Preclinical Antiviral Profiling of AB-161, an Oral HBV Inhibitor that Destabilizes HBV RNA and Suppresses HBsAg

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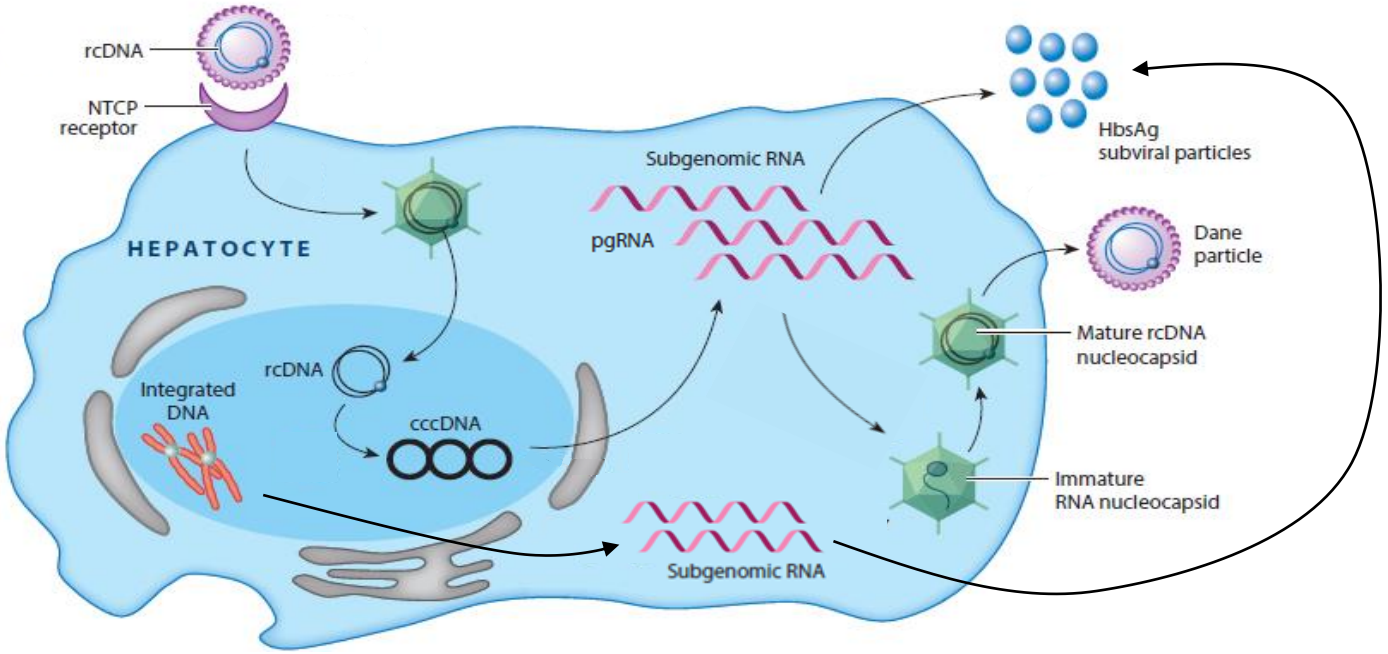
Persistence of Hepatitis B Virus

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 within host chromosomes, another source for HBsAg
- **HBsAg**
  - Contributes to immune exhaustion
  - Reduction of HBsAg by RNAi associated with signs of immune activation*
- **HBV RNA destabilizer**
  - Small-molecule oral approach to reduce HBV RNA and HBsAg

*Ganchua *et al* Reduction of HBsAg mediated by RNAi therapeutic AB-729 in chronic hepatitis B patients is associated with T cell activation and a decline in exhausted CD8 T cells. 2022 EASL.

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Scientific Rationale

HBV Hijacks PAPD5/7-Complex to Stabilize Viral Transcripts

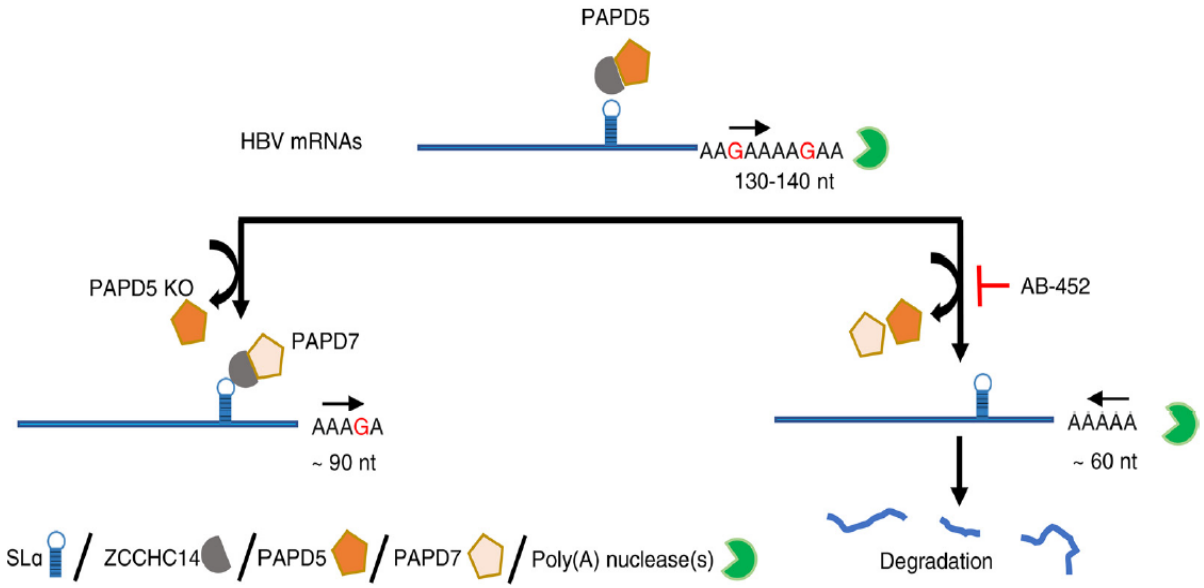

FIG 8 Proposed model illustrating the interplay between HBV *cis* element SLα and the host factors PAPD5 and PAPD7 in maintaining HBV RNA integrity and stability.

Liu *et al* 2021 J Virology

- HBV RNA contains a highly conserved stem-loop sequence (SLα) within its post-transcriptional regulatory element
- HBV RNA stability depends on the SLα interaction with the PAPD5/7-complex[1-4]
  - PAPD5 and PAPD7 are non-canonical poly(A) RNA polymerase proteins
  - 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-molecules targeting PAPD5/7 (AB-452, AB-161)
  - Degrade HBV RNA, reduce HBsAg and viral replication

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

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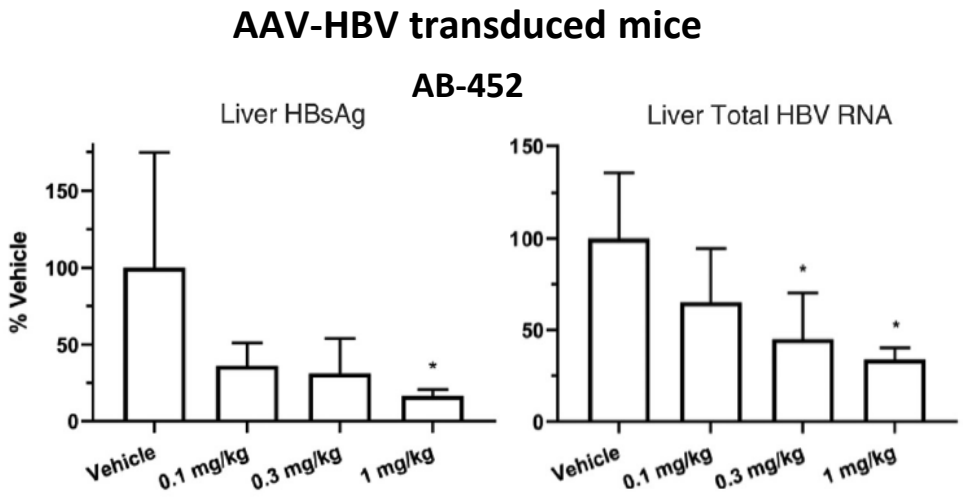
Next Generation HBV RNA Destabilizer

- AB-452: first-generation HBV RNA destabilizer
  - Potent *in vitro* and *in vivo* antiviral activity
  - 28-day toxicology studies well tolerated, but peripheral neuropathy observed during 13-week dosing in dog
  - Halted further development to focus on next generation effort
- Strategy for next generation HBV RNA destabilizer
  - Antiviral profile consistent with MOA
  - Antiviral effect driven by liver centricity and reduce systemic exposure
  - AB-161 selected as next generation HBV RNA destabilizer



Liu *et al* 2021 JV

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-161	2.3	27	>50	8.5	5.9	2.1 to 4.5 (GT A - H)	1.9 to 2.0

- AB-161 inhibits 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 HBV genome (partial genome encoding HBsAg)
- AB-161 shows board genotype coverage
- AB-161 retains activity in HBV mutants resistant to nucleos(t)ide analogs

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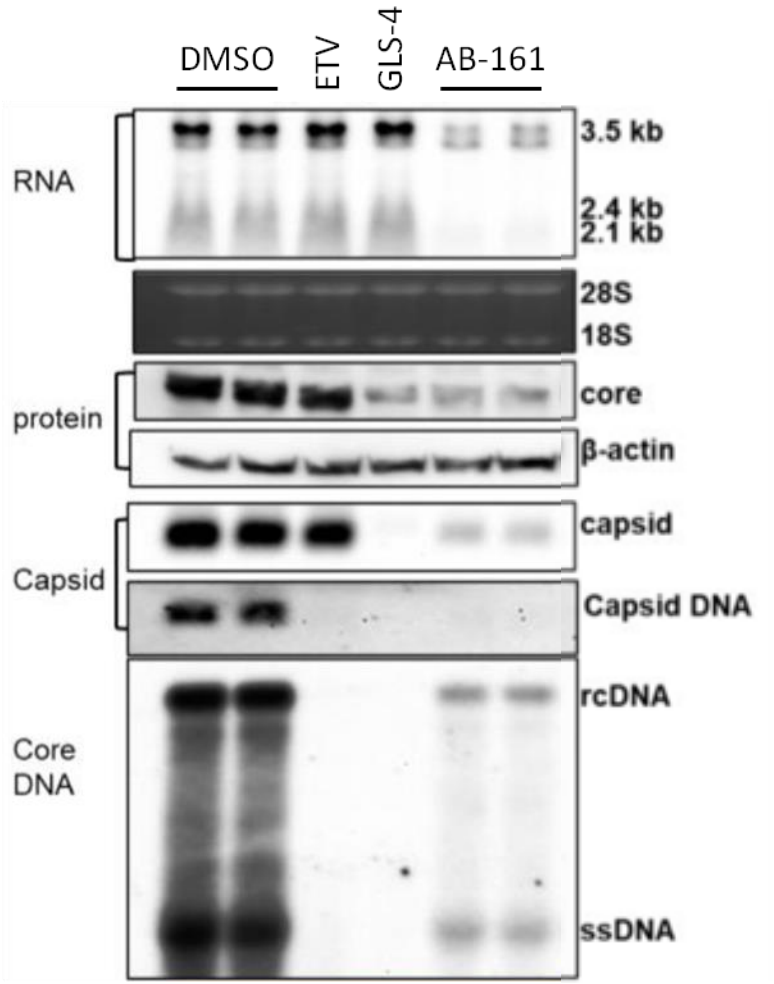
HBV RNA Destabilizer, but Not NUC or CAM, Reduces HBV RNA

AB-161 Interferes with Multiple Steps within Viral Life Cycle



- AB-161
  - Reduces pgRNA (3.5 kb) and sRNA (2.4 and 2.1 kb)
  - Reduces core protein, capsid particles, and HBV DNA
  - Differentiated from NUC and CAM
- Nucleoside analog (NUC)
  - ETV: only inhibits HBV DNA
- Capsid assembly modulator (CAM)
  - GLS-4 (CAM-A): induces formation of aberrant capsids
  - Reduces core, capsids, and viral replication
  - No effect on HBV RNA

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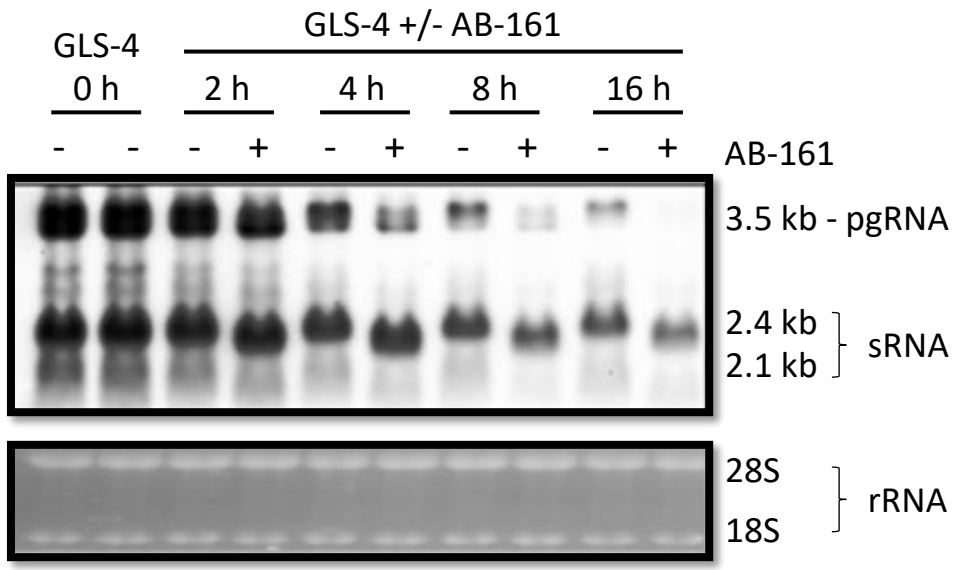
Time Dependent HBV RNA Degradation by AB-161



- 2 hour post addition of AB-161: rapid shortening of both pgRNA and subgenomic RNA (sRNA)
- Accelerated degradation of transcribed HBV RNA in the presence of AB-161 over time
- Combination of AB-161 and a CAM could enhance pgRNA degradation

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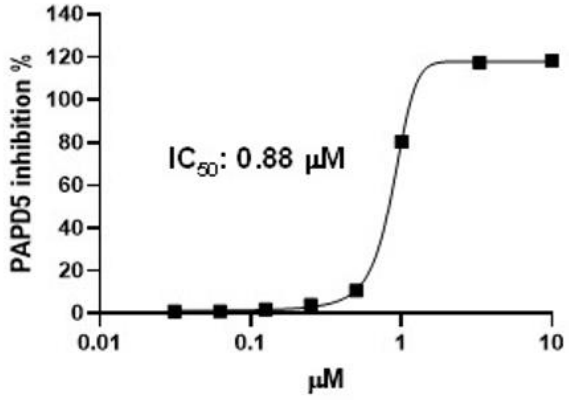
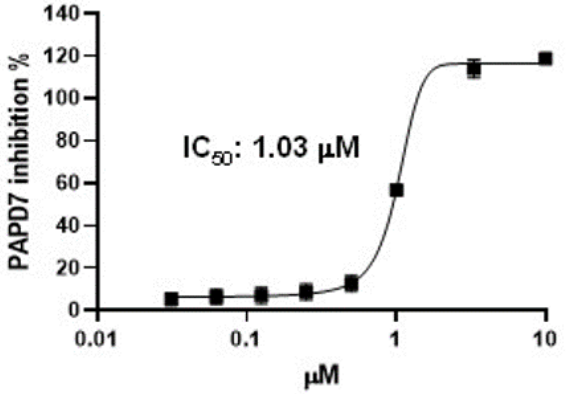
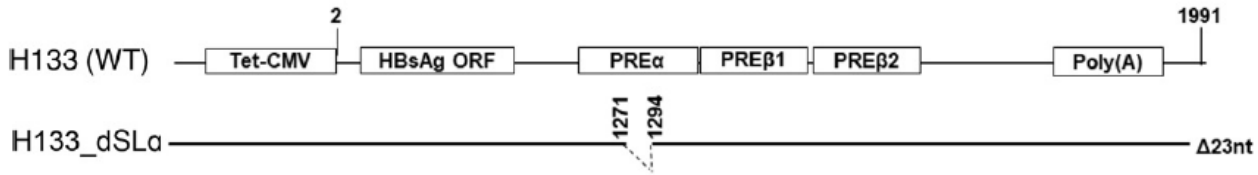
Mechanism of Action: AB-161 Targets PAPD5/7

Inhibition of HBsAg Dependent on SLα within HBV RNA

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







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

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

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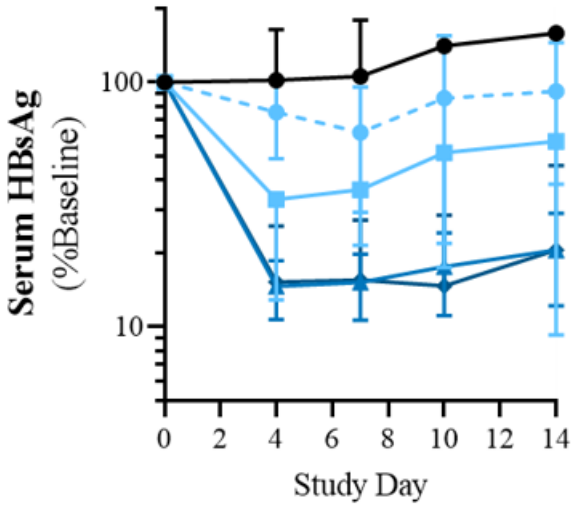
AB-161 Reduces HBsAg in AAV-HBV Mouse Model

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)
- Serum HBsAg reduction achieved when fraction unbound C_{24h} > EC_{90} in liver

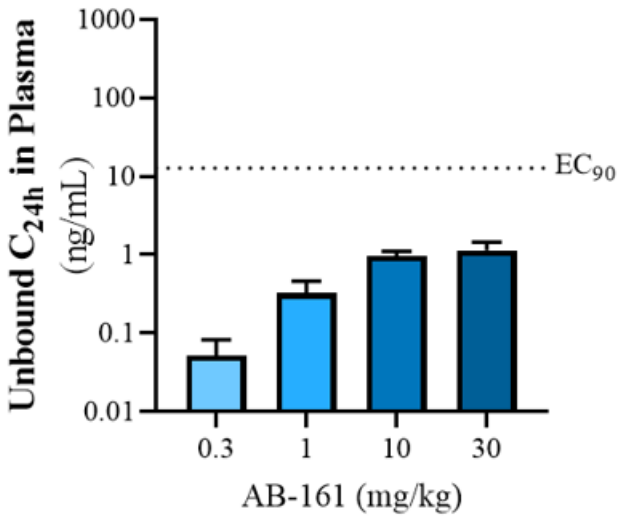
AAV-HBV mouse

AB-161 QD for 14 days

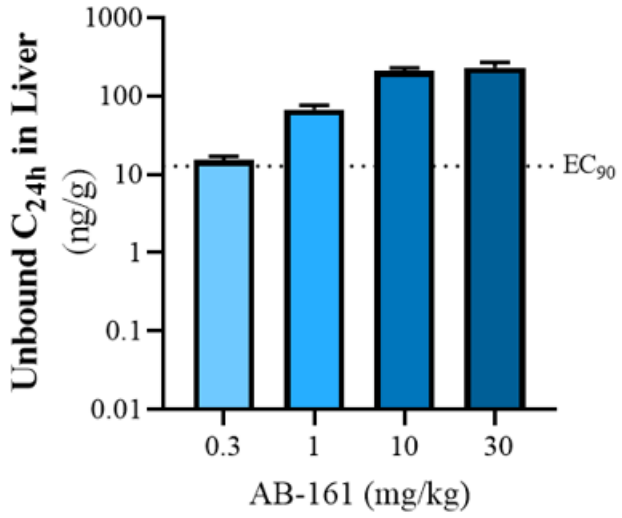


Fraction Unbound Concentrations (C_{24h})

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



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



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

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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 repeat dose toxicology studies
  - No liver enzyme biomarker changes noted
  - No clinical observations, clinical chemistry, or organ weight changes
- Dog 60-day non-GLP repeat dose toxicology studies
  - No peripheral neuropathy effects at comparable plasma exposures to AB-452 where findings were observed
- AB-161: next generation molecule that completed IND-enabling studies

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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 HBV RNA degradation and reduces viral proteins through targeting PAPD5/7
  - 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 being evaluated in Phase 1 clinical study

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Backup Slides

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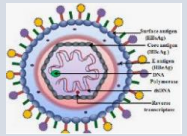
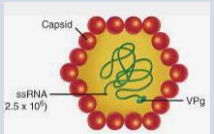
PAPD5 and PAPD7: Biology

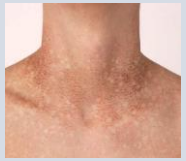
- PAPD5/7, also known as TENT4B/4A (terminal nucleotidyltransferases)
  - Non-canonical polyA polymerases: polyadenylate aberrant RNA precursors for degradation
  - Members of a family of 11 human TENTs (Yu and Kim 2020 Nature Rev Mol Cell Bio)
- TENTs may function redundantly to maintain host RNA metabolism
  - PAPD4 *in vivo* KO studies: no overt changes in animal behaviors (Kakanishi *et al* 2007 BBRC, Mansur *et al* 2017 RNA)
  - PAPD5/7 *in vitro* inhibition, KD/KO studies in liver cells: lack of global gene expression changes or cellular toxicities (Mueller *et al* 2018 Hep, Liu *et al* 2021 JV); further safety effects to be monitored in toxicology and pharmacology studies

PAPD5/7: Therapeutic indications





- **Hepatitis B infection**: PAPD5/7 regulate HBV transcripts stability[1,2]
- **Hepatitis A infection**: PAPD5/7 inhibits HAV *in vitro* and *in vivo*[3,4]
- **Dyskeratosis congenita (DC) and other telomere diseases**[5,6]
  - Shortened telomerase, cause by rare genetic mutations, limited treatment options
  - Inhibition of PAPD5 could be a therapy for patients with DC and other telomere pathologies



1. Mueller *et al* 2019 Hep, 2. Liu *et al* 2021 JV, 3. Kulsuptrakul *et al* 2021 Cell Rep, 4. Li *et al* 2022 PNAS, 5. Nagpal *et al* 2020 Cell Stem Cell, 6. Shukla *et al* 2020 Blood Advances

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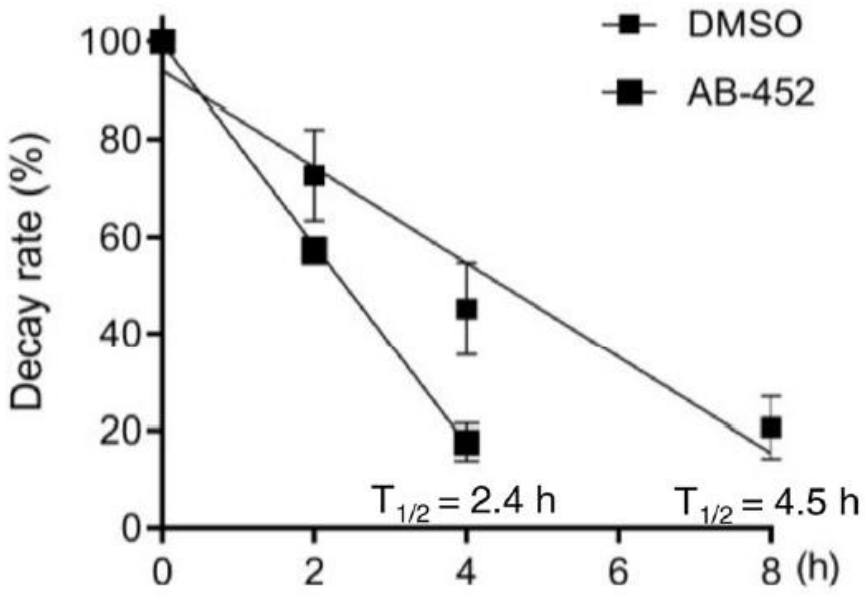
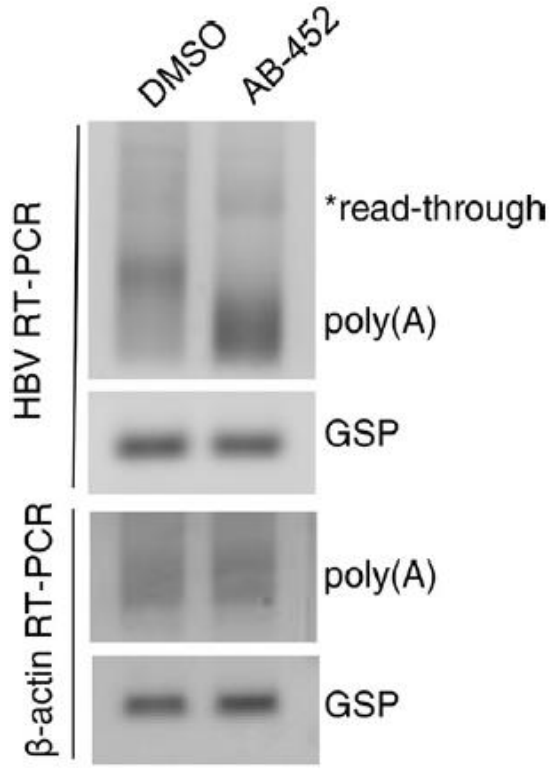
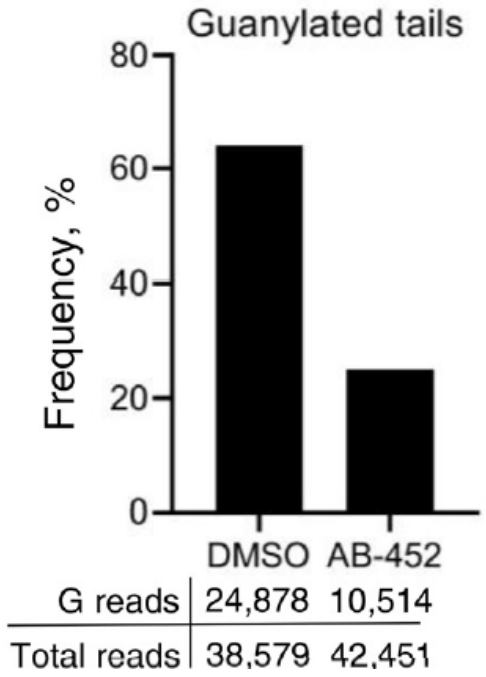
HBV RNA Destabilizer Triggers Faster Viral RNA Decay and Alters the Composition of HBV Poly(A) Tail *in vitro*

- HBV RNA half-life 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







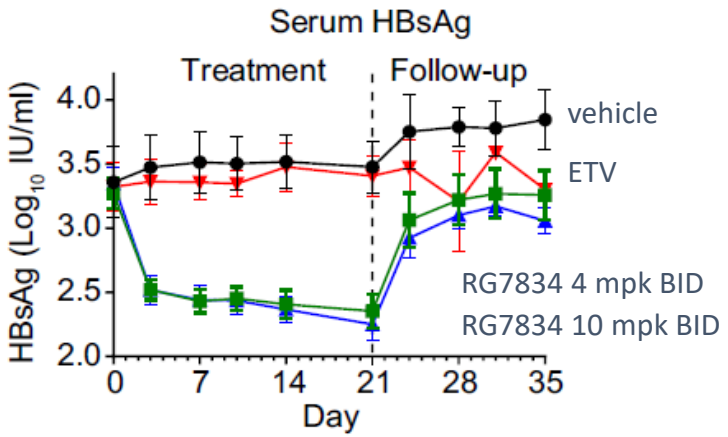
	DMSO	AB-452
G reads	24,878	10,514
Total reads	38,579	42,451

Liu *et al* 2021 JV

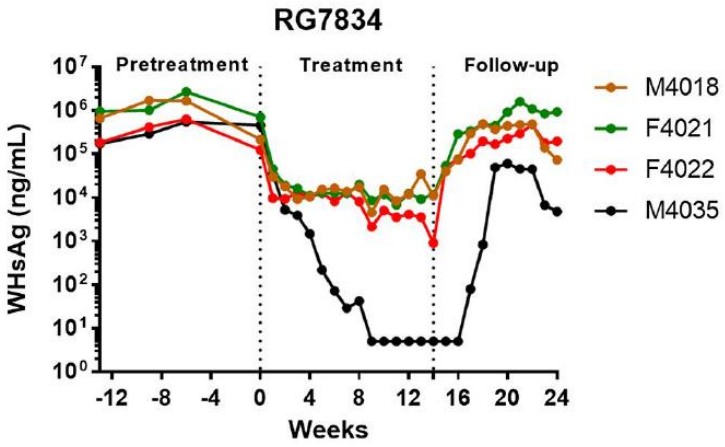
HBV RNA Destabilizers: Preclinical Proof-of-Concept

- 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]

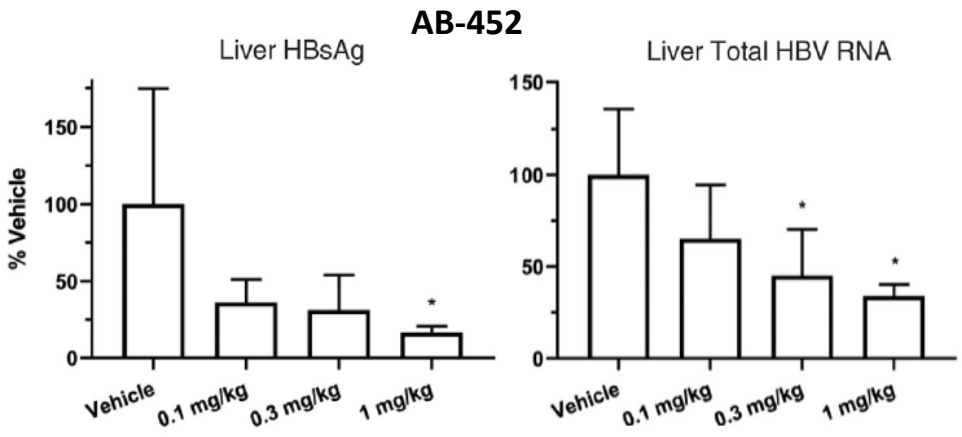
HBV infected humanized mice



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