Lack of Translation of Favorable Nonclinical Toxicology Profile of Antisense Oligonucleotide (ASO) ALG-020572 to Chronic Hepatitis B (CHB) Patients

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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 S antigen (HBsAg). ALG-020572 is a 5' GalNAc-conjugated antisense oligonucleotide (ASO) that was being developed for the treatment of CHB by targeting the HBsAg coding region of the Hepatitis B Virus (HBV) genome. Once ALG-020572 enters hepatocytes via uptake through asiaglycoprotein receptors (ASGPRs), the GalNAc moiety is rapidly cleaved to release the active ASO moiety, ALG-020579.

Methods

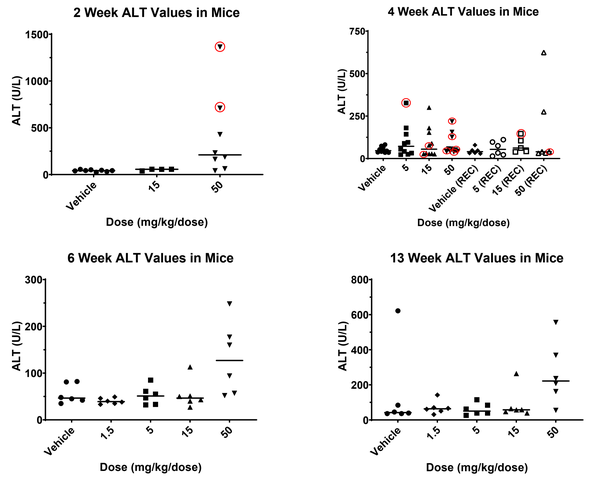
- ALG-020572 was formulated in water for injection.
- CD-1 mice and cynomolgus monkeys were dosed subcutaneously (SC), twice weekly (BIW) for two weeks and then weekly (QW) thereafter in 2-week (5 doses) and 4-week studies (7 doses) followed by chronic studies up to 20 weeks in mice or 24 weeks in monkeys (doses of 0, 5, 15, 50/30 mg/kg/dose). The toxicokinetic and toxicology assessments included clinical observations, clinical chemistry, hematology, coagulation, cytokines and complement (monkey only) and anatomic pathology.
- Following a favorable toxicology and toxicokinetic profile, a two-part, double-blind, randomized, placebo-controlled Phase 1 study (NCT05001022) was initiated for ALG-020572 to evaluate the safety, tolerability, PK and antiviral activity.
  - In Part 1, 32 healthy volunteers (N=6 active and 2 control/cohort) received single ascending subcutaneous (SC) doses of 50, 150, 300 and 480 mg ALG-020572, which were found to be well tolerated.
  - Part 2 was designed to evaluate multiple ascending SC doses of ALG-020572 in virologically suppressed HBeAg negative CHB subjects (ALG-020572 or placebo on Days 1, 4, 8, 11, 15, 22 and 29). A single cohort received 210 mg of ALG-020572 or placebo, SC, before the study was terminated.
  - Assessments included treatment emergent adverse events (TEAEs), vital signs, physical examination, electrocardiograms (ECG), laboratories, PK, and hepatitis B virus viral markers (in Part 2 only).

ALG-020572 demonstrated a favorable nonclinical safety profile

- ALG-020572 and ALG-020579 were neither mutagenic in the *in vitro* Ames assay nor clastogenic in the *in vitro* or *in vivo* MNT assays
- There were no cardiovascular, respiratory, or CNS findings in safety pharmacology or repeat-dose toxicity studies up to the highest doses tested of 50 mg/kg/dose
- In repeat-dose toxicity studies, ALG-020572 was well tolerated
  - In 2-week, 5-dose, range-finding studies in mice and monkeys, ALG-020572 was well tolerated with NOAELs at the highest dose tested of 50 mg/kg/dose BIW
  - In 1-month, 7-dose, GLP studies in mice and monkeys, ALG-020572 was well tolerated with the NOAELs at the highest doses administered of 50 mg/kg/dose BIW/QW
    - › In a pattern typical of rodent specific oligo-related inflammatory response[1], repeat-dose studies in mice demonstrated ALG-020572 attributed sporadic minimal to mild ALT/AST elevations with minimal non-adverse single-cell necrosis noted and no exacerbation at 1-month compared to 2-week study.
    - › Minimal to moderate histopathological changes were noted at the injection site, lymph nodes, liver and kidney were consistent with oligo-related uptake in these tissues[1] (mononuclear cell infiltration, basophilic granules within macrophages) with partial to complete recovery
    - › There were no adverse changes in hematology, serum chemistry or coagulation parameters, cytokines, or complement activation (monkey only) in either species up to 50 mg/kg/dose
  - Chronic toxicity studies were discontinued at 20 weeks (22 doses) in mouse and 24 weeks (26 doses) in monkey due to clinical safety findings and termination of the program
    - › No adverse toxicology findings were noted in either species
    - › No significant worsening of ALT noted in the mouse chronic study (Day 93); No ALT changes were noted in monkeys

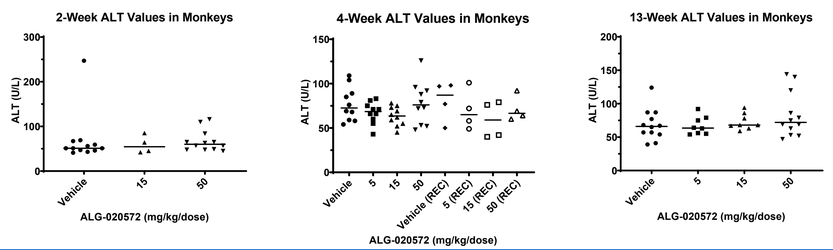
Mice: Sporadic ALT elevations with minimal liver histopathology

Mice: Dosing of ALG-020572 resulted in sporadic ALT elevation and minimal single cell necrosis (circled red) in individual mice that was not dose responsive and did not correspond to liver concentrations of ALG-020579. Longer term dosing resulted in dose responsive ALT elevations but no single cell necrosis



Study	Mouse Endpoint Assessed 24 hrs after last dose	5 mg/kg/dose	15 mg/kg/dose	50 mg/kg/dose
2 weeks (5 doses)	Minimal single-cell	ND	0/4	1/4 *
	Liver ALG-020579 concentration (µg/g)	ND	72.5	112*
4 weeks (7 doses)	Minimal single-cell	2/20	3/20	13/20
	Liver ALG-020579 concentration (µg/g)	18.2	28.1	64.1
20 weeks (22 doses)	Minimal single-cell	ND	ND	0/32
	Liver ALG-020579 concentration (µg/g)	ND	ND	110

Monkeys: No changes in liver enzymes or adverse liver histopathology observed



Study	Timepoint (hrs) after last dose	Liver ALG-020579 Concentration at 5 mg/kg/dose (µg/g)	Liver ALG-020579 Concentration 15 mg/kg/dose (µg/g)	Liver ALG-020579 Concentration 50 mg/kg/dose (µg/g)
2 weeks (5 doses)	Mean of 48 and 72	ND	601	1015*
4 weeks (7 doses)	48	243	548	995
24 weeks (26 doses)	48	ND	ND	1643~

*Results averaged from 2 studies, ~average of 3 animals; ND-not determined

- Rodents are generally more sensitive to pro-inflammatory and single cell hepatocellular effects of oligos which has not translated to safety risks in human[2,3]
- Greater elevations of ALT in mice with similar adverse liver histopathology have been observed with a previous Aligos compound which did not translate to humans up to 400 mg QW for 12 weeks[4]
- No ALT elevation or liver histopathology noted in monkeys at ~10-fold higher liver concentrations

ALG-020572 had a favorable nonclinical and single ascending dose (SAD) safety profile in healthy volunteers but was discontinued due to safety concerns following multiple doses in CHB patients[5]

- Single SC doses up to 480 mg well tolerated with no safety signals identified
- Multiple Doses: 4 of the 6 subjects who received 210 mg ALG-020572 experienced significant ALT flares (≥10xULN) after receiving 2-7 doses of ALG-020572. 2 of the 6 subjects also developed liver related symptoms including nausea, vomiting, and fatigue along with elevated total bilirubin values (peak 2xULN), with one subject briefly being hospitalized (SAE).
- Liver biopsies obtained in 3 of the 4 subjects with significant ALT flares showed varying histologic findings but all were assessed as being suggestive of DILI by Study review committee and ALT Flare Committee
- All subjects recovered with DILI related lab values returning to, or near to, baseline after discontinuation of study drug and no sequelae were observed over the following 4.5 months of follow-up
- No other concerning safety findings (TEAEs, laboratories, VS, EKG, physical examination) were observed

Lower ALG-020579 liver concentrations in CHB subjects than monkeys

Subject #	ALG-020572 210mg	ALG-020579 in liver (µg/g) 11-13 days after last dose	Fold Change compared to Monkey at NOAEL in 4-week study
#1	7 doses	164	6.1
#2	6 doses	276	3.6
#3	2 doses	588	1.7

- Liver biopsy samples from CHB subjects collected 11-13 days after last dose from 3 of 4 subjects with significant ALT flares (>10X ULN)
- Liver concentrations of ALG-020579 at time of biopsy were high (2-8-fold above projected liver EC_{90} levels) but remained 1.7-6.1-fold below the no observed adverse effect (NOAEL) liver concentrations observed in toxicology studies in monkeys. Given the time of biopsy was <1 liver t1/2, the concentrations at the time of adverse effect are estimated to be similar to the monkey NOAEL liver concentrations.
- ALG-020572 was not detected in liver, demonstrating effective cleavage to the active parent ASO
- Neither increases in ALT nor reductions in HBsAg correlated with liver ALG-020579 concentrations

ALG-020572 demonstrated a favorable nonclinical efficacy profile and clinical antiviral activity

- ALG-020579 potently inhibited HBsAg release in HepG2.2.15 cells
- In AAV-HBV mouse model, ALG-020572 dosing resulted in a dose responsive HBsAg of up to 1.2 log10 (IU/mL) reduction in S antigen at 10 mg/kg after 6 doses using the same regimen in the clinical study without ALT increases[6]
- In clinical studies, treatment with up to seven 210 mg ALG-020572 doses resulted in 0.3-1.63 log10 IU/mL HBsAg reductions, while subjects treated with placebo had no change in HBsAg levels[5]
  - No correlation between ALT elevation and HBsAg reduction was observed

Conclusions

- The clinical outcome of multiple doses ALG-020572 at 210mg in HBeAg negative CHB subjects was not predicted in nonclinical or SAD in healthy volunteer studies.
- Liver concentration of ALG-020579 in the MAD were up to 6-fold below the NOAEL in monkeys, generally considered the more clinically relevant species
- As rodents are generally more sensitive to pro-inflammatory and single-cell hepatocellular effects of oligonucleotides and effects in the liver of rodents have generally not translated to safety risks in humans, the relationship of ALG-020572-mediated effects observed in mice and the ALT flares seen in the MAD portion of the Phase 1 study is uncertain
- Development of ALG-020572 has been terminated
- Follow up nonclinical studies are ongoing, utilizing 3D human liver microtissue experiments to look for long-term toxicity (for results, see Misner et al, ACT Poster P518)

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