Pharmacodynamics of durable HBsAg suppression by AB-729 short interfering RNA correlates with pharmacokinetics of RNA-induced silencing complex (RISC) loading within liver

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INTRODUCTION

- AB-729 is a novel siRNA agent utilizing RNA interference (RNAi)
- It inhibits HBV replication, reduces all HBV transcripts, and lowers all HBV antigens including notably surface antigen (HBsAg)
- AB-729 mediates a long duration of HBsAg reduction observed in both preclinical HBV models as well as in CHB subjects after single dose administration[1]
- It is currently unclear whether 1) direct RNAi activity due to persistence of AB-729 in the liver; 2) repression of HBV due to awakened antiviral immune responses (see Poster #SAT397) or 3) a combination of both contribute to the prolonged duration of HBsAg reduction

OBJECTIVES

- To understand correlations of AB-729 pharmacodynamic (PD) effect with pharmacokinetic (PK) profile in the liver in a preclinical HBV mouse model, to better understand the mechanisms driving the prolonged duration of HBsAg reduction following AB-729 dosing in CHB patients

BACKGROUND

- AB-729 mediates HBV RNA degradation through RNAi activity

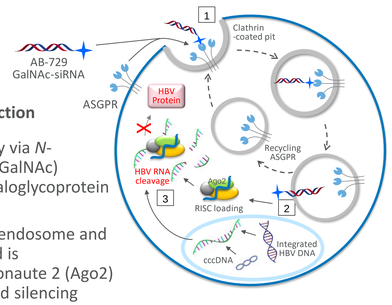


Fig 1: AB-729 mode of action

1. Liver cell-specific entry via *N*-Acetylgalactosamine (GalNAc) binding to ASGPR (asialoglycoprotein receptor)
2. AB-729 escapes from endosome and antisense guide strand is incorporated into Argonaute 2 (Ago2) protein in RNA-induced silencing complex (RISC)
3. Ago2 mediates sequence-specific cleavage of HBV RNAs guided by AB-729 antisense strand sequence, leading to HBV RNA degradation

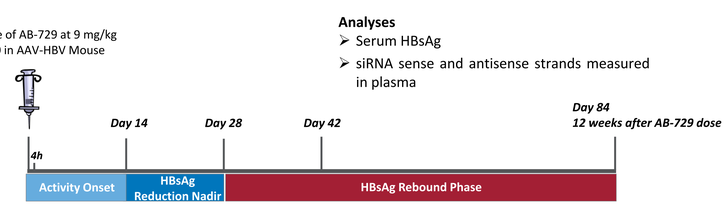
- Use of an AAV-HBV mouse model enables relation of AB-729 effects on HBV markers with RNAi activity and siRNA levels in plasma and liver.
  - HBV PD marker: Serum HBsAg reduction
  - RNAi activity marker: Degree of RISC-loaded AB-729 antisense in liver
  - AB-729 levels in plasma and liver

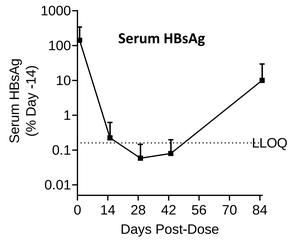
RESULTS

1. AB-729 pharmacokinetic profile in plasma does not correlate with HBsAg reduction

Single dose of AB-729 at 9 mg/kg on Day 0 in AAV-HBV Mouse

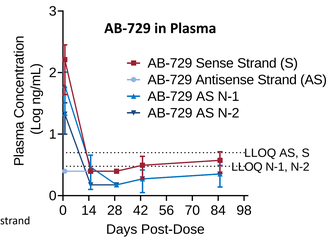
Analyses
- Serum HBsAg
- siRNA sense and antisense strands measured in plasma





Following a single dose of AB-729 at 9 mg/kg, maximum reduction of >3.2 $\log_{10}$ in HBsAg was observed at 4 weeks post-dose, followed by rebound after 6 weeks post-dosing.

- AB-729 presence in plasma is transient, with rapid elimination
- Nadir of HBsAg reduction does not correlate with PK profile of AB-729 in plasma



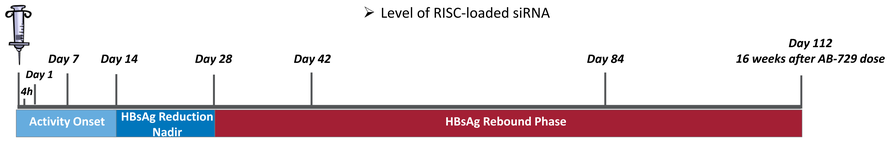
Values below lower limit of quantification (LLOQ) set as ½ LLOQ
AB-729 AS N-1 and AB-729 AS N-2 are active metabolites of AB-729 antisense strand

2. In liver, levels of AB-729 siRNA do not correlate with pharmacodynamic profile of HBsAg reduction

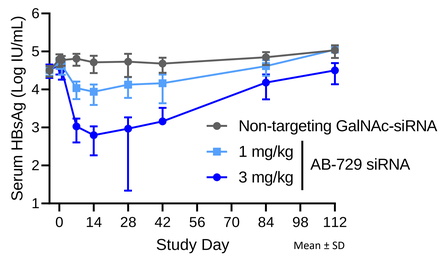
Single dose of AB-729 siRNA with surrogate GalNAc ligand at 1 mg/kg or 3 mg/kg or non-HBV Targeting GalNAc-siRNA at 3 mg/kg on Day 0

AAV-HBV Mouse
28 days post-AAV-HBV injection

Analyses
- Serum HBsAg
- Total siRNA in liver
- Level of RISC-loaded siRNA

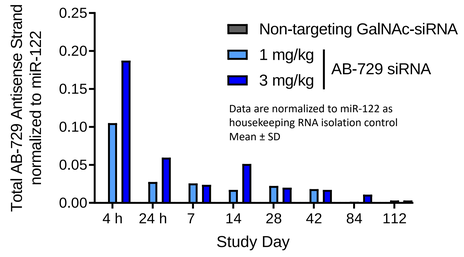


A Following a single dose of AB-729 siRNA at 1 mg/kg or 3 mg/kg, a dose responsive decline in serum HBsAg was observed.
- Maximum decline in HBsAg was observed at 2 weeks post-dose, followed by rebound after Day 28.
- At 1 mg/kg, mean maximum reduction in serum HBsAg was 0.9 $\log_{10}$ at Day 14, compared to baseline
- At 3 mg/kg, mean maximum reduction in serum HBsAg was 1.9 $\log_{10}$ at Day 14



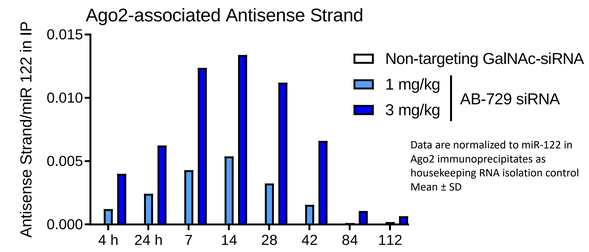
B Pharmacokinetics of total AB-729 siRNA levels in liver does not correlate with pharmacodynamic HBsAg reduction in this AAV-HBV mouse model.
- AB-729 siRNA levels in liver decline 4 h post-dosing
- By 16 weeks following single dose administration, 1.6 to 2.9% of AB-729 siRNA relative to C_{max} levels remains in the liver

In contrast, maximum knockdown of HBsAg occurs between 14-28 days following AB-729 siRNA administration, at a time when AB-729 siRNA levels are declining in the liver.



3. Pharmacokinetic profile of RISC-loaded AB-729 antisense strand in liver correlates with pharmacodynamic profile of HBsAg reduction

Degree of AB-729 antisense strand loading onto Ago2 in RISC complexes coincides with HBsAg reduction
- Ago2-associated AB-729 antisense strand increases over time, peaking at Day 14 and declining thereafter
- Peak of Ago2-associated AB-729 antisense strand at Day 14 coincides with nadir of HBsAg silencing
- Decline in Ago2-associated AB-729 antisense strand mirrors rebound in HBsAg observed after Day 28



CONCLUSIONS

- AB-729-mediated HBsAg reduction is associated with loading of AB-729 antisense strand onto the RISC complex in AAV-HBV mouse
  - This PK profile of RISC-loaded antisense strand is supportive of clinical dosing schedules being explored for AB-729 (every 4, 8 and 12 weeks)
- AB-729 pharmacodynamic effects in preclinical models do not directly correlate with pharmacokinetics of total siRNA levels in plasma or liver
- These preclinical data suggest that the direct-acting RNAi activity of AB-729 has a limited half-life and other mechanisms may account for prolonged HBsAg reduction in CHB patients

REFERENCES

1. Yuen MF, et al. AASLD TLM, November 13-16, 2020.
2. Lee ACH, et al. Poster FRI184. EASL International Liver Congress, April 12, 2019.

ACKNOWLEDGEMENTS

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METHODS

- AB-729 sense and antisense strands were quantitated by either LC-MS/MS or stem-loop quantitative reverse-transcription polymerase chain reaction (qRT-PCR)
- Ago2-associated AB-729 antisense strand was quantitated by anti-Ago2 antibody immunoprecipitation of liver lysates, followed by RNA isolation and stem loop qRT-PCR

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