ALN-KHK, an Investigational RNA Interference Therapeutic, Successfully Targets Hepatic Ketohexokinase in a Single Ascending Dose Study of Overweight to Obese Adults

Poster number: 859-P

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Conclusions

- **In this single ascending dose study involving overweight to obese adults, subcutaneous administration of ALN-KHK, an investigational subcutaneous RNA interference therapeutic, showed an encouraging safety and tolerability profile.**
- **Target engagement was observed based on increasing serum fructose, urine fructose, and suppression of serum fibroblast growth factor 21 (FGF21) in a dose-dependent manner following a fructose tolerance test (FTT).**
- **Results of this first clinical study of ALN-KHK support its further evaluation in patients with type 2 diabetes and suggest the potential for quarterly or biannual subcutaneous dosing.**
  - **Part B of the study has been initiated and will evaluate multiple doses of ALN-KHK in obese patients with type 2 diabetes.**

Introduction

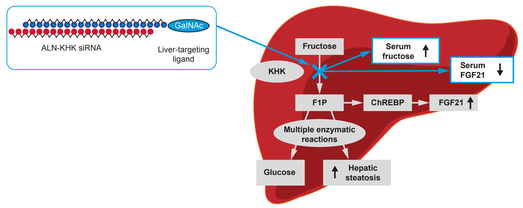
Background

- Rising global obesity rates are attributed in part to excess fructose consumption from sucrose and high-fructose corn syrup.[1]
  - Relative to glucose, fructose is preferentially metabolized in the liver and can contribute to development of hepatic steatosis, which is strongly associated with hepatic insulin resistance and type 2 diabetes.[1,2]
  - Ketohexokinase (KHK) is an enzyme involved in the initial step of fructose metabolism that phosphorylates fructose to fructose-1-phosphate (F1P).
- Reducing hepatic KHK expression through small interfering RNA (siRNA)-mediated inhibition may decrease hepatic lipogenesis and increase insulin sensitivity, thus improving glycemic control in obese individuals with type 2 diabetes.

ALN-KHK

- ALN-KHK is an *N*-acetylgalactosamine (GalNAc)-conjugated, investigational, subcutaneously administered RNA interference therapeutic designed to decrease hepatic KHK expression (**Figure 1**).
- Preclinical studies have demonstrated that single subcutaneous doses of ALN-KHK provide potent and durable reduction in KHK protein.[3]

Figure 1. Mechanism of Action of ALN-KHK



ChREBP, carbohydrate response element binding protein; F1P, fructose-1-phosphate; FGF21, fibroblast growth factor 21; GalNAc, *N*-acetylgalactosamine; KHK, ketohexokinase; siRNA, small interfering RNA.

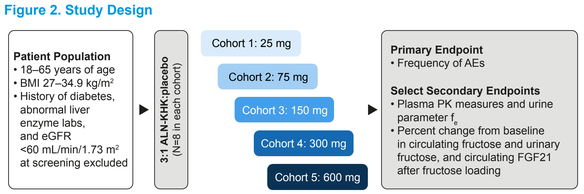
Aim

- To evaluate the safety, tolerability, and pharmacology of ALN-KHK in overweight to obese adults.

Methods

- Part A of this Phase 1/2 trial (NCT05761301) is a single ascending dose study of healthy overweight to obese adults (body mass index of 27–34.9 kg/m^2) who were randomized to treatment with a single subcutaneous dose of ALN-KHK or placebo (**Figure 2**).
- Participants were observed for a 3-month double-blind period. After completion of the Month 3 visit, patients treated with ALN-KHK were observed for up to 6 months for safety assessments. The primary and select secondary endpoints are shown in **Figure 2**.
- Target engagement was assessed through response to an FTT. For the FTT, after an overnight fast of 12–14 hours, participants consumed 75 g of fructose dissolved in water.
  - Fructose ingestion leads to an increase in circulating serum fructose and an associated increase in urinary fructose.
  - Hepatic FGF21 is induced through the metabolism of fructose to F1P, which leads to an increase in circulating levels of the hepatokine FGF21; therefore FGF21 was used as a marker of target engagement.[4] Based on its mechanism of action, treatment with ALN-KHK was expected to decrease circulating FGF21 following fructose ingestion.

Figure 2. Study Design



AE, adverse event; BMI, body mass index; f_e, fraction excreted; FGF21, fibroblast growth factor 21; PK, pharmacokinetics.

Results

Patient Population

- In total, 40 participants enrolled in the study, and cohorts were generally well balanced in terms of age and key disease-related baseline characteristics (**Table 1**).
  - One participant in the 150 mg cohort discontinued the study early owing to a personal reason. A second participant in this cohort was lost to follow-up after Day 85 of the study.

Table 1. Patient Baseline Demographics

Demographic	Placebo (N=10)	ALN-KHK 25 mg (N=6)	ALN-KHK 75 mg (N=6)	ALN-KHK 150 mg (N=6)	ALN-KHK 300 mg (N=6)	ALN-KHK 600 mg (N=6)	ALN-KHK Total (N=30)	Total (N=40)
Mean age, years (SD)	42.8 (7.2)	49.7 (9.2)	46.5 (16.0)	46.0 (11.9)	45.0 (14.2)	44.0 (14.3)	46.2 (12.5)	45.4 (11.5)
Male, n (%)	7 (70.0)	6 (100.0)	3 (50.0)	5 (83.3)	2 (33.3)	4 (66.7)	20 (66.7)	27 (67.5)
Race, n (%)								
White	9 (90.0)	6 (100.0)	4 (66.7)	4 (66.7)	4 (66.7)	4 (66.7)	22 (73.3)	31 (77.5)
Black or African-American	1 (10.0)	0	2 (33.3)	1 (16.7)	1 (16.7)	2 (33.3)	6 (20.0)	7 (17.5)
More than one race	0	0	0	1 (16.7)	1 (16.7)	0	2 (6.7)	2 (5.0)
Mean BMI, kg/m² (SD)	29.9 (1.6)	30.3 (2.2)	28.6 (1.0)	28.7 (0.9)	29.2 (1.2)	31.1 (2.2)	29.6 (1.8)	29.7 (1.7)
Mean HbA1c, % (SD)	5.3 (0.2)	5.2 (0.2)	5.9 (0.3)	5.4 (0.3)	5.2 (0.2)	5.5 (0.3)	5.5 (0.4)	5.4 (0.3)

BMI, body mass index; HbA1c, glycated hemoglobin; SD, standard deviation.

Primary Endpoint: Safety and Tolerability

- Adverse events (AEs) were reported in 40% of participants receiving placebo and 33.3% of participants receiving ALN-KHK (**Table 2**).
  - All AEs were mild, nonserious, and recovered or resolved.
- Two events (injection-site reactions) in the ALN-KHK 300 mg cohort were considered related to study drug; both cases were mild, transient, and resolved within 24 hours.

Table 2. Frequency of AEs

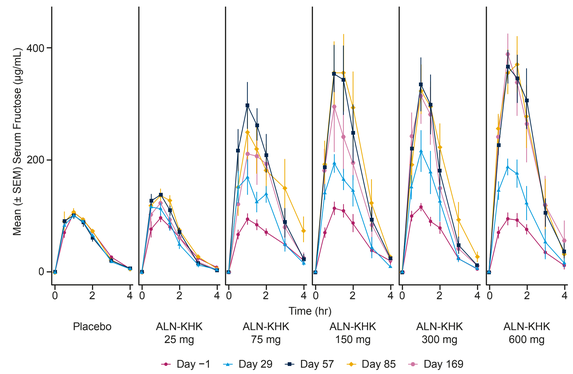
Patients with AEs, n (%)	Placebo (N=10, PY=2.7)	ALN-KHK 25 mg (N=6, PY=3.0)	ALN-KHK 75 mg (N=6, PY=2.8)	ALN-KHK 150 mg (N=6, PY=2.5)	ALN-KHK 300 mg (N=6, PY=4.1)	ALN-KHK 600 mg (N=6, PY=2.8)	ALN-KHK Total (N=30, PY=15.2)
At least 1 AE	4 (40.0)	2 (33.3)	2 (33.3)	1 (16.7)	5 (83.3)	0	10 (33.3)
At least 1 treatment-related AE	0	0	0	0	2 (33.3)	0	2 (6.7)
Most common AEs[a]							
Diarrhea	2 (20.0)	0	2 (33.3)	0	3 (50.0)	0	5 (16.7)
Headache	1 (10.0)	0	1 (16.7)	0	3 (50.0)	0	4 (13.3)
Abdominal discomfort	1 (10.0)	0	0	1 (16.7)	1 (16.7)	0	2 (6.7)
ISR	0	0	0	0	2 (33.3)	0	2 (6.7)
Nausea	0	0	1 (16.7)	0	1 (16.7)	0	2 (6.7)
Vomiting	0	0	0	0	2 (33.3)	0	2 (6.7)

[a]Most common AEs are those occurring in >5% of patients.
AE, adverse event; ISR, injection site reaction; PY, patient-years.

Secondary Endpoint: Assessment of Plasma and Urine Pharmacokinetics

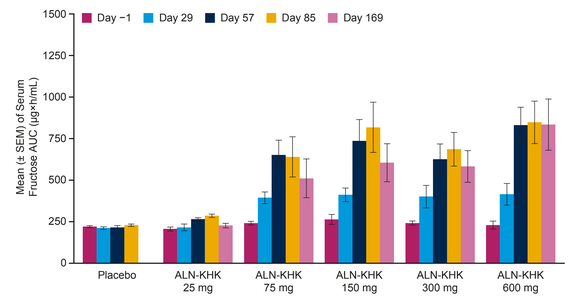
- Peak plasma ALN-KHK was observed 3–10 hours after administration, and the elimination half-life was 5–8 hours. Concentrations of ALN-KHK in plasma were dose-proportional across the 25–600 mg dose range.
- Urinary excretion of ALN-KHK was less than 25%.

Figure 3. Mean Serum Fructose After FTT



FTT, fructose tolerance test; hr, hour; SEM, standard error of the mean.

Figure 4. Serum Fructose AUC in Response to FTT Over Time



AUC, area under the concentration–time curve; FTT, fructose tolerance test; SEM, standard error of the mean.

Secondary Endpoint: Change from Baseline in Serum Fructose After Fructose Tolerance Test

- With ALN-KHK 600 mg, mean increases of serum fructose from Day −1 (baseline) of greater than 2.5-fold were sustained to Day 169 (**Figure 3; Figure 4**)
- At the lowest ALN-KHK dose (25 mg), there was no significant increase in area under the concentration–time curve (AUC) for serum fructose. An increase in AUC was observed with the 75 mg and higher doses. The AUC at each dose was highest at Day 57 and Day 85. The effect was sustained to Day 169 with the 600 mg dose (**Figure 4**).

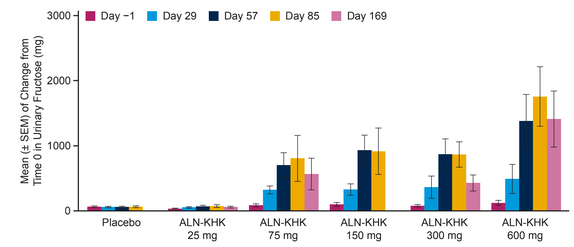
Secondary Endpoint: Change from Baseline in Urinary Fructose After Fructose Tolerance Test

- An increase in urinary fructose excretion was observed with the 75 mg and higher doses of ALN-KHK. With the 600 mg dose, mean (± SEM) change in urinary fructose at Day 85 compared with Day −1 was 1633 (± 431) mg (**Figure 5**).
- Mean urinary fructose excretion fraction of the total 75 g fructose load peaked at Day 85 for ALN-KHK 600 mg at 2.3%.

Secondary Endpoint: Change from Baseline in Circulating FGF21 After Fructose Tolerance Test

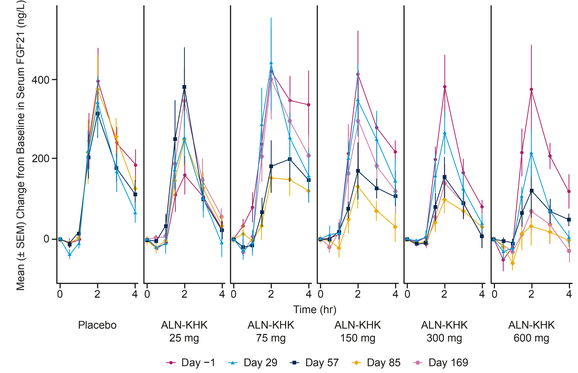
- The mean suppression of serum FGF21 after FTT evident from change from baseline increased in a dose-dependent manner (**Figure 6**). Basal FGF21 level was not affected (data not shown).
- Mean reductions in FGF21-positive incremental AUC from Day −1 became more prominent with increasing doses of ALN-KHK (≥75 mg) through Day 85, with partial recovery at Day 169 (**Figure 7**).

Figure 5. Change from Time 0 in Urinary Fructose in Response to FTT Over Time



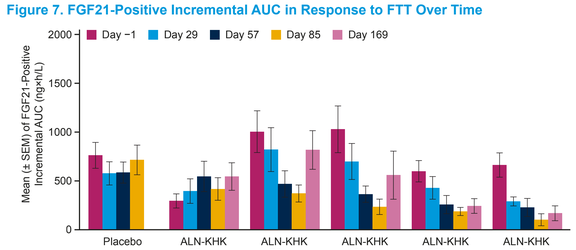
For the 150 mg dose cohort, the Day 169 visit values were missing owing to technical issues.
FTT, fructose tolerance test; SEM, standard error of the mean.

Figure 6. Mean Absolute Change from Baseline in Serum FGF21 after FTT



FGF21, fibroblast growth factor 21; FTT, fructose tolerance test; hr, hour; SEM, standard error of the mean.

Figure 7. FGF21-Positive Incremental AUC in Response to FTT Over Time



AUC, area under the concentration–time curve; FGF21, fibroblast growth factor 21; FTT, fructose tolerance test; SEM, standard error of the mean.

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DISCLOSURES

EMF, **TN**, **KLB**, **XL**, **YW**, **LN**, and **FK** are employees of and report being shareholders in Alnylam Pharmaceuticals.
GM reports being a principal investigator at Altasciences.

Thank you to the patients, their families, investigators, study staff, and collaborators for their participation in this study.
Acknowledgments: Editorial assistance provided by PharmaGenesis Cardiff, Cardiff, UK, was funded by Alnylam Pharmaceuticals in accordance with Good Publication Practice (GPP 2022) guidelines.

Funding: This study was funded by Alnylam Pharmaceuticals