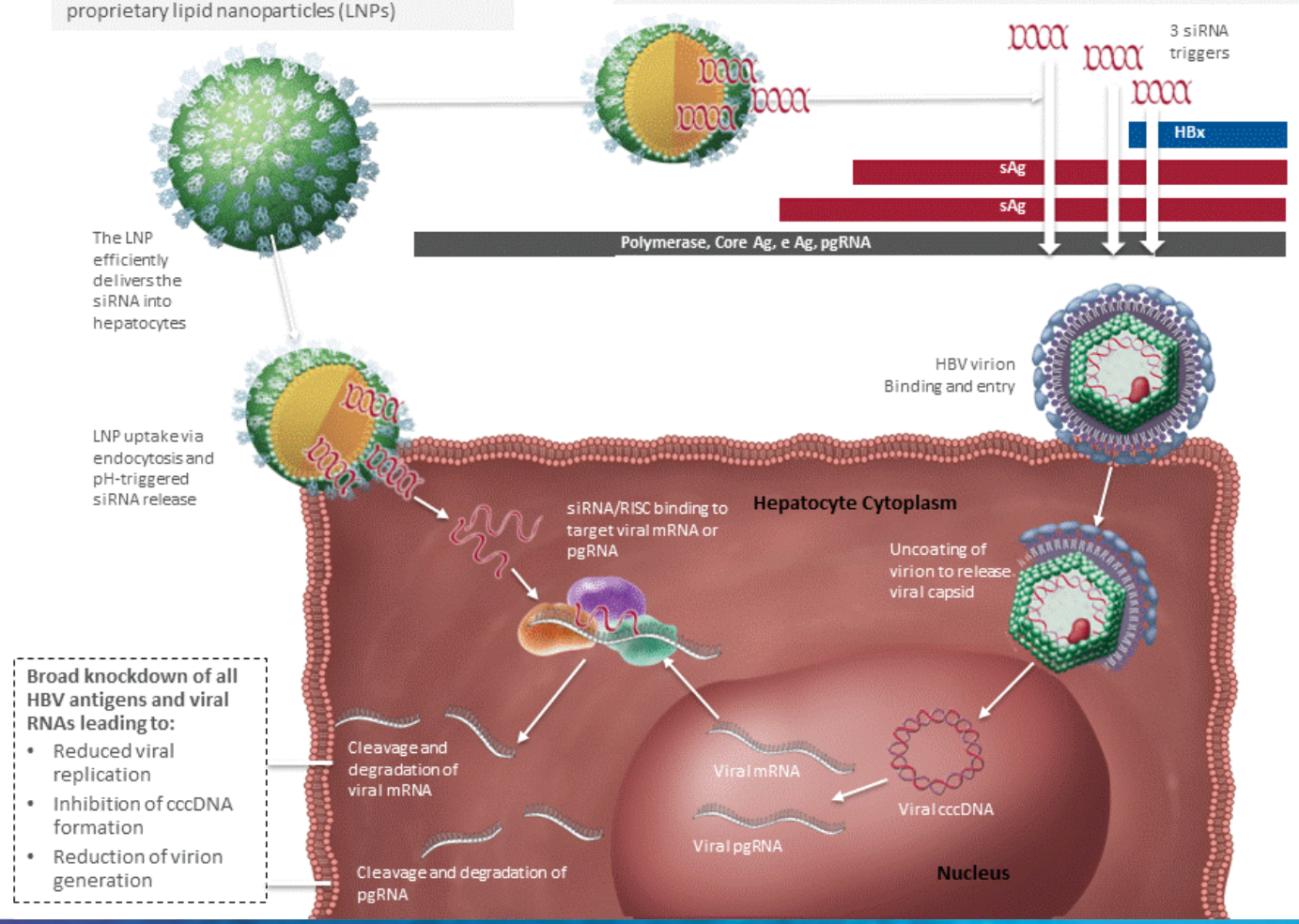
1. Inhibit Production
   - Transcription
   - Translation
2. Inhibit Secretion



siRNA and LNP Delivery

1. **ARB-1467 is a novel antiviral agent** in which 3 anti-HBV siRNA "triggers" are packaged inside proprietary lipid nanoparticles (LNPs)

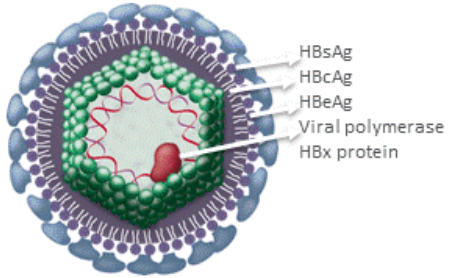
2. **The 3 siRNA triggers within ARB-1467** are designed to target all 4 viral RNA transcripts encoded by the HBV genome at sites that are highly conserved across HBV genotypes



Broad knockdown of all HBV antigens and viral RNAs leading to:

- Reduced viral replication
- Inhibition of cccDNA formation
- Reduction of virion generation

3. **By targeting all 4 viral RNA transcripts**, ARB-1467 inhibits production of all HBV viral antigens (Ag), the viral polymerase, HBx protein, and pre-genomic RNA



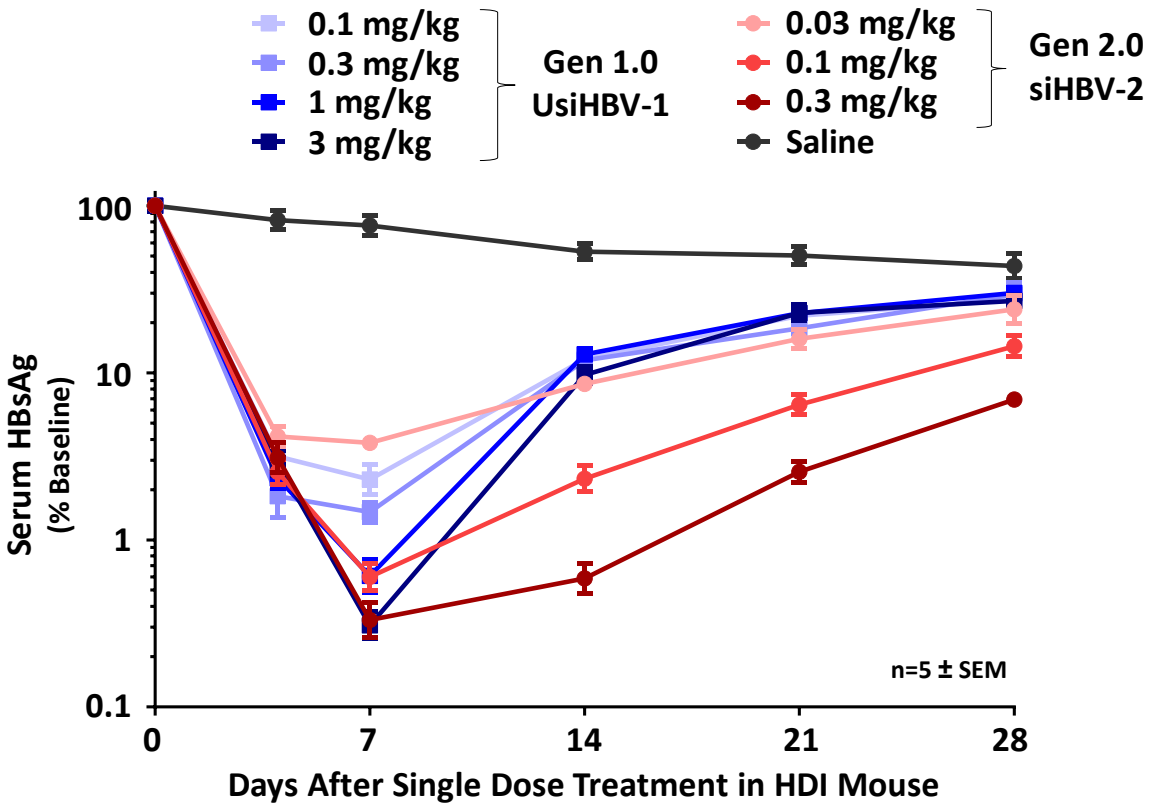
4. **Through knockdown of all HBV viral proteins,** it is anticipated that the 3 siRNA triggers within ARB-1467 will inhibit viral replication, remove viral immune suppression and reawaken the immune system

5. **ARB-1467 is suitable for use:**

- Across HBV genotypes
- Regardless of HBeAg status
- Regardless of treatment status
- In combination with currently approved and experimental agents due to complementary MOA, potentially leading to improved outcomes

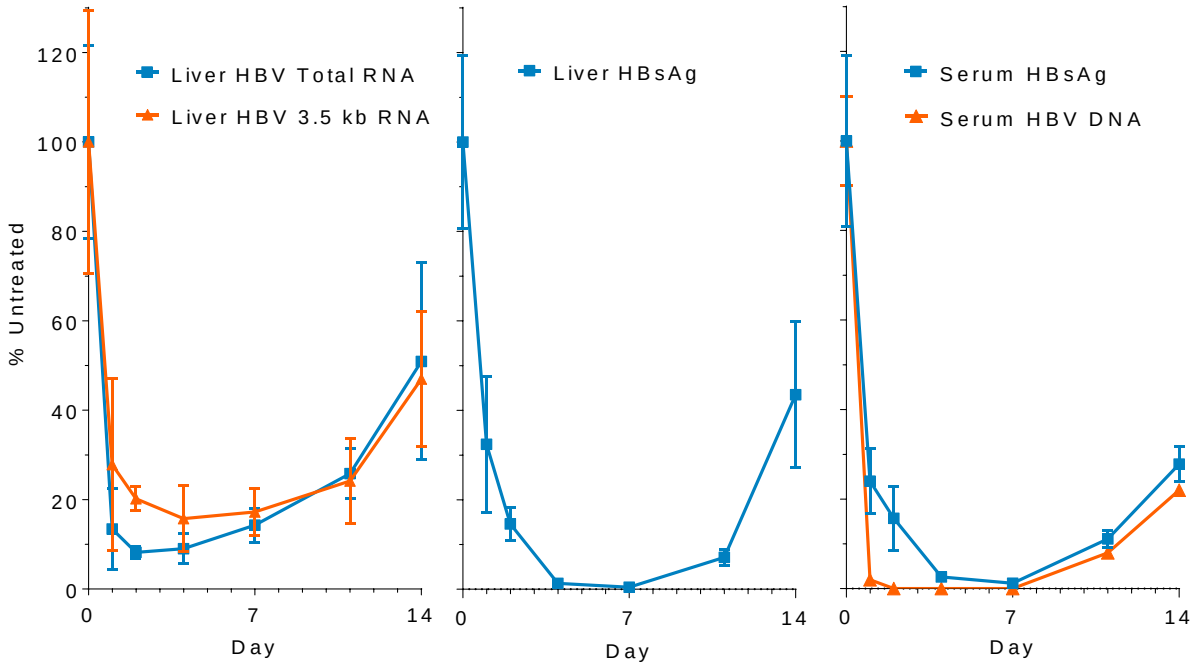
*RISC = RNA-Induced Silencing Complex
**pgRNA = pre-genomic RNA

HBsAg Knock Down Using siRNAs



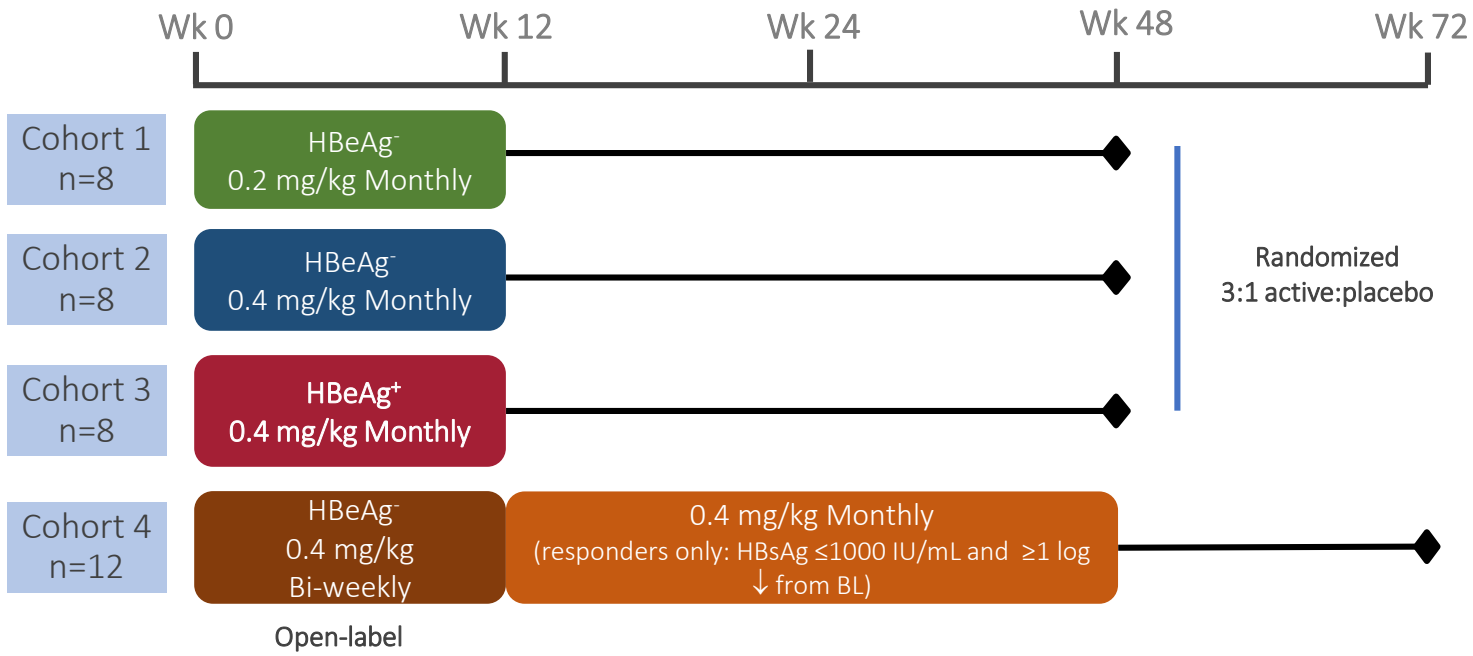
Gen 1.0 = ARB-1467

Gen 2.0 = ARB-1740



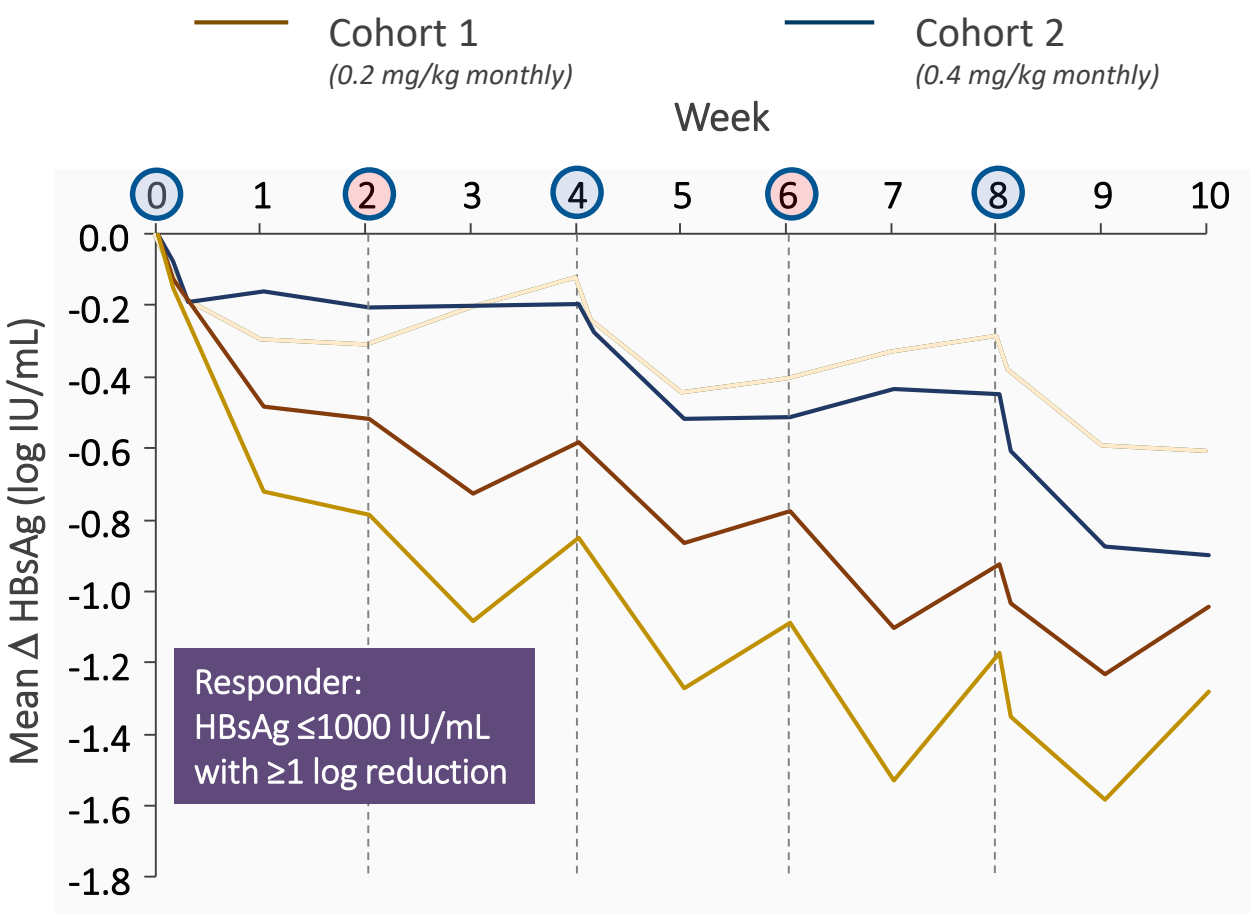
Study Design

Chronic HBV Patients on Stable Nucleos(t)ide Therapy



- ARB-1467 or placebo given as a 2-hour IV infusion
- Broad inclusion criteria
  - Non-cirrhotic, chronic HBV infection receiving NA therapy with ETV or TDF for ≥ 12 months
  - HBsAg ≥ 1000 IU/mL, HBV-DNA negative
  - ALT or AST ≤ 2x ULN
  - Fibroscan ≤ 9 kPa
- Pre-medications given the evening prior and 30 minutes prior to each infusion

Overall HBsAg Declines by Cohort (HBeAg-)



Mean Change from Baseline HBsAg

Dosing Day: All Groups Bi-weekly only (Cohort 4)

n (%)	Cohort 1 HBeAg- 0.2 mg/kg Monthly n=6	Cohort 2 HBeAg- 0.4 mg/kg Monthly n=6	Cohort 3 HBeAg+ 0.4 mg/kg Monthly n=6	Cohort 4 HBeAg- 0.4 mg/kg Bi-weekly n=12	Placebo n=6
HBV genotype[b]					
B	0	1 (17)	0	1 (8)	0
C	4 (67)	1 (17)	4 (67)	8 (67)	4 (67)
D	2 (33)	3 (50)	0	0	1 (17)
C/D	0	1 (17)	1 (17)	0	1 (17)
Undetermined[c]	0	0	1 (17)	3 (25)	0

Overall Safety: Treatment-Emergent Adverse Events

	Cohort 1	Cohort 2	Cohort 3	Cohort 4	
n (%)	HBeAg- 0.2 mg/kg Monthly n=6	HBeAg- 0.4 mg/kg Monthly n=6	HBeAg+ 0.4 mg/kg Monthly n=6	HBeAg- 0.4 mg/kg Bi-weekly n=12	Placebo n=6
Any AE *Drug-related*	5 (83) *3 (50)*	5 (83) *4 (67)*	2 (33) *2 (33)*	8 (67) *4 (42)*	5 (83) *2 (33)*
Grade 3–4 AEs	1 (17)	0	0	0	0
Serious AEs	1 (17)[a]	0	0	0	0
Discontinuation due to an AE	0	1 (17)[b]	0	1[c]	0
Grade 3–4 lab[d] abnormalities	4 (67)	5 (83)	4 (67)	9 (75)	4 (67)

[a]Left cochleovestibular deficit, not related to study treatment.

[b]Discontinued after the 2nd dose due to acute HEV super-infection and "HBV blip"(HBV-DNA 88 IU/mL)[1].

[c]Discontinued after the 3rd dose due to mild infusion reaction, arthralgia and hair loss.

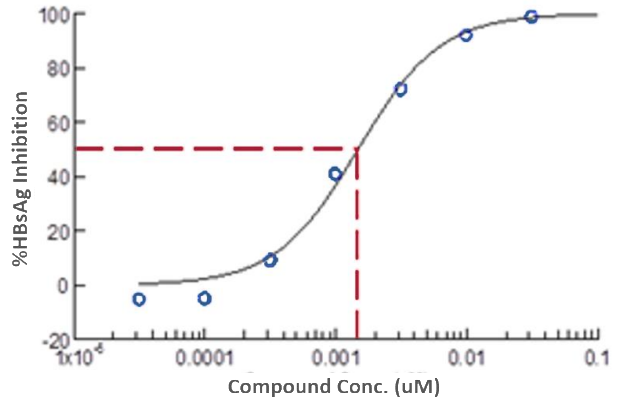
[d]Isolated ↑ glucose, ↓ lymphocytes and ↓ phosphate in all groups including placebo.

- Most AEs to date have been mild and transient
- 17/18 (94%) subjects in Cohorts 1–3 received all three monthly doses
- 11/12 (92%) in Cohort 4 received all five bi-weekly doses

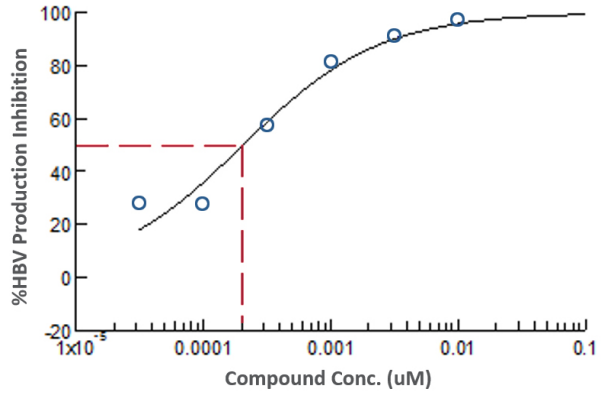
Streinu-Cercel A, *et al. J Hepatol* 2017;66(suppl 1):S688–S689.

AB-452 is a Potent Small Molecule HBV RNA Destabilizer That Inhibits Both HBsAg and HBV DNA Production

Potency Model	EC_{50} (µM)	CC_{50} (µM)	Endpoint
HepG2.2.15	0.0015	>50	HBsAg/ELISA
HepG2.2.15	0.0028	>50	HBeAg/ELISA
HepG2.2.15	0.0002	>50	HBV DNA/qPCR
PHH	0.0087	>1	HBsAg/ELISA
PHH	0.0088	>1	HBeAg/ELISA
HepG2/NTCP	0.0097	ND	HBsAg/ELISA
HepG2/NTCP	0.0036	ND	HBeAg/ELISA



AB-452 is a potent HBsAg inhibitor in HepG2.2.15 cells with an EC_{50} value of 1.5 nM



AB-452 is a potent inhibitor of HBV DNA in HepG2.2.15 cells with an EC_{50} value of 0.2 nM

AB-452 – Genotype Coverage and Viral Selectivity

Genotype Coverage

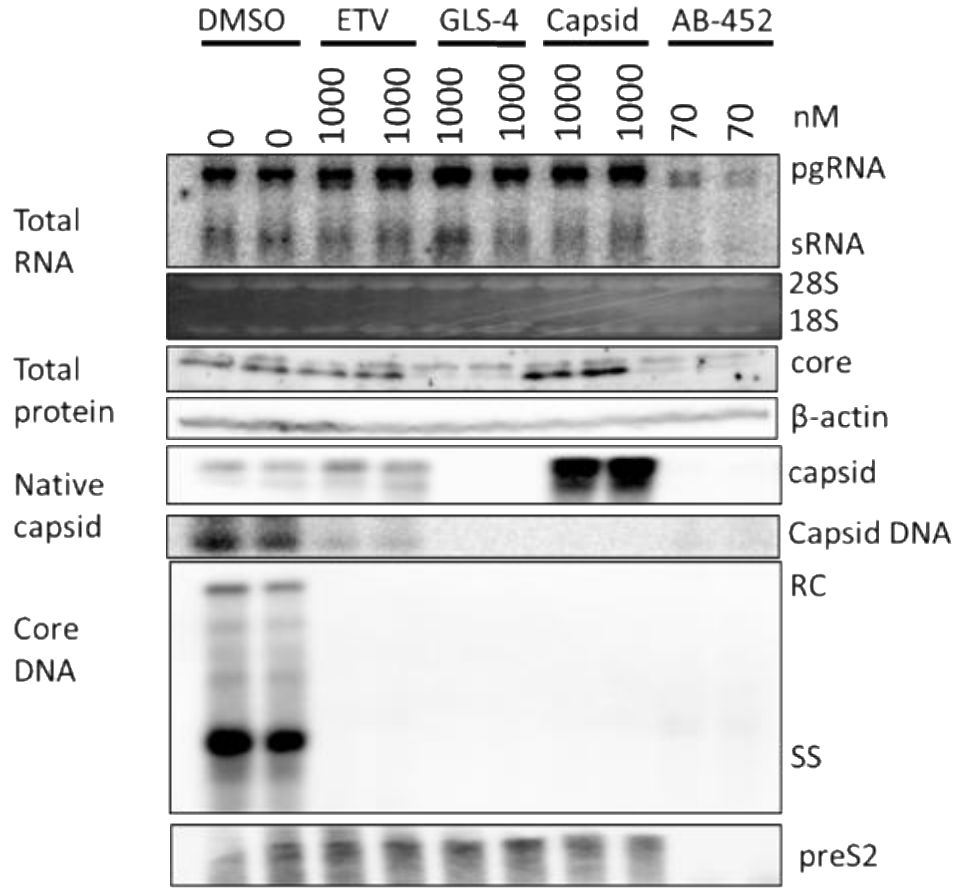
Genotype	HBsAg EC_{50} (µM)
A	0.0013
B	0.0018
C	0.0020
D	0.0008

Viral Selectivity

Virus	Family	Genome	AB-452		Host Cell Line
			EC_{50} (µM)	CC_{50} (µM)	
HCV	*Flaviviridae*	(+) ssRNA	>30	>30	Huh7
WNV	*Flaviviridae*	(+) ssRNA	>30	>30	VERO
RSV	*Paramyxoviridae*	non-segmented (-) ssRNA	>30	>30	HEp2
IFA	*Orthomyxoviridae*	segmented (-) ssRNA	>30	>30	MDCK
HIV	*Retroviridae*	ssRNA to DNA	>30	>30	CEMSS
HSV	*Herpesviridae*	dsDNA	>30	>30	VERO
hCMV	*Herpesviridae*	dsDNA	>30	>30	MRC5
DENV	*Flaviviridae*	(+) ssRNA	>30	>30	Huh7
HRV 1A	*Picornaviridae*	(+) ssRNA	>30	>30	H1/HeLa

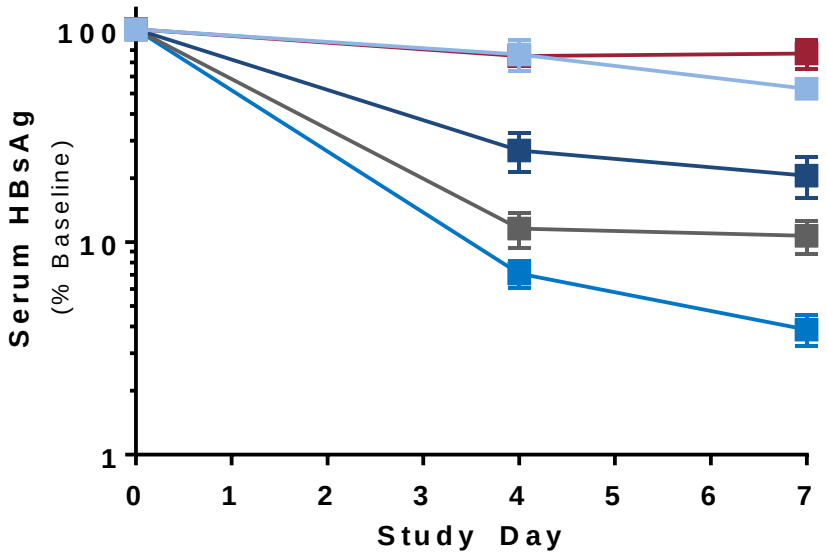
Multiple Aspects of the HBV Lifecycle Affected by AB-452

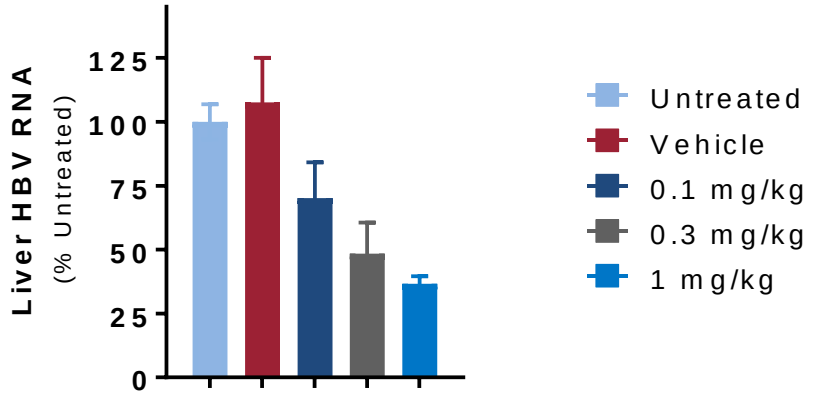
HBV RNA reduction leads to interference in viral gene expression, DNA replication, and virion assembly in HepG2.2.15 cells.



In Vivo HBsAg Inhibition with PO Administration of AB-452

- An immunocompetent mouse model of chronic HBV, infected with an AAV carrying a 1.2-fold overlength genome of genotype D
- Results are expressed as a percentage of individual animals' Day 0 pre-dose values. Data shown as mean ± SEM (n=5).
- AB-452 administered for 1 week, PO, BID
- Doses: 0.1, 0.3 and 1 mg/kg
- 1.4 log10 serum HBsAg reduction in a dose-dependent manner.
- Correlated well with liver HBV RNA levels.

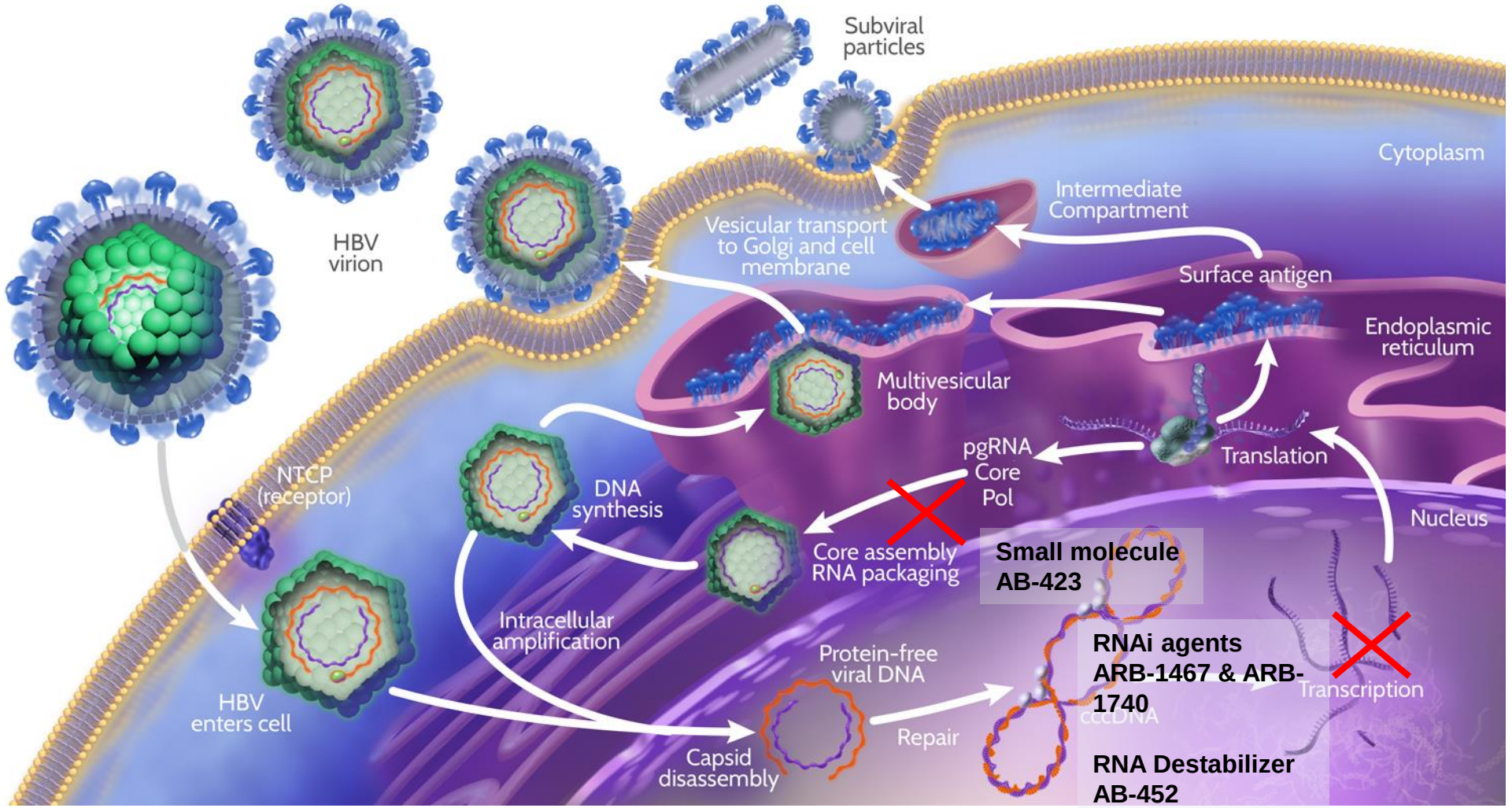




Combination Therapy

- General belief that no single approach will be sufficient to deliver a cure
- As in HCV and HIV combinations of drugs with different MOA will be the solution
- Which combination will deliver the ultimate “cure” is yet to be determined
- How to assess combinations preclinically that may guide clinical studies is developing

Preclinical Combination Studies



ARB-1467 & ARB-1740 (RNA interference)

- Three siRNAs encapsulated in a lipid nanoparticle delivery system

AB-423 (Core/Capsid Inhibitor)

- Oral small molecule

AB-452 (RNA Destabilizer)

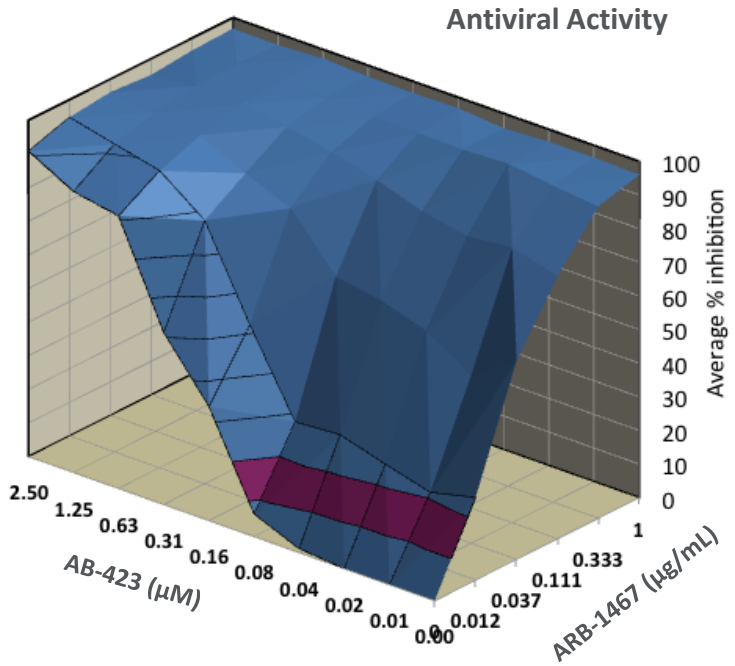
- Oral small molecule

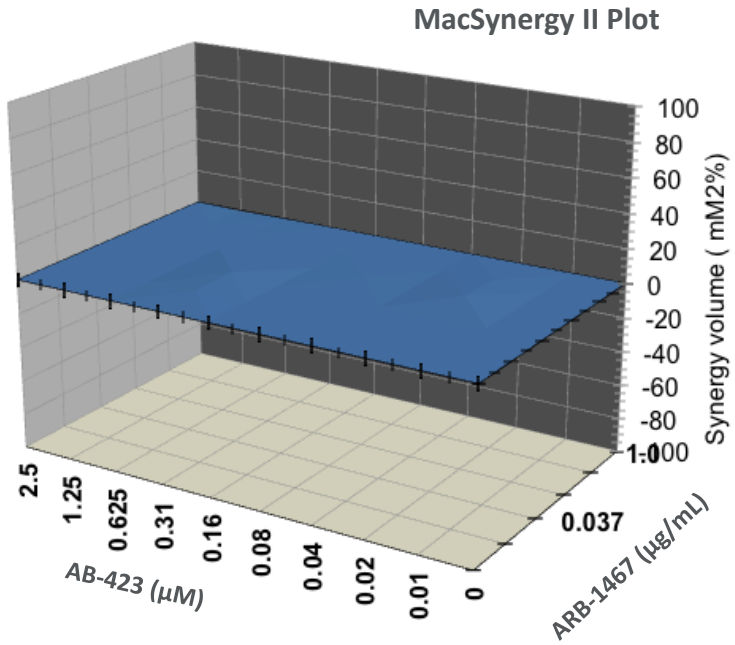
Pegylated Interferon

- Approved drug

In Vitro Combination Studies

Capsid Assembly Inhibitor AB-423 with siRNA ARB-1467





HBV rcDNA Synthesis by bDNA assay (AML-12-HBV 10 cells)

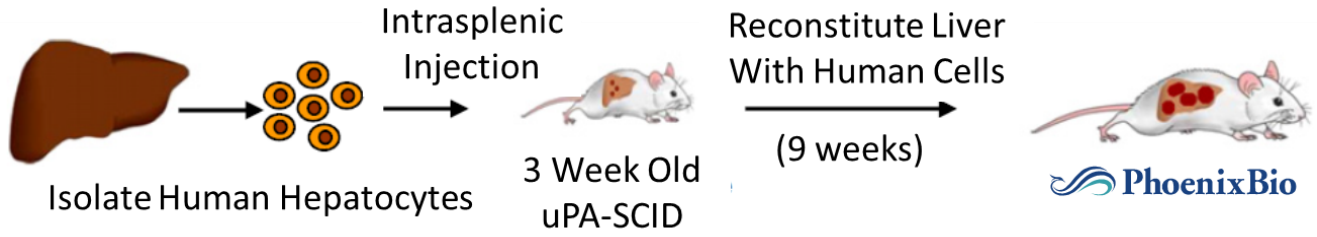
- Additive Interaction
- No Antagonism
- MacSynergyII Analysis
- EC50 values comparable to historical values
- No cytotoxicity detected by Cell TiterGlo assay in combination

SYNERGY PLOT (99.9%)	
Bonferroni Adj.	96%
SYNERGY	**6.96**
log volume	1
ANTAGONISM	**-0.81**
log volume	-0.12

HBV-Infected Chimeric Mouse

Humanized liver supports complete HBV life cycle

- **Stabilized chronic HBV infection**
- **Viral replication driven from accumulated cccDNA**



	Sub-type	HBsAg (log_{10} IU/mL)	HBV DNA (log_{10} copies/mL)
PXB Mouse (Gt C)	Hemizygous uPA	3.5 (2.8-3.8)	8.3 (7.7-8.5)
CHB Patient	HBeAg positive	4.0 (1.8-5.0)[1] 4.4 (±0.7)[2]	9.2 (±0.8)[2]
	HBeAg negative	3.2 (0.8-5.0)[1] 3.9 (±0.5)[2]	6.8 (±1.2)[2]

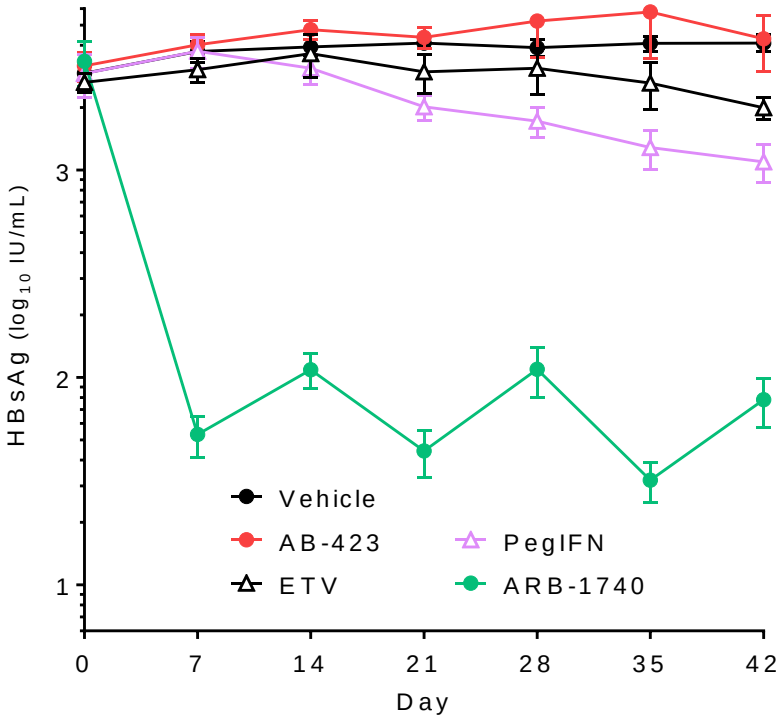
Reference 1: Seto et al. HEPATOLOGY 2013; 58: 923-931, Reference 2: P. Arends et al. Journal of Viral Hepatitis 2014

LNP siRNA + pegIFN

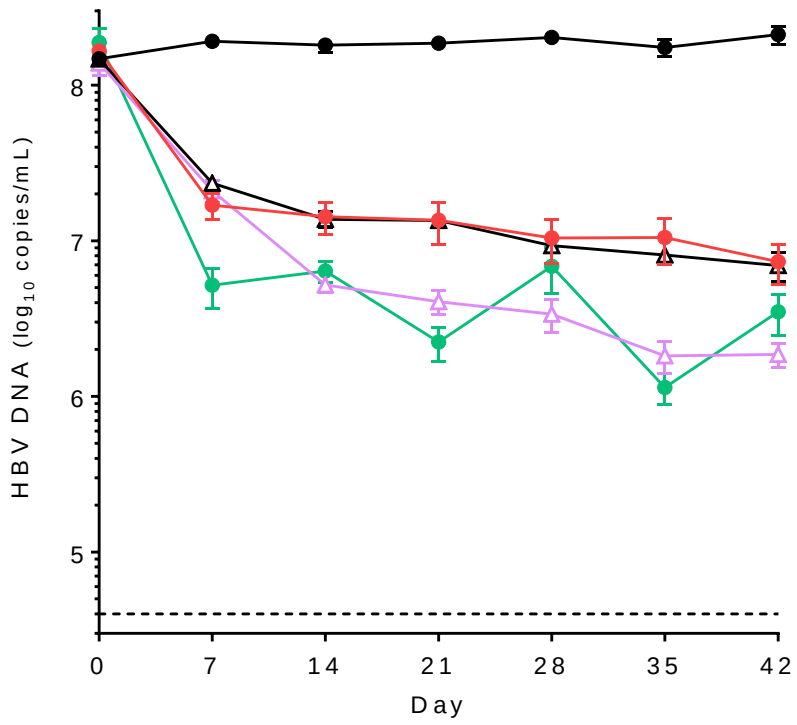
Preclinical study in infected humanized mouse model

- Each agent has stand-alone activity against HBV virus

Serum HBsAg



Serum HBV DNA



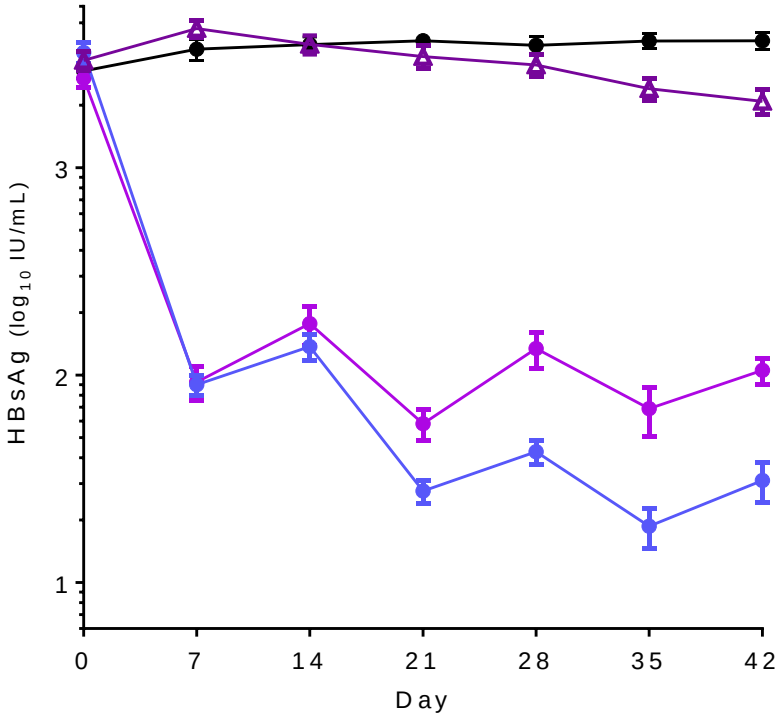
	Treatment for 6 weeks		
	Dosage	Route	Frequency
AB-423	100 mg/kg	PO	BID
ETV	1.2 µg/kg	PO	QD
PegIFN	30 µg/kg	SQ	2×/wk
ARB-1740	3 mg/kg	IV	biweekly

LNP siRNA + pegIFN

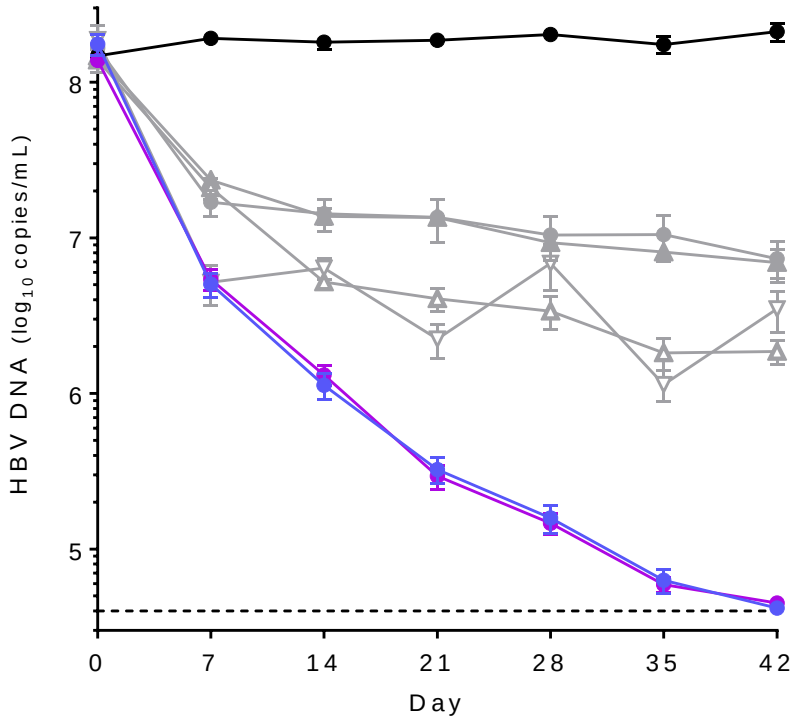
Preclinical study in infected humanized mouse model

- Triple combo containing pegIFN has additional benefit of greater antigen control

Serum HBsAg



Serum HBV DNA



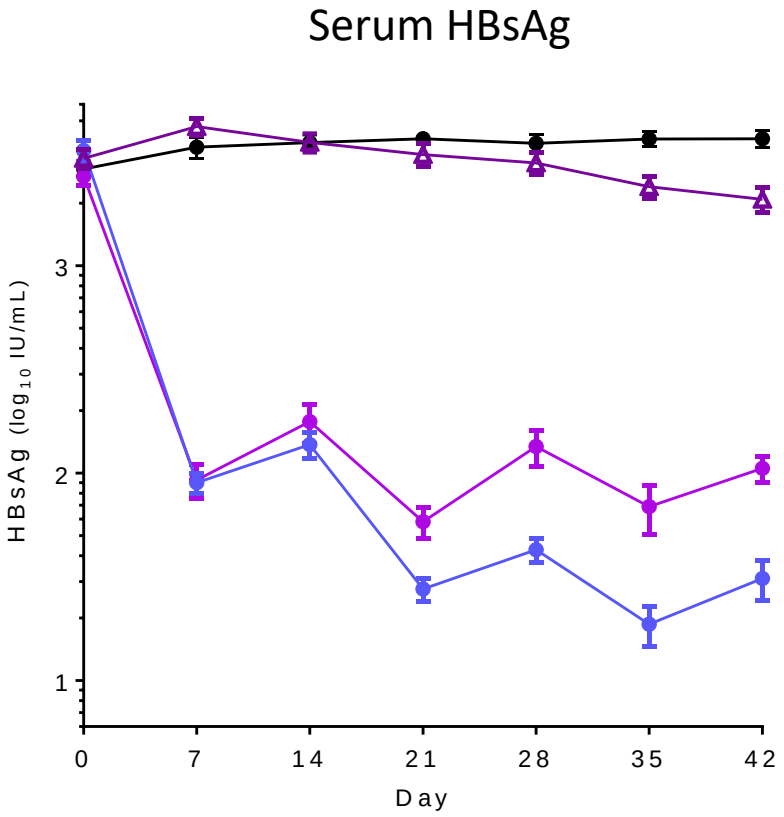
	Treatment for 6 weeks		
	Dosage	Route	Frequency
AB-423	100 mg/kg	PO	BID
ETV	1.2 µg/kg	PO	QD
PegIFN	30 µg/kg	SQ	2×/wk
ARB-1740	3 mg/kg	IV	biweekly

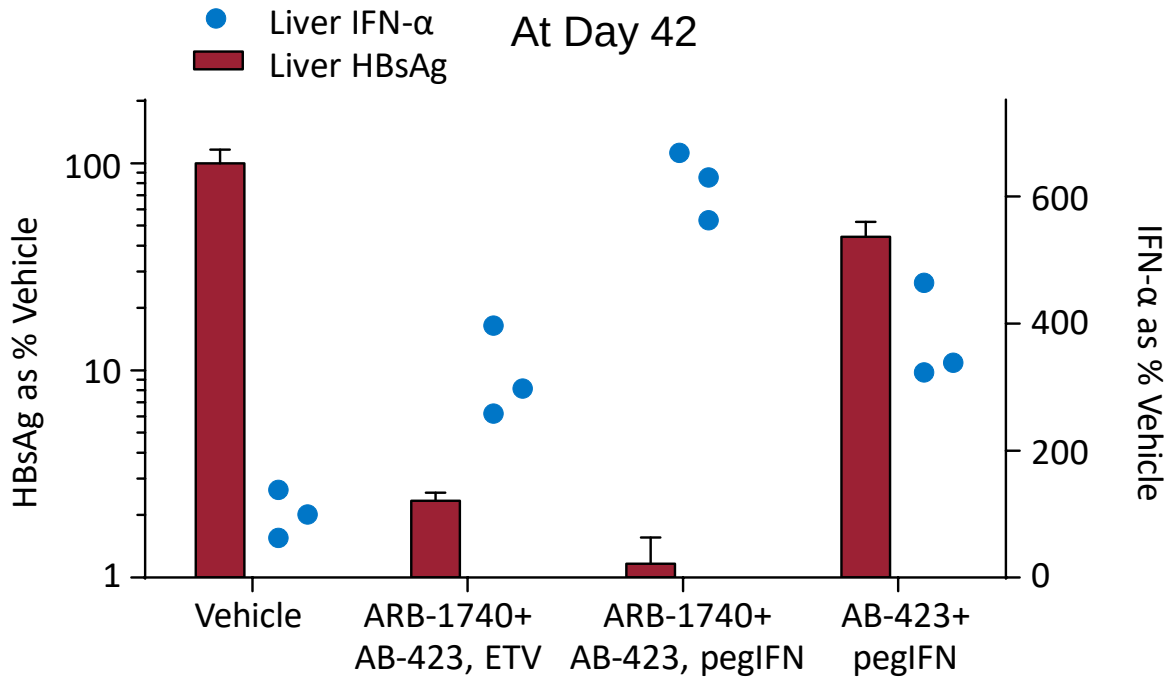
- Vehicle
- AB-423 + PegIFN
- ARB-1740 + AB-423 + ETV
- ARB-1740 + AB-423 + PegIFN

HBsAg Removal Correlated with ↑ Host Immune Response

Infected humanized mouse model

- HBsAg removal by ARB-1740 correlated with gain in human IFN-α expression
- In vivo human hepatocyte innate immune response was further potentiated by combining ARB-1740 with pegylated interferon

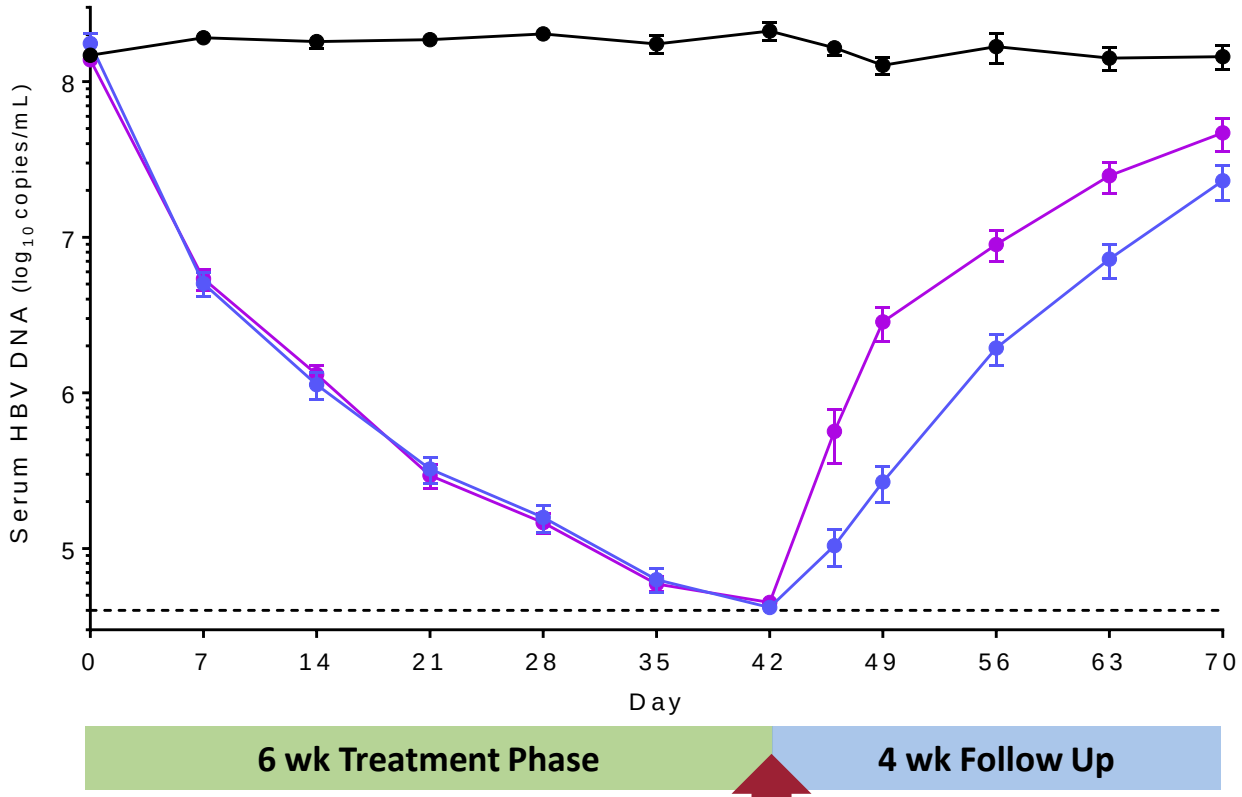


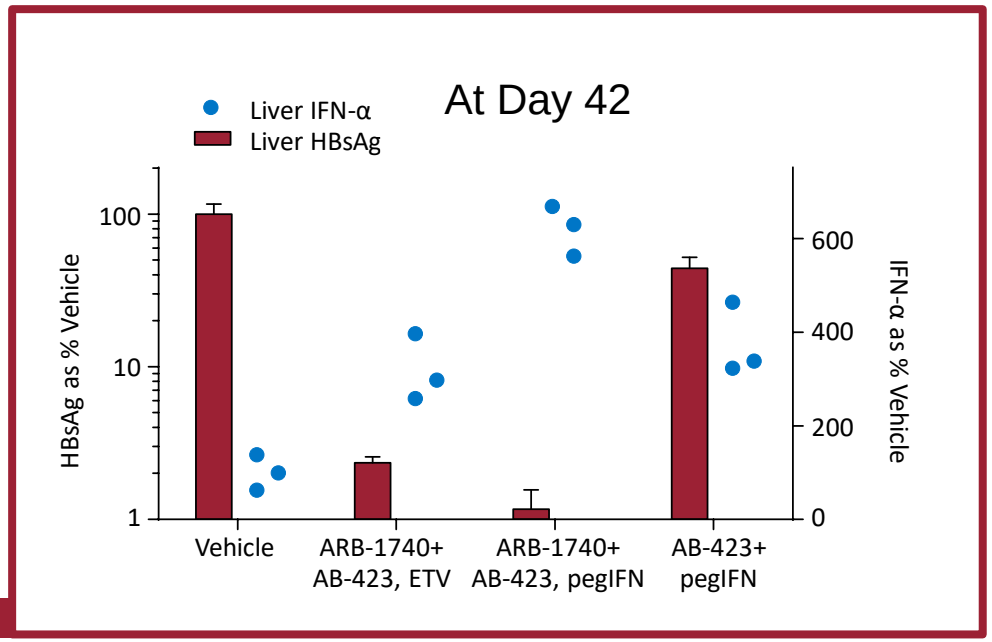


Slower Off-treatment Viral Rebound Correlated with ↑ Host Immune Response

Infected humanized mouse model

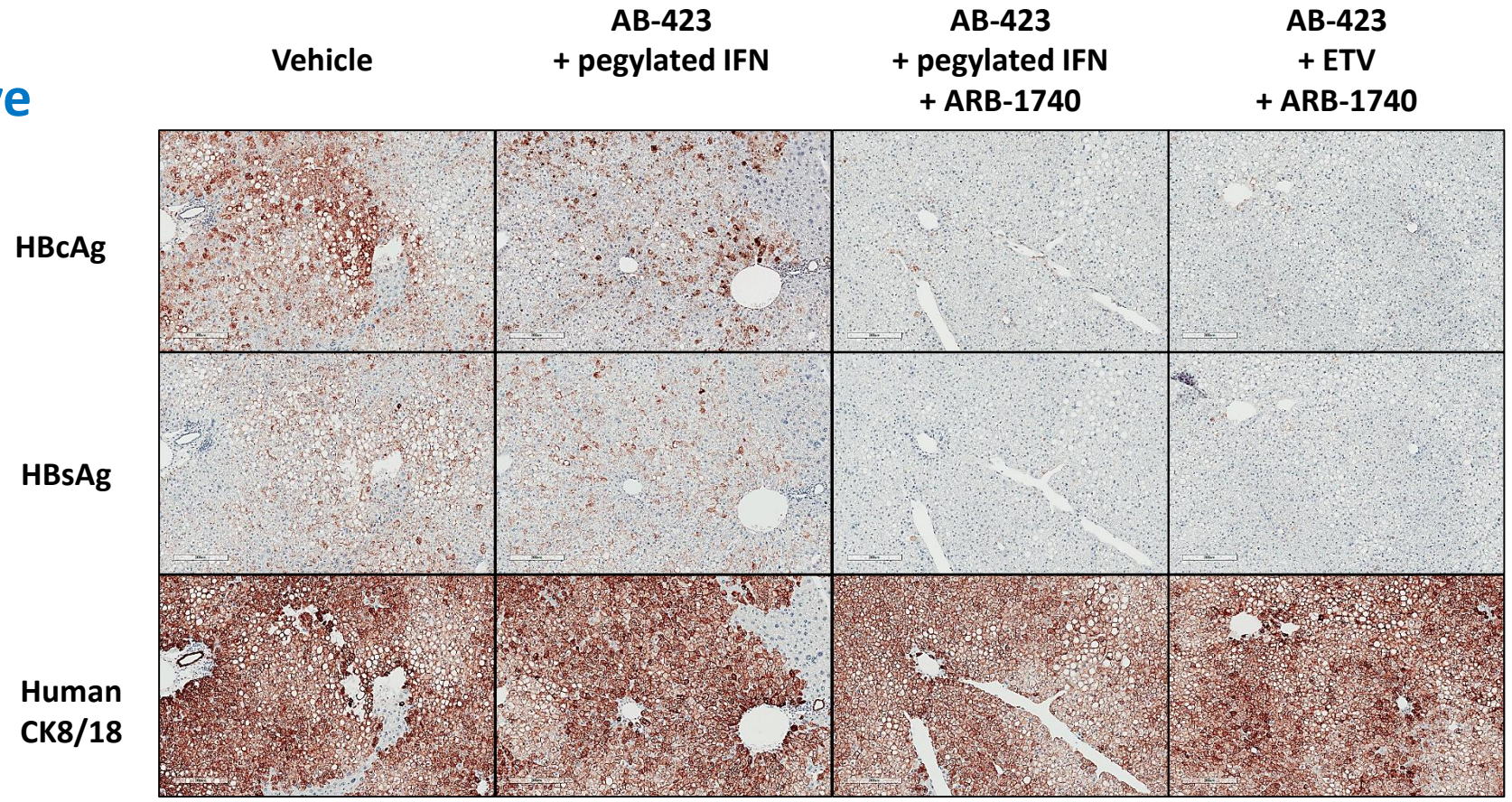
Model lacks T and B cells (adaptive immunity)





ARB-1740
Inhibits Production of All HBV Proteins

Removal from liver, a key immunosuppressive environment



- Liver HBV antigens at end of 6-week treatment

RNA Destabilizer AB-452 in Combination with siRNAs In Vitro

AB-452 shows additive to synergistic effects when combined with HBV LNP siRNA agents *in vitro*

Inhibitor A	Inhibitor B	Cell Culture Model	Conclusion*
AB-452	ARB-1467**	HEPG2.2.15 (HBsAg)	Additive to Synergistic
AB-452	ARB-1740**	HEPG2.2.15 (HBsAg)	Additive

***MacSynergy II Analysis; Bliss Independence Model[1]**

****ARB-1467 and ARB-1740 are HBV LNP siRNA agents**

Summary

- In chronically infected HBV patients, HBsAg is believed to play a significant role in control of the host immune response
- HBV functional cures are associated with the loss of HBsAg
- siRNA / LNP modalities have been shown to reduce HBsAg levels in the clinic
- Novel small molecule RNA destabilizers show potential to significantly reduce levels of HBsAg in addition to reduction of HBV DNA and other viral antigens
- Combinations of either an siRNA/LNP or RNA destabilizer agent with other MOA agents have shown additive or synergistic anti-HBV effects in preclinical models.

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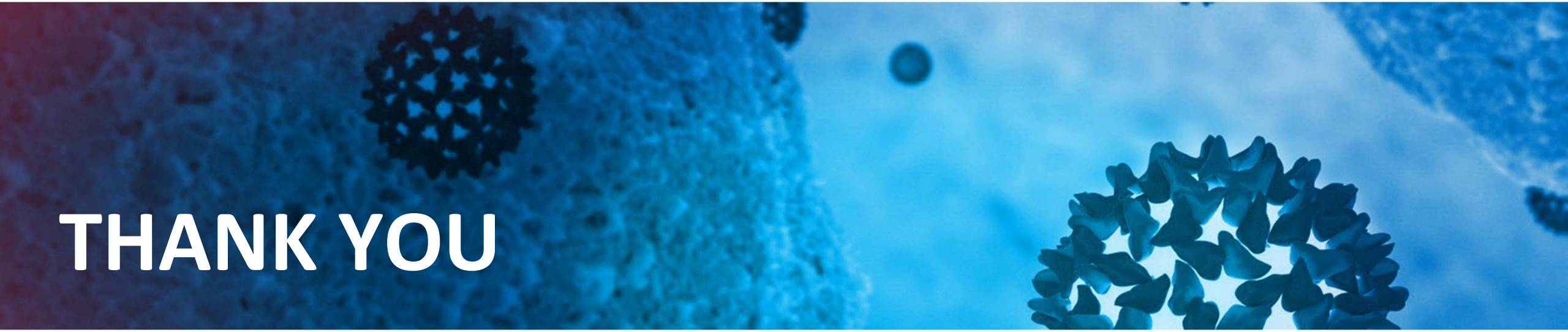
Arbutus BIOPHARMA



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