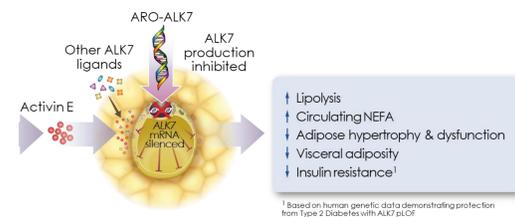


ARO-ALK7, a RNAi Therapeutic Targeting Adipose ALK7 Expression for Obesity

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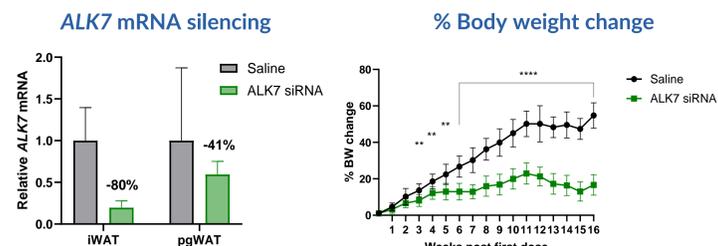
INTRODUCTION

- While incretin-based therapies are the current frontline pharmacotherapeutics for obesity and metabolic outcomes, issues concerning significant loss of lean mass, adverse GI events at high dose levels, and disproportional fat mass gain after cessation of the therapies remain a challenge for many patients
- Human genetic studies support ALK7 as a therapeutic target with pLOF ALK7 variants associated with 1) reduced WHRadjBMI, 2) protection from type 2 diabetes, 3) reduced risk of cardiovascular disease
- ALK7 signaling suppresses lipolysis, increasing adipocyte size and lipid content
- We evaluated the potential therapeutic benefits of ALK7 silencing by siRNA designed to specifically target adipose tissue

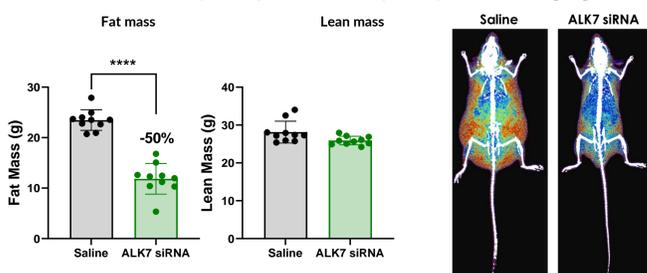


PHARMACOLOGICAL STUDIES OF ALK7 siRNA IN A DIET-INDUCED OBESE (DIO) MOUSE MODEL

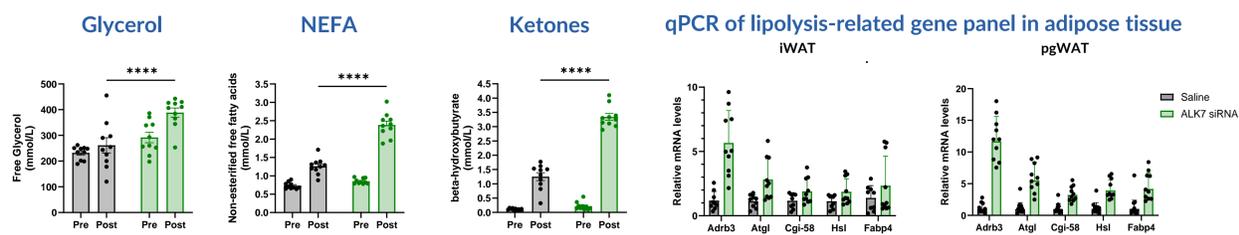
1. ALK7 silencing in adipose tissue suppresses body weight gain and improves body composition in DIO mice



Body composition analysis by DEXA imaging

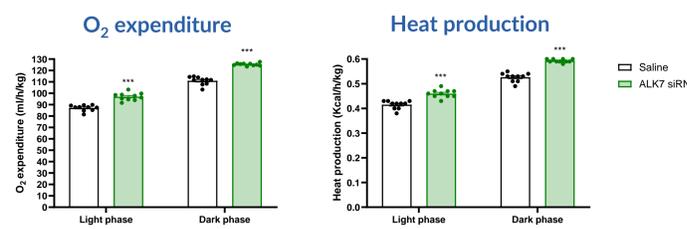


2. Body fat loss is mechanistically attributed to enhanced lipolysis and increased energy expenditure



Serum collected prior to and 30 min after challenge with CL316,243, a β_3 -adrenergic receptor agonist (IP, 1 mg/kg)

- Animals (N=10/group) with reduced ALK7 expression had increased circulating levels of glycerol, NEFAs, and ketones (Mean \pm SEM) and exhibited upregulation in the expression of lipolytic genes



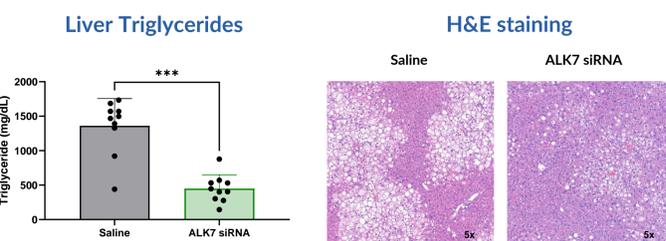
At week 8, energy expenditure measurements (Mean \pm SEM) were obtained from C57Bl/6 DIO mice (N=10/group) caged in a TSE Phenomaster system over 2 dark and 1 light cycle. ALK7 siRNA treated group exhibited:

- Increased levels of O₂ consumption and heat production
- No change in food intake

C57Bl/6 DIO mice (male, age = 18 weeks at initiation) received chronic treatment with a mouse surrogate ARO-ALK7 (3 mg/kg weekly), which resulted in:

- ALK7 mRNA silencing efficacy of 80% in inguinal white adipose tissue and 40% in perigonadal white adipose tissue at study terminus (week 16)
- A significant suppression of up to 39% body weight reduction relative to saline controls
- Approximately 50% reduction in fat mass with preservation of lean mass by DEXA imaging analysis

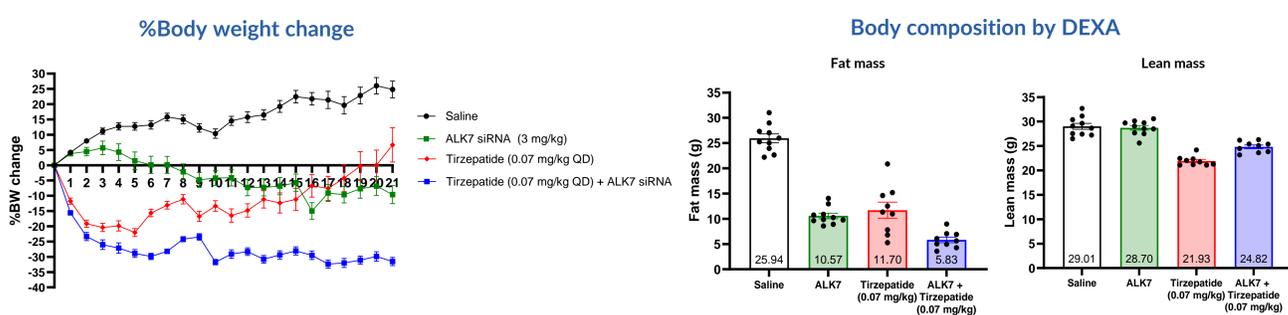
3. Increased lipid mobilization due to ALK7 silencing lead to reduced hepatic steatosis



At study terminus, neutral lipid contents in the livers of the animals were analyzed. ALK7 siRNA treated animals demonstrated greater reduction in hepatic lipid accumulation by:

- Triglyceride concentration via biochemical assay
- Histologic analysis using liver sections stained with hematoxylin and eosin (H&E)

4. ALK7 silencing with tirzepatide enhances therapeutic benefits of tirzepatide monotherapy

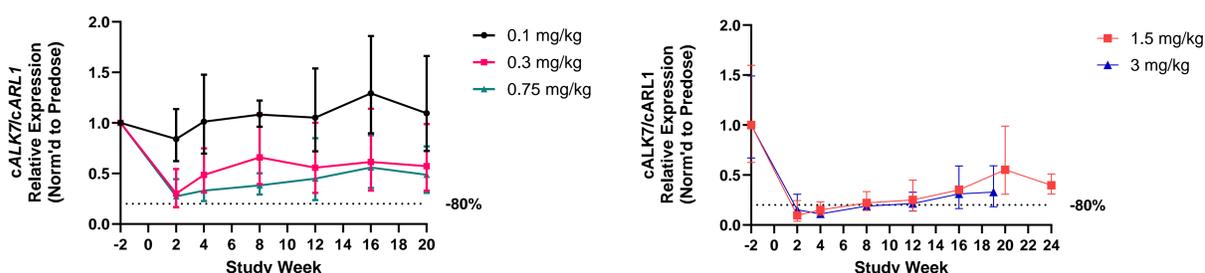
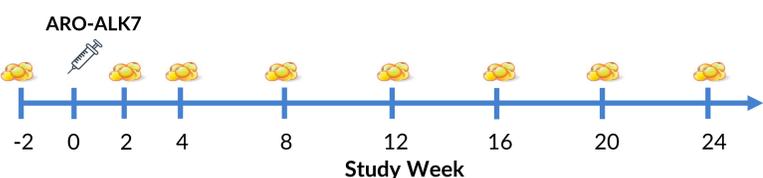


C57Bl/6 DIO mice (male, age = 20 weeks) were treated with either 1) saline weekly, 2) ALK7 surrogate siRNA 3 mg/kg weekly, 3) tirzepatide (0.21 mg/kg QD), 4) tirzepatide (0.07 mg/kg QD), or 5) tirzepatide (0.07 mg/kg QD) and ALK7 surrogate siRNA 3 mg/kg weekly (N=10/group)

- Co-treatment of tirzepatide and ALK7 siRNA at optimal dose levels had an additive effect on body weight and fat mass reductions
- ALK7 siRNA ameliorated the significant loss of lean mass associated with tirzepatide

PHARMACODYNAMIC STUDIES OF ARO-ALK7 IN NON-HUMAN PRIMATES

5. A single subcutaneous dose of ARO-ALK7 achieves deep and durable silencing of ALK7 mRNA expression in subcutaneous adipose tissue



- The dynamic range (0.1 - 0.75 mg/kg) of ARO-ALK7 was evaluated in cynomolgus monkeys (N=4/group)
- A single dose of ARO-ALK7 resulted in a dose-dependent reduction of ALK7 mRNA expression

- The duration of ARO-ALK7 was evaluated in cynomolgus monkeys (N=4/group)
- A single 3 mg/kg ARO-ALK7 dose resulted in ~89% ALK7 mRNA silencing in adipose tissue after 1 month, and ~75% silencing for up to 12 weeks

TOXICOLOGICAL STUDY OF ARO-ALK7 IN HAN WISTAR RATS

6. ARO-ALK7 is well-tolerated subcutaneously in Han Wistar rats

ARO-ALK7 administered as a single dose of 30, 60, or 120 mg/kg (Day 15 necropsy), or repeat doses of 60 mg/kg on Day 1 and 29 (Day 43 necropsy)

- Histopathological findings of vacuolated macrophage infiltrates at injection site/lymph nodes (\geq 60 mg/kg), liver Kupffer cell hypertrophy (\geq 30 mg/kg)
- No adverse or dose-limiting findings were identified

CONCLUSIONS

- Chronic treatment of DIO mice with a surrogate of ARO-ALK7 suppressed body weight gain by 39%, reduced fat mass by 50%, and preserved lean mass compared to saline controls
- ALK7 surrogate RNAi-treated DIO mice exhibited enhanced lipolysis and increased energy expenditure
- Co-treatment of the ARO-ALK7 surrogate with tirzepatide in DIO mice enhanced body weight reduction and fat loss relative to tirzepatide monotherapy
- In NHPs, a single subcutaneous dose of ARO-ALK7 resulted in a dose-dependent and durable reduction in ALK7 mRNA expression in subcutaneous fat
- ARO-ALK7 was well tolerated in Han Wistar rats in non-GLP toxicological studies