Prediction of In Vivo Toxicity and Clinical Effects Using Antisense Oligonucleotides in an In Vitro Model Utilizing 3D InSight™ Human Liver Microtissues

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Background

Current standard of care for chronic hepatitis B (CHB) viral infection can effectively inhibit viral DNA replication but fail to reduce Hepatitis B surface antigen (HBsAg). ALG-020572 and ALG-020576 are both 5' GalNAc-conjugated antisense oligonucleotides (ASOs) that were being developed for the treatment of CHB by targeting the HBsAg coding region of the Hepatitis B Virus (HBV) genome. Once these ASOs enter hepatocytes via uptake through asiaglycoprotein receptors (ASGPRs), the GalNAc moiety is rapidly cleaved to release the active ASO moieties. Hepatoxicity has been previously associated with ASOs across multiple therapeutic areas. Therefore, we sought to examine whether an in vitro model utilizing human 3D liver microtissues (hLiMTs) could be applied to evaluate ASO-mediated cytotoxicity, thus providing a more relevant model to assess hepatotoxicity in humans. Here we will present data extending the utility of the model, along with assessment of additional ASOs beyond those developed at Aligos for the treatment of CHB.

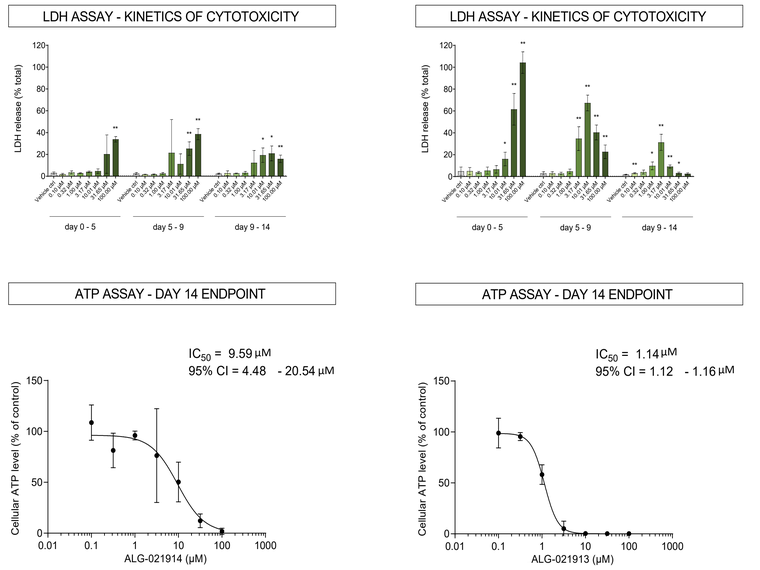
Methods

- ASOs were selected based on published data or experimental data generated by Aligos; ASOs that showed signs of hepatotoxicity in either preclinical species or in humans were selected along with ASOs that had no hepatotoxicity
- All ASOs were synthesized by Aligos and formulated in sterile water at 2 mM and experiments were performed blinded using ALG numbers
- 3D InSight™ human liver microtissues (hLiMT_674 production) were cultured in the absence of BSA and were maintained for 14 days (N ≥ 3 per concentration tested)
- The top concentration of ASOs tested was 100 µM, with half-log dilutions down to 0.1 µM. ASOs were dosed on Days 0, 5, and 9
- Media was collected on Days 0-5, 5-9, and 9-14 for assessment of LDH release (Promega Cat. No. J2380)
- Intracellular ATP levels were measured by CellTiter-Glo® (Promega, Cat. No. G9243) on Day 14 to assess cytotoxicity

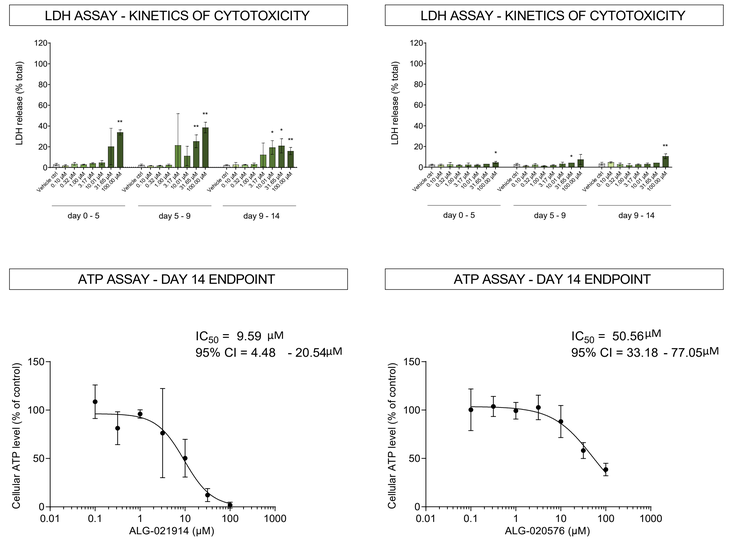
Positive and negative control ASOs were selected for testing based on in vivo toxicity and/or clinical findings

Compound	Target/ Indication	ALG #	GalNAc	In Vivo Toxicity	Clinical Effects
EZN-4176[1]	Androgen Receptor for prostate cancer	ALG-021914	No	Tumor growth inhibition in mice at 60 mg/kg with no ↑ALT reported	↑ALT/AST (~40%) at 10 mg/kg QW (~600 mg); clean at 0.5, 1, 2, 4, 6.5 mg/kg QW and reduced effects at 10 mg/kg with ↓frequency
Eplontersen[2]	hATTR	ALG-021911	Yes	No findings reported	Not reported; currently in Phase 3
ION558807[3,4]	*CXCL12* for Oncology	ALG-021913	No	Toxic in mice at 17 and 52 mg/kg; ↑caspase 3/7 activity (IC_{50} = 61 nM)	Unknown
GSK-836/ Bepirovirsen[5]	CHB	ALG-020001	No	No findings noted in AAV-HBV mouse model up to 10 mg/kg (Aligos)	No ↑ALT noted at 150 mg; 2 patient at 300 mg (ALT flare?)
ALG-020572[6,7]	CHB	ALG-020572	Yes	No findings noted in mice or monkeys up to 50 mg/kg	↑ALT noted in CHB patients at 210 mg
ALG-020579[6,7] (parent ASO of ALG-020572)	CHB	ALG-020579	No	No findings noted in mice or monkeys up to 50 mg/kg	↑ALT noted in CHB patients at 210 mg
ALG-020576[8]	CHB	ALG-020576	Yes	No findings noted in mice or monkeys up to 50 mg/kg	Not tested
ALG-021544[8]	CHB	ALG-021912	No	↑ALT noted in mice at 30 mg/kg	Not tested

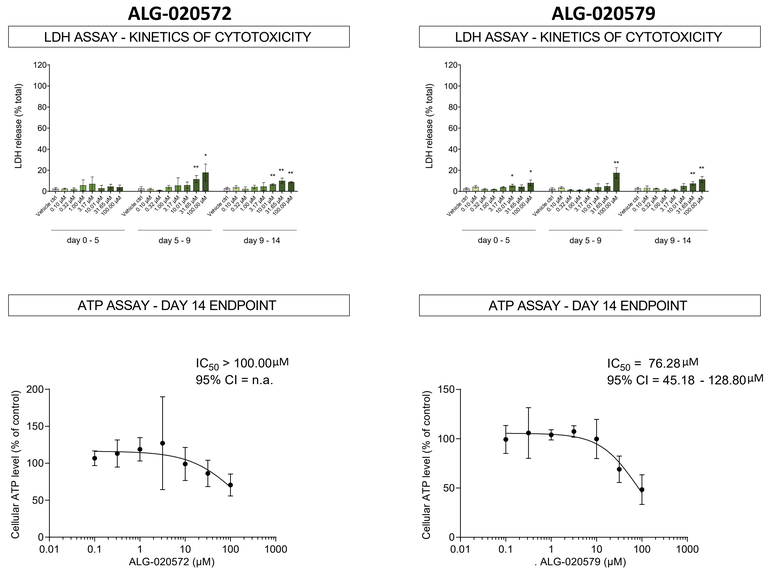
EZN-4176 and ION558807 had significant effects on LDH release and cytotoxicity



Toxicity in hLiMTs confirmed that chemical modifications on a specific sequence (ALG-021914) could reduce toxicity by 5-fold (ALG-020576)



ALG-020572 and ALG-020579 showed differential effects on hLiMTs toxicity where greater effects were noted with the unconjugated ASO, ALG-020579



- In liver, ALG-020579, the unconjugated ASO, had high concentrations in mice, monkey, and human[6,7] where as ALG-020572, the GalNac conjugated ASO, was BQL
  - The clinical outcome following multiple doses ALG-020572 at 210mg in HBeAg negative CHB subjects was not predicted in nonclinical studies or single ascending does in healthy volunteer studies up to 480 mg; development of ALG-020572 has been terminated
  - Utilization of 3D human liver microtissues may offer greater sensitivity to toxicity induced by ASOs

Compound	LDH Release	ATP IC50 (µM)	In Vivo Toxicity	Clinical Effects
EZN-4176[1]	**Yes**	**9.6**	**Unknown**	**Yes**
Eplontersen[2]	**No**	**>100**	**No**	**No**
ION558807[3,4]	**Yes**	**1.1**	**Yes**	**Unknown**
GSK-836/ Bepirovirsen[5]	**No**	**>100**	**No**	**No**
ALG-020572[6,7]	*Minimal*	*>100*	*No*	*Yes*
ALG-020579[6,7] (parent ASO of ALG-020572)	**Minimal**	**76.3**	***No***	**Yes**
ALG-020576[8]	*No*	*50.6*	*No*	-
ALG-021544[8]	**Yes**	**8.95**	**Yes**	-

- Generally good concordance between LDH release and ATP levels, where more potent ASOs showed effects on both parameters
- ASOs with IC_{50}'s less than 100 µM also showed signs of hepatotoxicity either preclinically or in humans

Conclusions

- Across the 8 ASOs assessed in these studies, generally good correlation between cytotoxic effects in 3D human liver microtissues and either preclinical or human outcomes of hepatotoxicity
- Low compound requirement, fast turnaround time, and free uptake of ASOs (no transfection is required) using human cells make the model attractive to reduce compound attrition due to cytotoxicity
- Utility of assay to identify potential hepatotoxicity requires additional testing of ASOs to truly determine value of the model but may be useful as an early screen to reduce the number of preclinical animal studies and ASO attrition

References

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