Preclinical Development of ALN-KHK, an Investigational RNAi Therapeutic for Type 2 Diabetes Mellitus

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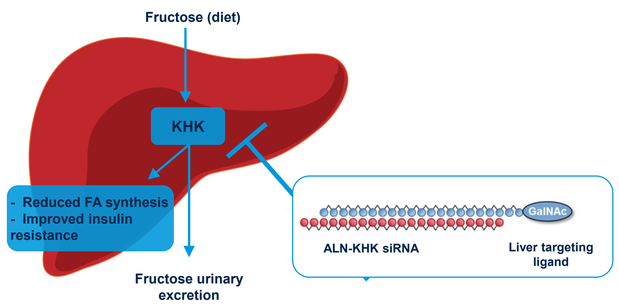
Conclusions

- **ALN-KHK demonstrated suppression of liver ketohexokinase (KHK) mRNA and protein in non-human primates (NHPs) after a single subcutaneous (SC) injection, with potent and durable reduction in KHK protein (94.1% lowering through day 85 at the 10 mg/kg dose).**
- **Reduction of liver KHK protein was associated with elevated circulating fructose and reduced circulating fibroblast growth factor 21 (FGF21) in NHPs after oral fructose bolus.**
- **ALN-KHK had acceptable safety profile and was well tolerated in GLP toxicology studies, supporting ongoing first-in-human studies in overweight and obese healthy volunteers (ClinTrials ID: NCT05761301).**

Background and Rationale

KHK inhibition as a therapeutic approach for T2DM

Figure 1. Therapeutic Hypothesis



ALN-KHK is delivered to hepatocytes by GalNAc conjugation to specifically target hepatocyte-expressed KHK mRNA. Reduction of hepatic KHK is expected to result in reduced fructose metabolism in the liver and thus increased circulating fructose and fructose excretion in the urine. Reduction of fructose metabolism in the liver should reduce FA synthesis in the liver and lead to improved insulin resistance in individuals with T2DM.

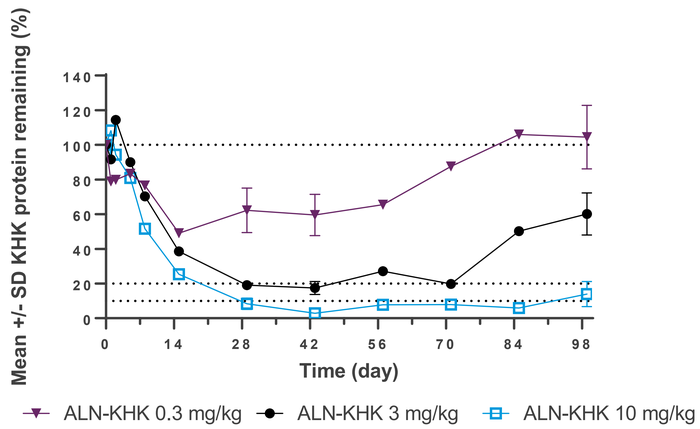
- Fructose metabolism in the liver results in fatty acid (FA) synthesis and is associated with liver steatosis and insulin resistance in animal models[1,2].
- Increased fructose consumption from added high fructose corn syrup is implicated in T2DM and metabolic syndrome.
- Ketohexokinase (KHK) is the first enzyme in fructose metabolism, responsible for phosphorylating fructose to fructose-1-phosphate (F1P).
- Lowering hepatic KHK expression by targeting the KHK mRNA with siRNA should reduce fatty acid (FA) synthesis and thus reduce insulin resistance and improve glycemic control in obese individuals with T2DM.
- Individuals with homozygous mutations in KHK are healthy supporting the safety of knocking down KHK.

Results

ALN-KHK Pharmacodynamics in NHPs

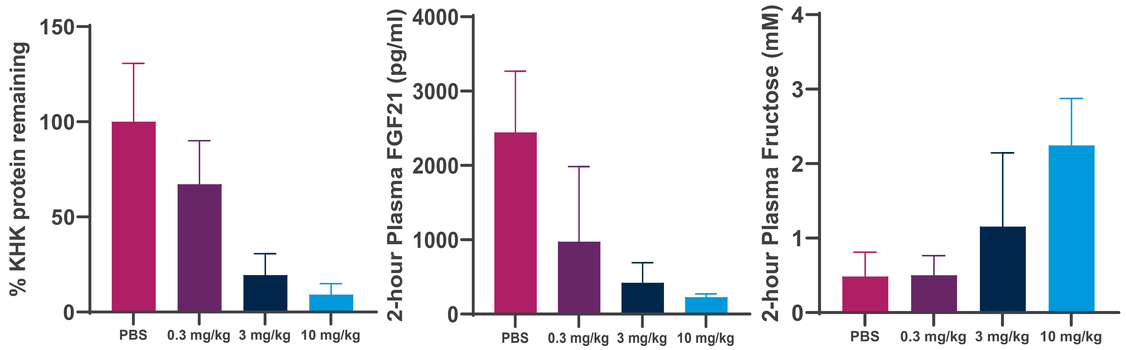
- A single subcutaneous dose of ALN-KHK in cynomolgus macaques maximally reduced liver KHK protein expression by 94.1% through day 85 at the 10 mg/kg dose.
  - 3 mg/kg dose maximal protein reduction was 82.6%; 0.3 mg/kg dose maximal protein reduction was 50.9%.
  - KHK liver mRNA reduction was similar to protein reduction (data not shown).
- Pharmacodynamic profile supports infrequent dosing in the clinic.
- ALN-KHK selection was based on in vitro assessment of a large set of GalNAc-siRNAs targeting human and cynomolgus macaque KHK transcripts.

Figure 2. Suppression of liver KHK protein in cynomolgus macaques after a single subcutaneous (SC) injection of ALN-KHK



KHK liver protein was quantified from liver biopsies by LC-MS/MS after a single subcutaneous dose of ALN-KHK at 0.3 mg/kg, 3 mg/kg, or 10 mg/kg. Percent protein remaining is normalized to pre-dose biopsies and is reported as mean +/- standard deviation (SD). Baseline KHK liver protein values were 3775-5489 ng/g.

Figure 3. Reduction of liver KHK protein resulted in a dose dependent increase in circulating fructose and decrease in circulating fibroblast growth factor 21 (FGF21)



Oral fructose bolus (30g) was given to cynomolgus macaques 30 days following a single SC injection of ALN-KHK. KHK liver protein was quantified from terminal liver biopsies by LC-MS/MS. Circulating fructose was quantified by LC-MS/MS and circulating FGF21 was quantified by ELISA (R&D). Reduction of KHK protein (compared to placebo group) was 32.8%, 80.6%, and 90.9% at 0.3 mg/kg, 3 mg/kg and 10 mg/kg, respectively. Blood was collected at baseline and 0.5, 1, 2, 3, 4 and 5 hours fructose bolus. Shown is the plasma fructose and plasma FGF21 at 2 hours post-fructose bolus.

Reduction in KHK protein resulted in increased circulating fructose and decreased circulating FGF21

- ALN-KHK targeting of liver KHK in cynomolgus macaques resulted in a 4.6-fold increase of plasma fructose at 2 hours post-fructose bolus in the 10 mg/kg dosed group.
- ALN-KHK targeting of liver KHK in cynomolgus macaques resulted in a 10.7-fold decrease in circulating FGF21 levels 2 hours post-fructose bolus in the 10 mg/kg group.
  - Increased circulating FGF21 is observed in humans in response to oral fructose bolus[3].
  - Induction of FGF21 by fructose is thought to be mediated by carbohydrate-responsive element-binding protein (ChREBP) and protein F1 (F1P)[4].
- Circulating fructose and FGF21 will be used as biomarkers of hepatic KHK silencing in the ALN-KHK first-in-human study.

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Disclosures: Authors report being employees and stockholders at Alnylam Pharmaceuticals.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FA, fatty acid; FGF21, fibroblast growth factor 21; GalNAc, N-Acetylgalactosamine; IND, investigational new drug; KHK, ketohexokinase; LC-MS/MS, liquid chromatography–mass spectrometry; mRNA, messenger RNA; SC, subcutaneous; siRNA, small interfering RNA; T2DM, Type 2 Diabetes Mellitus; SD, standard deviation

References: [1]Softic, 2017; [2]Andres-Hernando, 2020; [3]Dushay, 2015; [4]Fisher, 2016.

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