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Pharmacodynamic durability of ALG-125755, a GalNAc-conjugated siRNA, correlated with total and RNA induced complex (RISC) bound siRNA in mouse liver

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ALIGOS THERAPEUTICS

BACKGROUND AND AIMS

For the functional cure of chronic hepatitis B, a sustained loss of hepatitis B surface antigen (HBsAg) is required. Targeted small interfering RNAs (siRNAs) have recently demonstrated significant clinical reduction of HBsAg. ALG-125755 is a novel N-acetylgalactosamine (GalNAc)-conjugated siRNA currently in clinical development.[1,2,3] Here we demonstrate the mechanism of action of ALG-125755 and correlate the durable pharmacodynamics to total and RNA induced silencing complex (RISC) bound siRNA in mouse liver in the adeno-associated virus (AAV)-HBV mouse efficacy model.

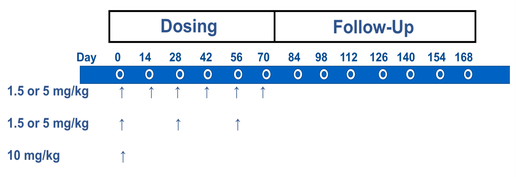
METHOD

To confirm the mechanism of action of ALG-125755, argonaute-2 (AGO-2) degradation of the target S-region HBV RNA sequence induced by the antisense strand (AS), ALG-125736, was qualitatively measured using denaturing polyacrylamide gel electrophoresis.

Total and RISC-bound siRNA quantification was performed in the harvested livers from a previously reported AAV-HBV mouse efficacy study, where a 10 mg/kg single dose or repeat doses up to 70 days at 1.5 or 5 mg/kg every other week (Q2W) or every four weeks (Q4W) demonstrated significant and durable decline in serum HBsAg.[3] Weekly (Days 1-70) or biweekly blood collection for HBsAg and HBeAg readouts.

Liver samples (Days 14, 28, 70, each prior to the dose, and postdose timepoints at Days 98 and 168) from the single dose and repeat (Q2W) dose groups were analyzed by liquid chromatography-high resolution accurate mass method for total siRNA and immunoprecipitation/reverse transcription-quantitative polymerase chain reaction method for RISC-bound siRNA. The study design is shown below:

STUDY DESIGN EFFICACY IN AAV-HBV MOUSE



RESULTS

MECHANISM OF ACTION OF ALG-125755 DEMONSTRATED IN VITRO AND IN VIVO

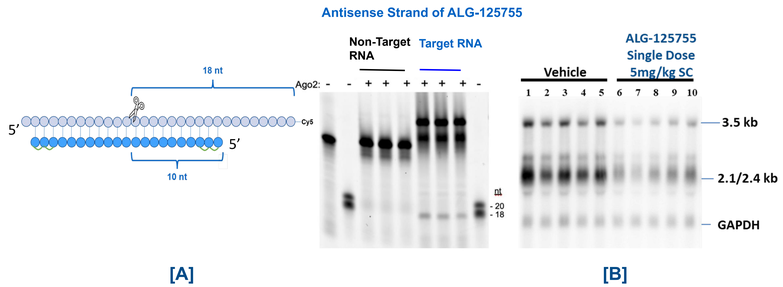


Figure 1: (A) Cleavage of complementary target RNA mediated by antisense strand of ALG-125755 associated with human AGO2; (B) Cleavage of HBV RNA through RNAi in mouse livers 42 days following a single SC dose of ALG-125755 at 5 mg/kg

(A) IN VITRO

- The antisense strand of ALG-125755 efficiently induced cleavage of a chemically synthesized target RNA in an in vitro assay with purified human AGO2
- The results demonstrate that the mechanism of action of ALG-125755 in reducing HBsAg expression is through RNAi degradation of HBV RNA mediated by AGO2[1]

(B) IN VIVO

- The engagement of ALG-125755 to AGO2 and on-target activity of the siRNA in reducing HBV RNA levels in mouse liver following a single SC dose at 5 mg/kg was confirmed by Northern blot analysis for HBV RNA. The results confirmed that the underlying mechanism of viral antigen reduction is the degradation of the liver HBV RNA 2.1/2.4 kb encoding HBsAg and 3.5 kb HBV RNA encoding other viral proteins HBeAg[1]

GOOD CORRELATION OF PHARMACODYNAMIC DURABILITY WITH TOTAL AND RISC-BOUND siRNA IN AAV-HBV MICE

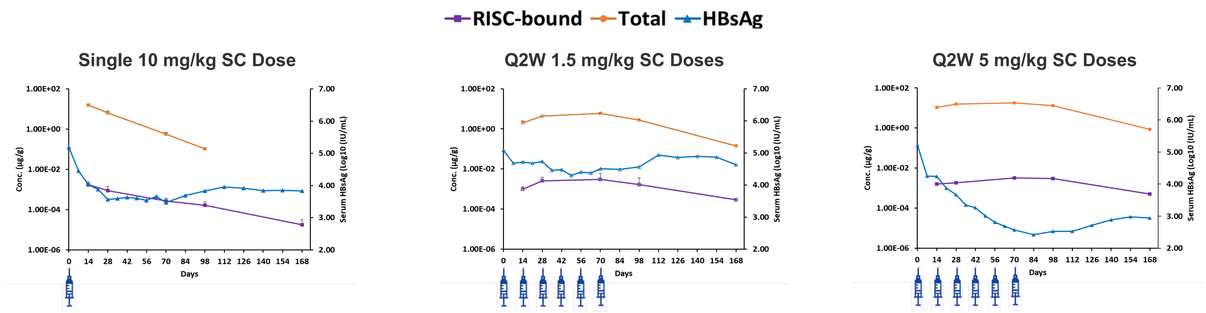


Figure 2: Concentration-time profile in liver of total siRNA and RISC-bound siRNA, and serum HBsAg levels following subcutaneous administration of a single 10 mg/kg, or repeated Q2W at 1.5 and 5 mg/kg (six doses each) in AAV-HBV mice

- A dose dependent and sustained (≥70 days post last dose) reduction of serum HBsAg, the PD marker observed with ALG-125755 in AAV-HBV mice
- Following a single dose,
  - The half-life of RISC-bound siRNA (27.9 days) was two times longer than that of the total liver siRNA (11.8 days) supporting less frequent dosing
- Following repeat doses,
  - The increase in total and RISC-bound concentrations increased with dose
  - Sustained RISC-bound siRNA concentrations and total siRNA concentration were noted with quantifiable concentration ≥70 days post last dose (Day 168)
  - The kinetics of total and RISC-bound siRNA were generally similar
- The durability of the PD response tracked with both the total liver concentrations and with the RISC-bound concentration of the siRNA

CONCLUSIONS

- Binding of ALG-125755 to AGO-2 was demonstrated in vitro and in vivo, confirming that the mechanism of action for ALG-125755 is consistent with that of an siRNA
- Reductions in serum HBsAg levels was dose and dosing-regimen dependent and it was sustained for ≥70 days post-last dose ALG-125755 in AAV-HBV mice
- Pharmacodynamic response of HBsAg reduction and durability correlated with total siRNA and RISC-bound siRNA in mice liver
- The long half-life of the RISC-bound siRNA in mice indicates that dosing of ALG-125755 in human could be less frequent than monthly dosing
- Clinical development of ALG-125755 as a potential best-in-class HBV siRNA is ongoing; dosing in healthy volunteers was initiated in October 2022, and dosing in CHB patients in December 2022

REFERENCES

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