AB-161, an Oral HBV RNA Destabilizer to Suppress HBV RNA and HBsAg

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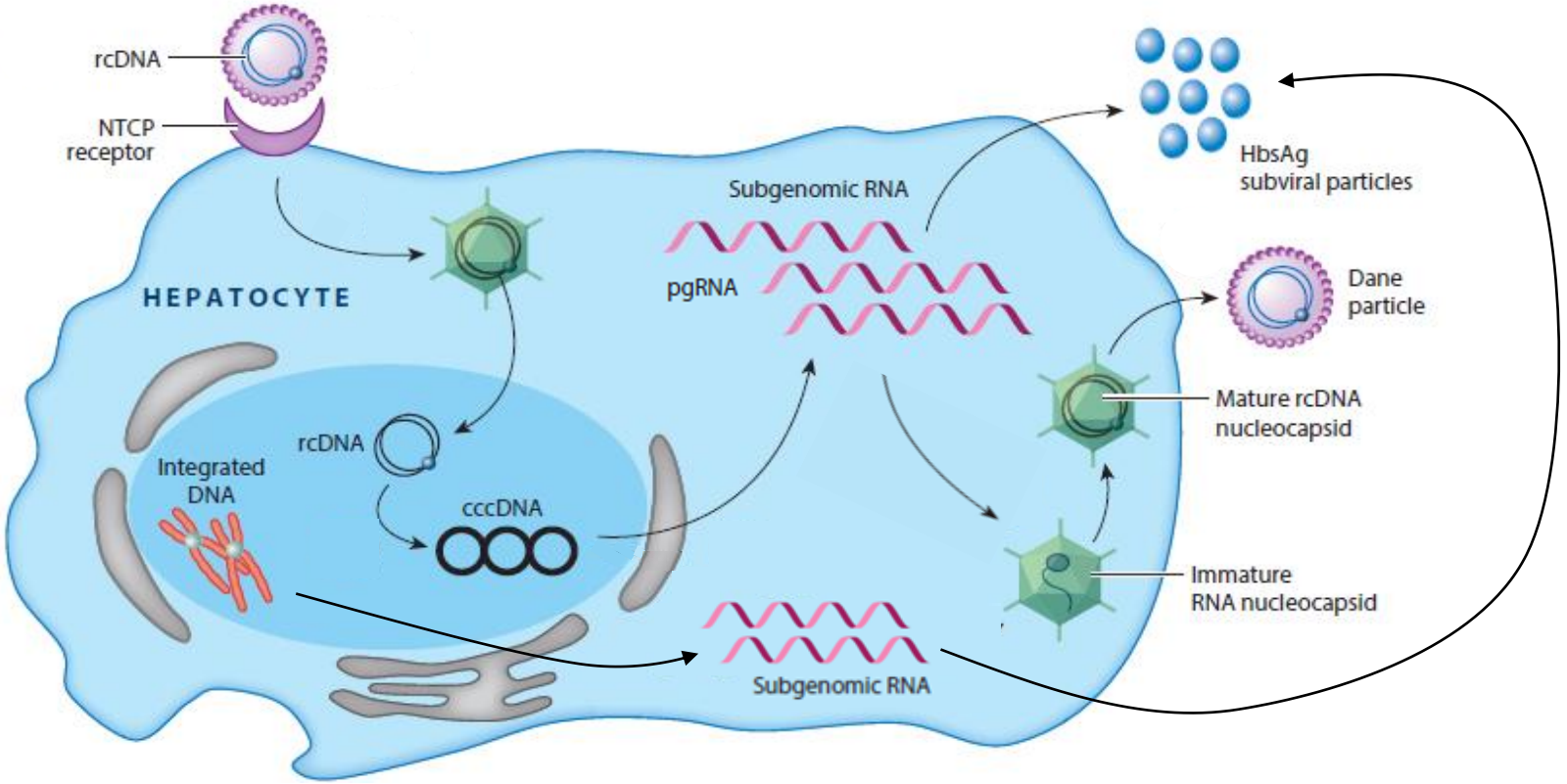
Persistence of Hepatitis B Virus

cccDNA and Integrated HBV DNA



HBV life cycle

adapted from: Naggie and Lok 2021 Annu Rev Med

- **Covalently closed circular DNA (cccDNA)**
  - Template for viral transcripts
  - Production of viral proteins including HBsAg
- **Integrated HBV DNA**
  - Partial HBV genome integrated into host chromosomes
  - Template for subgenomic HBV RNA, another source for HBsAg
- **HBsAg**
  - Contributes to immune exhaustion

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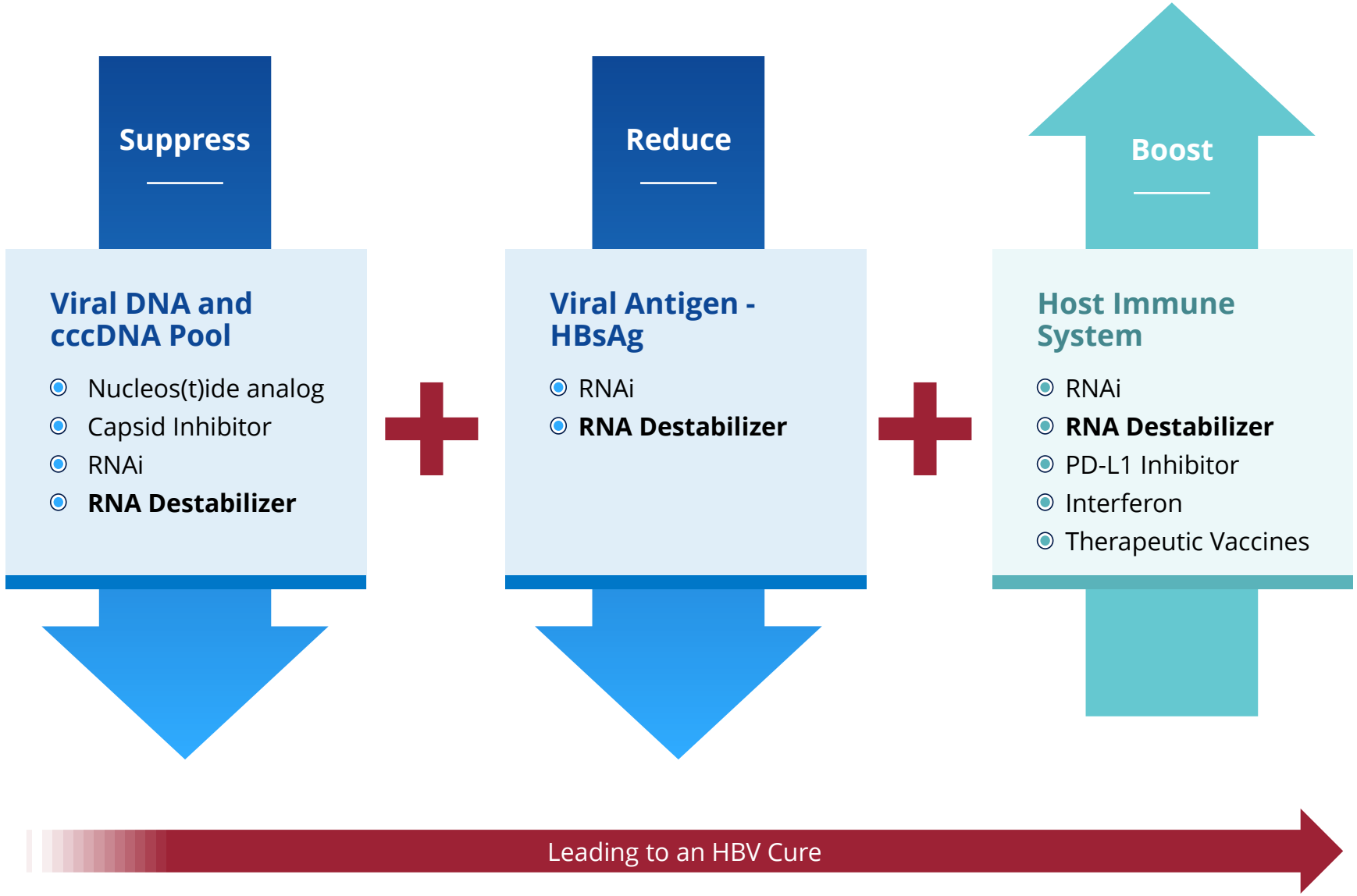
3-Pronged Approach to Therapeutic Success

- **Suppress** HBV DNA
- **Reduce** viral antigens
- **Boost** host immune response

Therapeutic success will **require a combination of agents** with complementary MOAs.



HBV Hijacks PAPD5/7-ZCCHC14 to Stabilize Viral Transcripts

Inhibitors targeting PAPD5/7 destabilize HBV RNA and reduce HBsAg

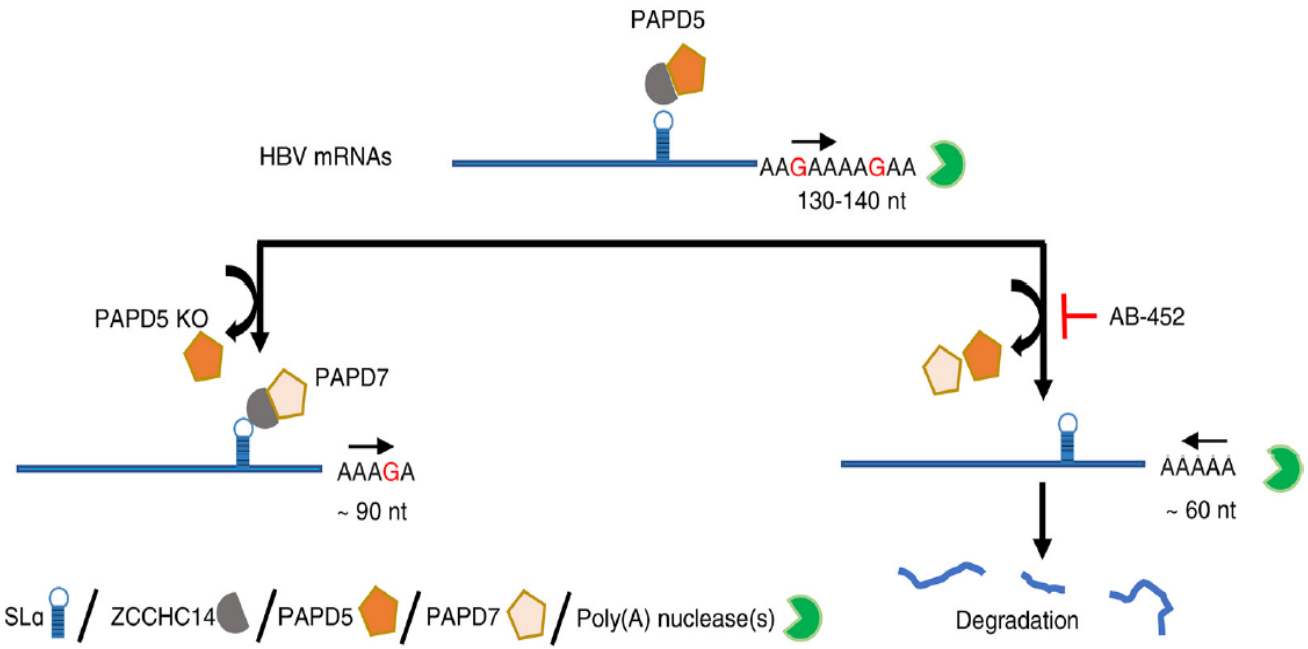


FIG 8 Proposed model illustrating the interplay between HBV *cis* element SLα and the host factors PAPD5 and PAPD7 in maintaining HBV RNA integrity and stability.

Liu *et al* 2021 J Virology

Scientific Rationale:

- HBV RNA contains a highly conserved stem-loop (SLα) sequence within its post-transcriptional regulatory element (PRE)
- HBV RNA stability depends on the SLα interaction with the PAPD5/7-complex[1-4]
  - PAPD5/7 recruitment onto HBV RNA leads to polyadenylation and guanylation within poly(A) tails
  - Mixed tailing stabilizes HBV RNA and prevents degradation by cellular nucleases
- Small-molecule inhibitors targeting PAPD5/7 (e.g. AB-452, RG7834)
  - Degrade HBV RNA, reduce HBsAg and viral replication

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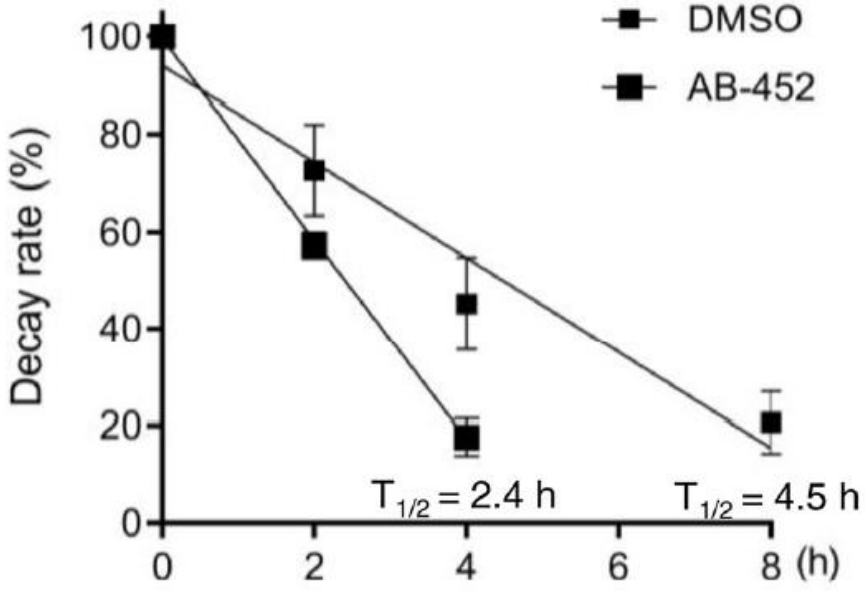
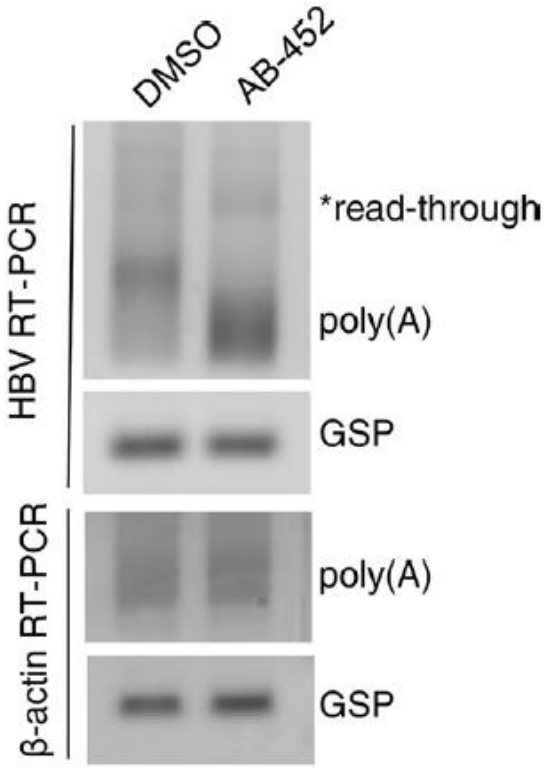
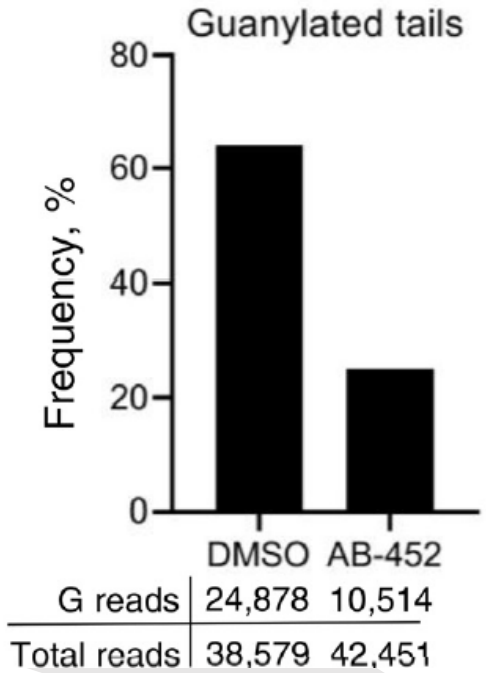
1. Zhou *et al* 2018 Antiviral Research, 2. Mueller *et al* 2019 Hep, 3. Kim *et al* 2020 Nature Structural & Mol Bio, 4. Liu *et al* 2021 JV

HBV RNA Destabilizer Triggers Faster Viral RNA Decay and Alters the Composition of HBV Poly(A) Tail *in vitro*

- HBV RNA half-live was reduced from 4.5 h to 2.4 h in the presence of AB-452, a first generation HBV RNA destabilizer
- Faster HBV RNA decay was due to shortening of the viral transcripts' poly(A) tail and reduction of intermittent guanosine incorporation







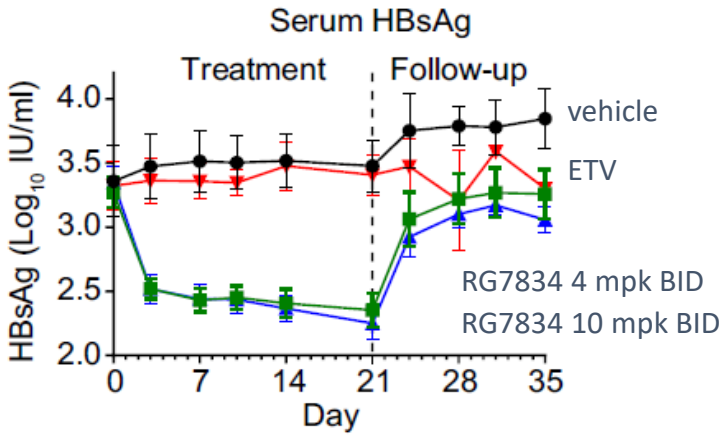
	DMSO	AB-452
G reads	24,878	10,514
Total reads	38,579	42,451

Liu *et al* 2021 JV

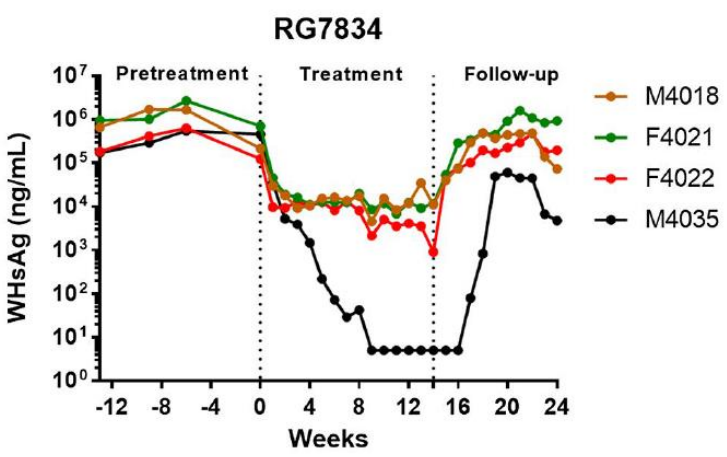
HBV RNA Destabilizers: Preclinical Proof-of-Concept

- HBV RNA destabilizers, represented by RG7834 and AB-452, reduce HBsAg in multiple animal models
  - HBV humanized mice: RG7834 (a reported DHQ RNA destabilizer) reduced HBsAg by 0.91 and 1.1 log when dosed at 4 mpk and 10 mpk BID, respectively[1]
  - WHV woodchucks: RG7834 reduced WHsAg by 2.57 log when dosed at 10 mpk BID[2]
  - AAV-HBV mice: AB-452 reduced HBsAg by 0.98 log when dosed at 1 mpk BID[3]

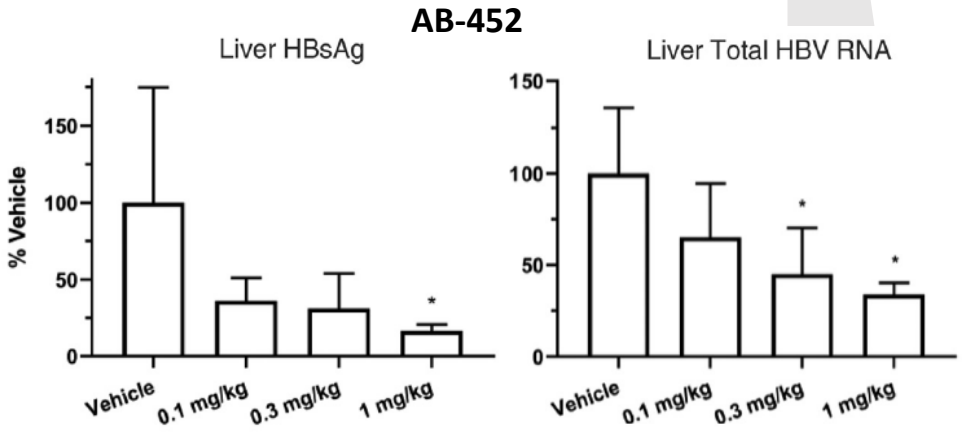
HBV infected humanized mice



WHV infected woodchucks



AAV-HBV transduced mice



1. Mueller *et al* 2018 J Hep, 2. Menne *et al* 2020 Hep Comm, 3. Liu *et al* 2021 JV

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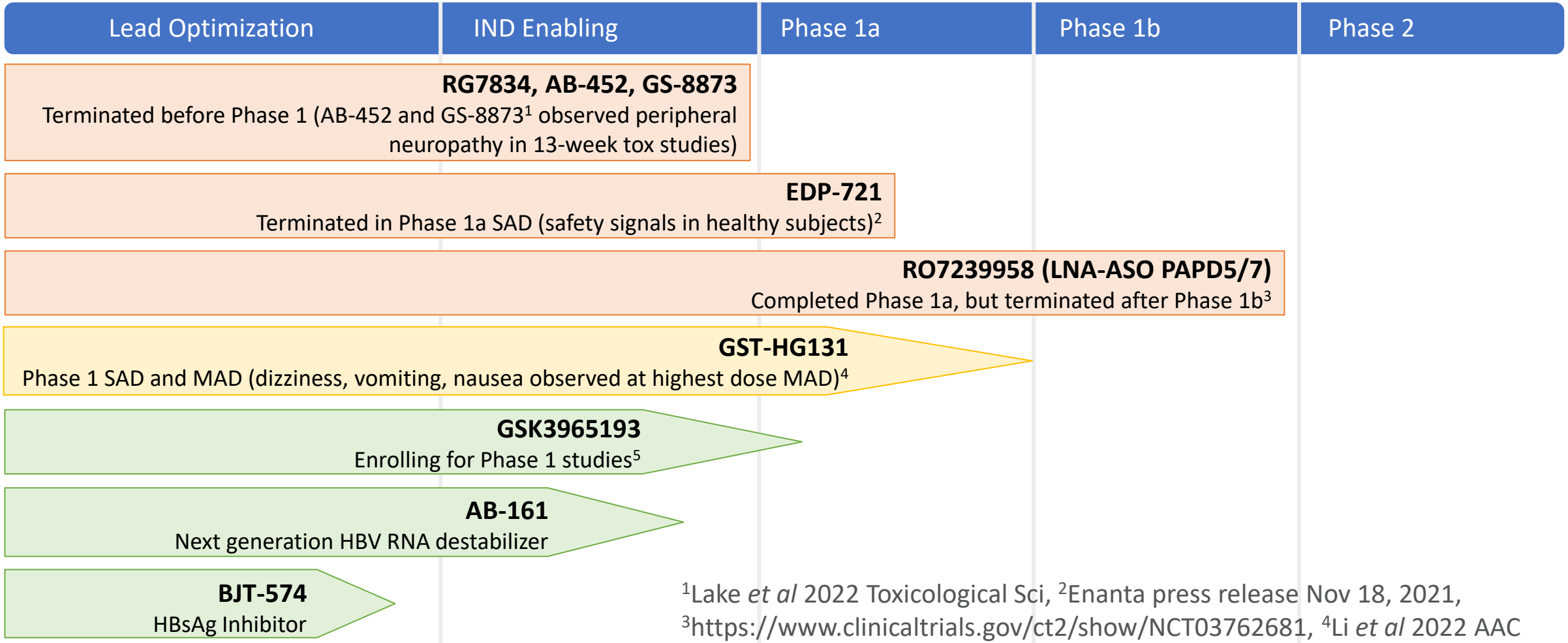
Development of HBV RNA Destabilizers



[1]Lake *et al* 2022 Toxicological Sci, [2]Enanta press release Nov 18, 2021, [3]https://www.clinicaltrials.gov/ct2/show/NCT03762681, [4]Li *et al* 2022 AAC [5]https://www.clinicaltrials.gov/ct2/show/NCT05330455

AB-161: Next Generation HBV RNA Destabilizer

- Strategy for next generation HBV RNA destabilizer
  - Maintain antiviral potency
  - Differentiated chemistry
  - Liver centricity
  - Reduce systemic exposure
- AB-161: selected as next generation HBV RNA destabilizer
  - Antiviral potency
  - Mechanism of action studies
  - Preclinical POC in AAV-HBV infected mice
    - Liver concentrations drive antiviral efficacy

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AB-161: Potent Antiviral Activity *In Vitro*

Compound	HepG2.2.15 HBsAg			HBV primary human hepatocytes (PHHs)	PLC/PRF/5 cells (integrated HBV)	HBV genotypes	NUC^R mutants
	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (μM)	HBsAg EC_{50} (nM)	HBsAg EC_{50} (nM)	HBsAg EC_{50} (nM)	HBsAg EC_{50} (nM)
AB-452	1.3	8.9	>50	6.6	2.4	1.2 to 2.3 (GT A - D)	1.3 to 4.4
AB-161	2.3	27	>50	8.5	5.9	2.1 to 4.5 (GT A - H)	1.9 to 2.0

- AB-161 inhibited HBsAg with single digit nM EC_{50} values across multiple HBV cell models
  - HepG2.2.15: stably replicative HBV
  - HBV PHHs: cccDNA dependent HBV replication
  - PLC/PRF/5: integrated partial HBV genome expressing HBsAg
- AB-161 active across GT A to H and retains activity in mutants resistant to nucleos(t)ide analogs
- Selective against HBV: panel of DNA and RNA viruses from different cell lines EC_{50} and $CC_{50} > 30$ μM

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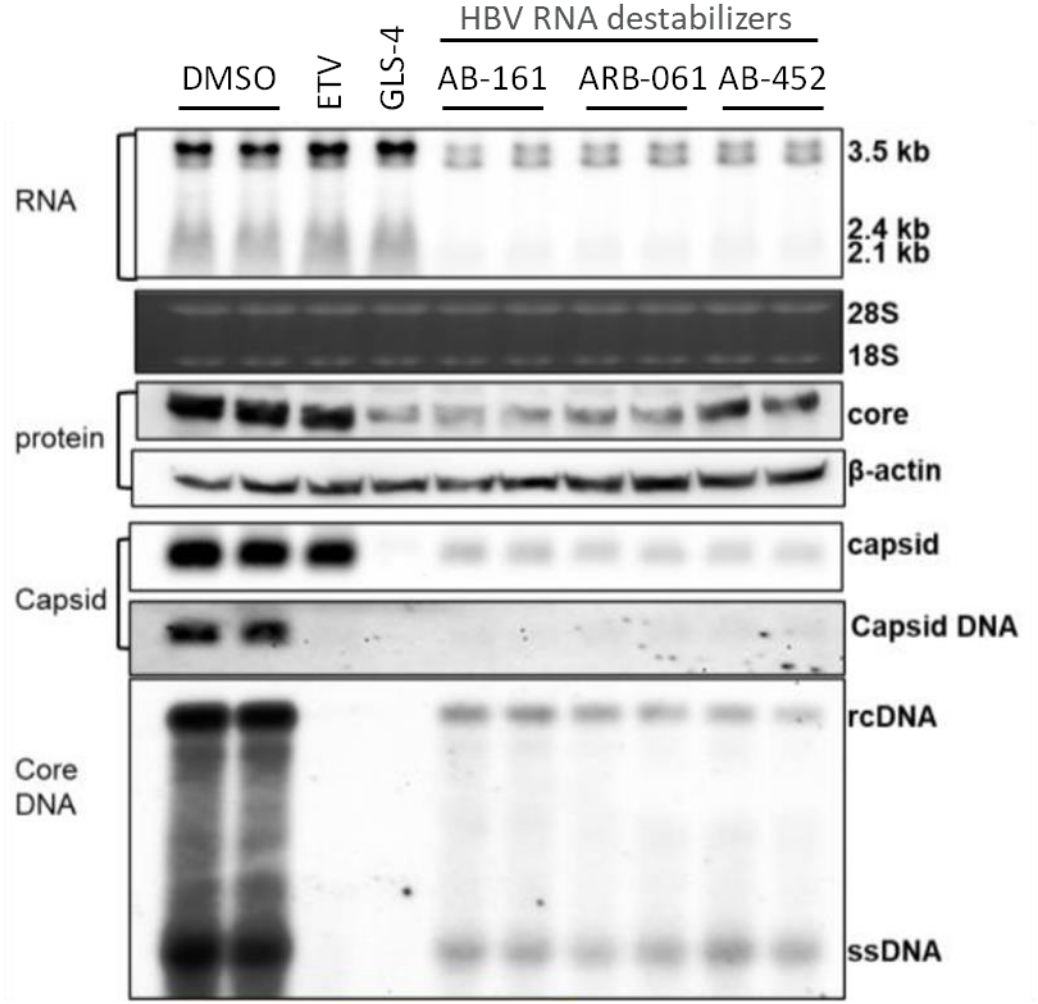
RNA Destabilizers, but Not NUCs or CAMs, Reduce HBV RNA

Multiple steps within viral life cycle suppressed by AB-161



- Nucleoside analog (NUCs)
  - ETV only inhibits HBV DNA
- Capsid assembly modulator (CAMs)
  - GLS-4 (Class I CAM): induces formation of aberrant capsids
  - Reduces core, capsids, and viral replication
  - No effect against HBV RNA
- HBV RNA destabilizers
  - AB-161, ARB-061, AB-452
  - Reduce core protein, capsids, and HBV DNA
  - Reduce pgRNA (3.5 kb) and sRNA (2.4 and 2.1 kb)
  - Differentiated MOA from NUCs and CAMs

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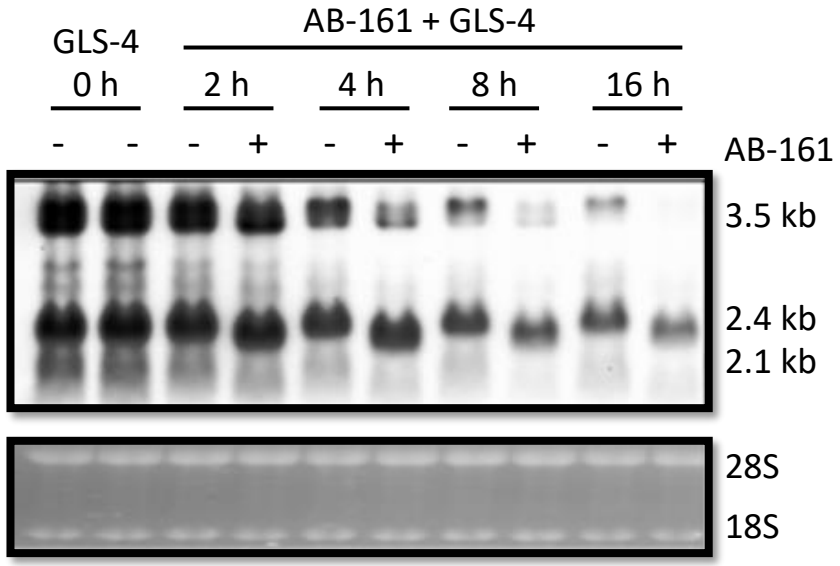
Time Dependent HBV RNA Degradation by AB-161

- AB-161 induces shortening and degradation of pgRNA and subgenomic HBV RNA, starting at 2 hours post treatment
- Combination of AB-161 and a CAM enhances pgRNA degradation



HepAD38 cells induced for HBV transcription and treated with GLS-4 to prevent pgRNA encapsidation, prior to the addition of AB-161

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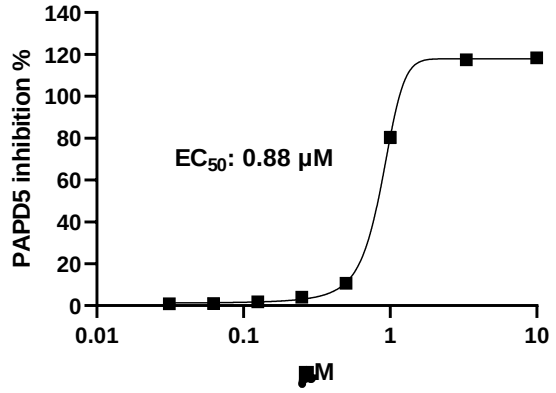
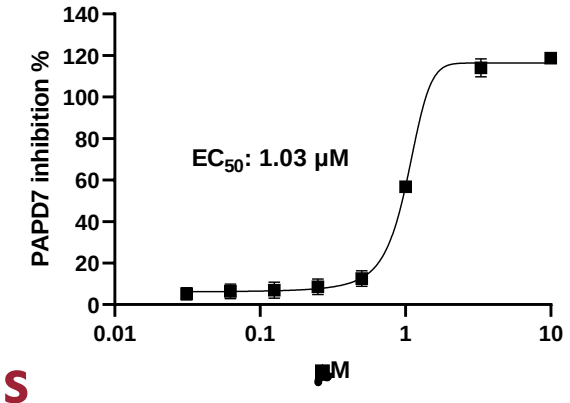
Mechanism of Action: AB-161 Targets PAPD5/7

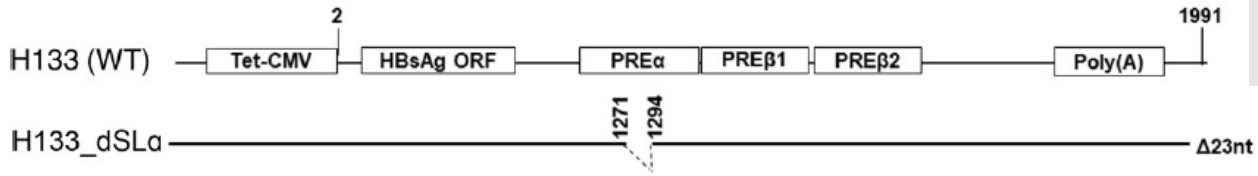
Inhibition of HBsAg is dependent on SLα within HBV RNA

- AB-161 inhibited enzymatic activity of recombinant PAPD5 and PAPD7





- Deletion of SLα (dSLα) with HBV PRE impaired HBsAg inhibition by AB-161
- Results consistent with HBV RNA stabilization being dependent on viral RNA-PAPD5/7 interaction through SLα



CMV-HBsAg	AB-161 EC_{50} (nM)	AB-452 EC_{50} (nM)
WT	2.5	2.8
dSLα*	>100	>100

*dSLα: stem-loop alpha deletion mutant

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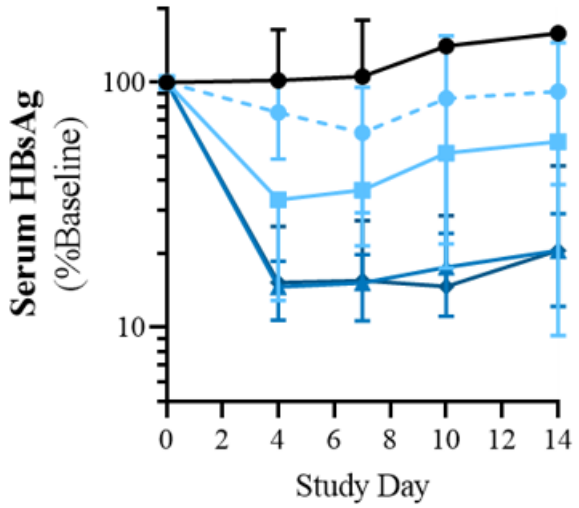
AB-161 Reduces HBsAg in AAV-HBV Mouse Model

Compound concentration in liver drives efficacy

- AB-161 effective as a once-daily dose in AAV-HBV mouse model (0.3, 1, 10, 30 mg/kg QD)
  - Dose-dependent reduction of HBsAg, also observed with BID dosing (0.3 and 1 mg/kg BID)
- HBsAg reduction achieved when fraction unbound C_{24h} > EC_{90} in liver

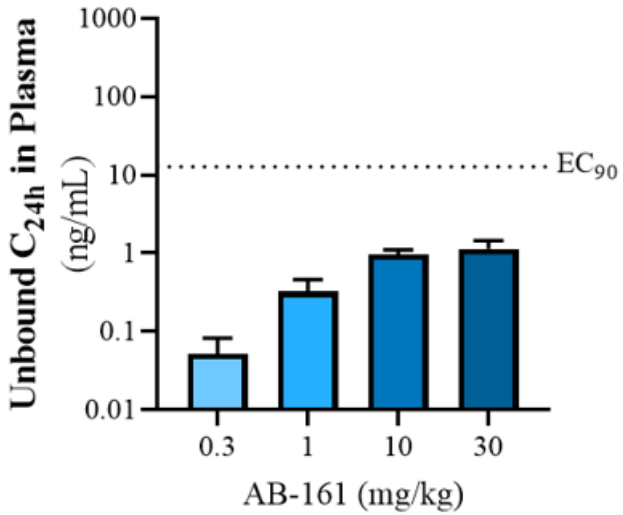
AAV-HBV mouse

AB-161 QD for 14 days

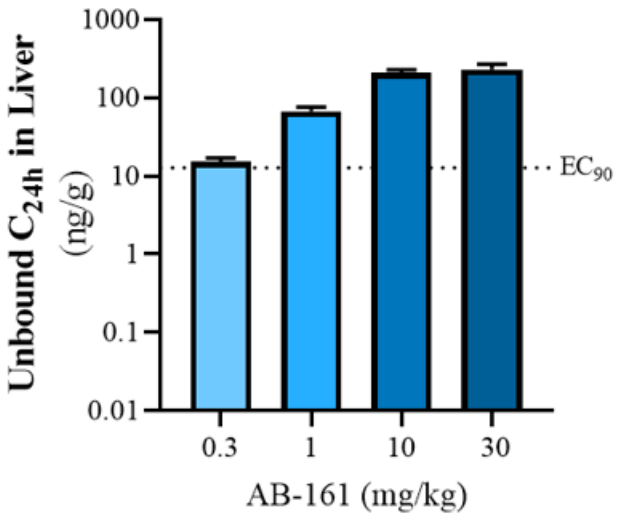


Fraction Unbound Concentrations (C_{24h})

$[AB\text{-}161]_{free}$: plasma



$[AB\text{-}161]_{free}$: liver



Vehicle · 0.3 mg/kg · 1 mg/kg · 10 mg/kg · 30 mg/kg

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AB-161: Preclinical Liver Centric Profile and Toxicology Assessment Support Further Development

- High liver concentrations and favorable liver-to-plasma ratios
- Biodistribution: highest exposures in liver compared to other organs
- Rat 14-day non-GLP repeat dose study
  - No liver enzyme biomarker changes noted
  - No clinical observations, clinical chemistry, or organ weight changes
- Dog 60-day non-GLP repeat dose study
  - No peripheral neuropathy effects at comparable exposures to AB-452 where findings were observed

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Conclusions

- AB-161, next generation HBV RNA destabilizer, effectively reduced HBV RNA and HBsAg in multiple HBV models *in vitro* and *in vivo*
  - HepG2.2.15 cells, HBV infected HepG2-NTCP and PHHs, PLC/PRF/5 cells
  - AAV-HBV mouse model: liver concentrations drive HBsAg reduction
- Mechanism of action studies
  - AB-161 mediates viral transcripts degradation, reduces viral proteins and viral replication
  - Targets PAPD5/7, protein complex that is recruited onto HBV RNA Stem-loop sequence to stabilize HBV RNA
  - HBV SLα critical for AB-161 antiviral activities
- Preclinical pharmacokinetics and repeat dose studies show favorable liver centricity and lack of peripheral neuropathy profiles
- AB-161 is currently completing IND-enabling studies

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