Preliminary human safety and PK of the liver-targeted mitochondrial protonophore TLC-6740

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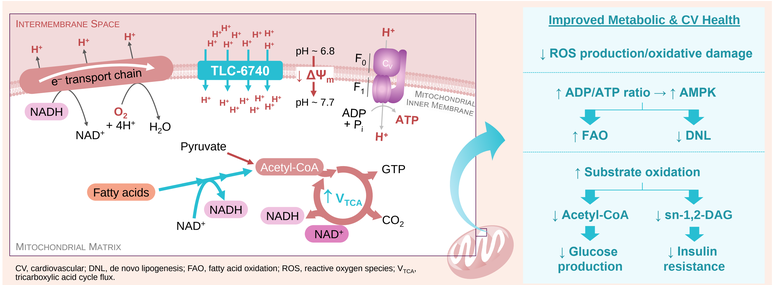
Introduction

Metabolic Benefits of Mitochondrial Protonophores

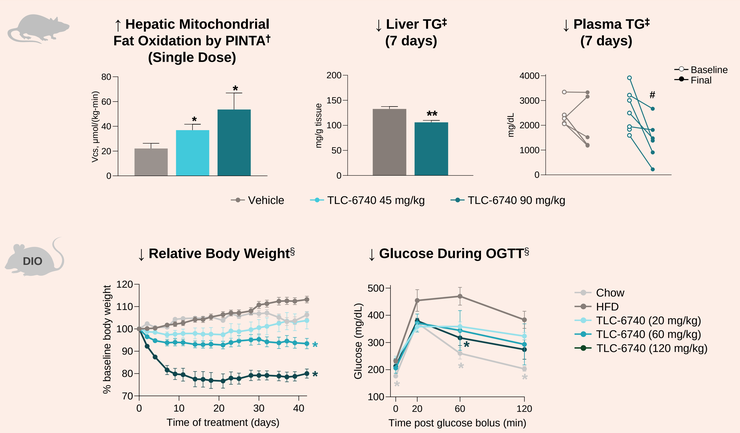
- Futile cycling of protons across the inner mitochondrial membrane by uncoupling dissociates oxidative phosphorylation from ATP synthesis, resulting in enhanced TCA cycle flux and β-oxidation to meet cellular energy demands[1]
- Mitochondrial uncoupling via synthetic protonophores (eg, 2,4-dinitrophenol [DNP]) is a validated approach for weight loss; however, safety concerns due to excessive systemic uncoupling (eg, hyperthermia) have limited clinical development[2]
- Mild mitochondrial uncoupling has multiple potential metabolic benefits[1]
- Various approaches to increase the therapeutic window of DNP (eg, controlled-release formulations and prodrugs) have shown promise[3,4]



CV, cardiovascular; DNL, de novo lipogenesis; FAO, fatty acid oxidation; ROS, reactive oxygen species; V_{TCA}, tricarboxylic acid cycle flux.

TLC-6740 is a Potent, Liver-Targeted Protonophore[6]

- TLC-6740 is a novel mitochondrial protonophore with distinct structure and pharmacology to DNP and its derivatives
- High hepatic extraction (~40x liver-to-plasma ratio) mediated by OATP transporters reduces maximal plasma concentrations and results in a greater therapeutic margin
- Increased *in vitro* potency compared with DNP (~6 to 18-fold) may enhance efficacy
- In rodent models, TLC-6740 increases hepatic TCA cycle flux, and dose-dependently decreases body weight, liver and plasma triglycerides, and improves glucose tolerance without increasing body temperature
- TLC-6740 is in clinical development for the treatment of obesity and obesity-associated disorders (eg, diabetes, NASH)



† Hepatic mitochondrial fat oxidation (citrate synthase flux [Vcs]) assessed by PINTA[5] in male, overnight fasted, Sprague-Dawley rats given a single oral dose of TLC-6740 or vehicle.[6]
‡ Liver and plasma triglycerides (TG) assessed in high-fat, high-cholesterol diet-fed Zucker Diabetic Fatty (ZDF) rats dosed orally with TLC-6740 or vehicle for 7 days.[6]
§ Body weight and plasma glucose during oral glucose tolerance test (OGTT) assessed in diet-induced obese (DIO) mice fed a high-fat diet for 18 weeks and treated with TLC-6740 or vehicle in diet for 6 weeks.[6]
*p<0.05 vs vehicle; #p≤0.05 vs baseline, within the same group.

Objectives

- To evaluate the safety, tolerability, and pharmacokinetics (PK) of single ascending doses of TLC-6740 in healthy subjects
- To simulate expected plasma and liver concentrations of TLC-6740 upon multiple dosing based on single-dose PK in healthy subjects

Methods

Study Design

* Admission occurred on Day -2 or Day -1 based on participation in an exploratory substudy for measurement of resting energy expenditure (data to be described separately as study remains blinded).

- Double-blind, placebo-controlled, SAD and MAD study in healthy subjects (NCT05822544)
  - Age 18–55 years; body mass index (BMI) 19–35 kg/m^2
  - No significant medical conditions by history, physical examination, or laboratory evaluation
- For each SAD cohort, 6 subjects were randomized to receive single doses of TLC-6740 (3, 6, 15, 30, 60, or 120 mg) and 2 to receive placebo in the fasted state
  - MAD portion of the study is ongoing and will be described separately
- Safety and tolerability assessments, including adverse event (AE) monitoring, laboratory tests, physical examinations, and electrocardiographic (ECG) evaluations were performed throughout the study
- Intensive PK sampling over 72 hours post Day 1 dose
  - TLC-6740 plasma concentrations determined using validated liquid chromatography-tandem mass spectrometry assay
  - PK parameters estimated via noncompartmental methods using Phoenix® WinNonlin® 6.2.1 and 8.3.4 (Certara, LP, Princeton, NJ) and will be disclosed upon study completion and unblinding
- Multiple dose simulations performed to estimate liver and plasma concentrations of TLC-6740 over 7 days of dosing based on Day 1 PK from SAD cohorts using Phoenix 64 version 8.3.3.33 (Certara)
  - Hepatic concentrations estimated based on liver-to-plasma ratios of TLC-6740 in preclinical species (ie, rat, dog, and non-human primate), in which liver and plasma exposures are highly correlated (r^2=0.84; unpublished data)
  - Based on preclinical data, a trough TLC-6740 liver concentration >5 μM is considered efficacious (unpublished data)
- As the study remains blinded, all data (except PK) include subjects on placebo

Results

SAD Subject Enrollment and Demographics

	3 mg: n=8	6 mg: n=8	15 mg: n=8	30 mg: n=8	60 mg: n=8	120 mg: n=8
Age, y	35 (21, 47)	27 (20, 38)	25 (20, 49)	26 (20, 43)	27 (19, 51)	30 (19, 52)
Male	1 (13)	5 (63)	6 (75)	3 (38)	2 (25)	3 (38)
Race*						
White	5 (63)	5 (63)	7 (88)	7 (88)	5 (63)	7 (88)
Pacific Islander	2 (25)	0 (0)	2 (25)	2 (25)	0 (0)	1 (13)
Asian	0 (0)	3 (38)	0 (0)	1 (13)	3 (38)	1 (13)
Black	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)	1 (13)
American Indian	1 (13)	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)
Hispanic ethnicity*	1 (13)	1 (13)	0 (0)	0 (0)	1 (13)	0 (0)
Weight, kg†	67.5 (63.4, 92.0)	74.8 (50.8, 104.5)	71.0 (60.3, 92.2)	77.0 (56.4, 90.6)	63.5 (55.8, 102.6)	74.7 (59.3, 111.6)
BMI, kg/m^2†	24.3 (23.9, 33.8)	23.5 (19.1, 28.9)	24.4 (21.6, 30.1)	26.9 (20.7, 30.6)	23.3 (20.6, 30.3)	25.3 (20.4, 32.6)

Data are median (min, max) or n (%). Each cohort includes 6 subjects treated with TLC-6740 and 2 subjects with placebo.
* Race and ethnicity are self-reported. In some subjects, multiple races were reported. Pacific Islander race includes Native Hawaiians and American Indian race includes Alaskan Natives.
† Weight and BMI measured on day of admission.

References: 1. Goedeke & Shulman, Molecular Metab 2021; 2. Poole FE, et al. JAMA 1934; 3. Perry RJ, et al. Science 2015; 4. Perry RJ, et al. Cell Metab 2014; 5. Perry RJ, et al. Nat Commun 2019; 6. Vijayakumar A, et al. AASLD 2022 (Abstract #2551).
Acknowledgements: We extend our thanks to all study subjects and participating investigators. This study was funded by OrsoBio, Inc.

Results

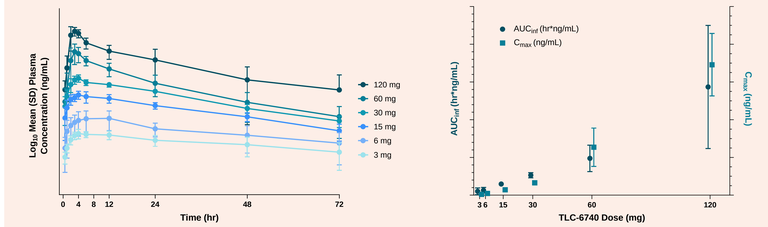
Safety and Tolerability

	3 mg: n=8	6 mg: n=8	15 mg: n=8	30 mg: n=8	60 mg: n=8	120 mg: n=8
Any TEAE	2 (25)	4 (50)	3 (38)	3 (38)	6 (75)	4 (50)
Rhinorrhea	0 (0)	1 (13)	1 (13)	0 (0)	2 (25)	0 (0)
Catheter site bruise	1 (13)	0 (0)	0 (0)	0 (0)	0 (0)	2 (25)
Headache	0 (0)	1 (13)	1 (13)	0 (0)	0 (0)	1 (13)
Upper respiratory tract infection	0 (0)	1 (13)	0 (0)	0 (0)	2 (25)	0 (0)
Grade ≥2 TEAE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Treatment-related TEAE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Serious TEAE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

TEAE, treatment-emergent AE. TEAEs that occurred in at least 3 subjects are listed by MedDRA Preferred Term. Each cohort includes 6 subjects treated with TLC-6740 and 2 subjects with placebo.

- Single doses of TLC-6740 (3 to 120 mg) or placebo were well tolerated
- All subjects completed dosing and the Follow-up visit on Day 15
- All TEAEs were deemed unrelated to treatment, non-serious, and mild in severity
- No clinically significant laboratory abnormalities or changes in vital signs, including body temperature, or ECG parameters

SAD Plasma Concentration Time Profiles and Linearity



- TLC-6740 was rapidly absorbed, with T_{max} occurring 3 to 12 hours postdose
- Mean TLC-6740 $t_{1/2}$ ranged from 17 to 46 hours
- Low plasma C_{max} consistent with liver targeting pharmacology based on active hepatic uptake
- Dose-dependent increases in plasma exposure

Multiple Dose Simulations of Plasma and Hepatic Exposures

- TLC-6740 is expected to reach steady state plasma and liver concentrations within 7 days
- TLC-6740 doses ≥6 mg are sufficient to achieve therapeutic liver exposures (>5 μM)
- Within the expected therapeutic dose range, projected steady-state plasma C_{max} will remain >80-fold below thermogenic exposures in dog toxicology studies, providing large safety margins for human use

Conclusions

- TLC-6740 was safe and well tolerated after single doses up to 120 mg in healthy subjects
- The prolonged half-life of TLC-6740 supports once-daily, oral dosing
- Based on projected plasma and liver concentrations and preclinical data, multiple dosing of TLC-6740 within this dose range is expected to:
  - Lead to low plasma C_{max} due to liver targeting, avoiding exposures associated with toxicity due to systemic uncoupling (eg, hyperthermia), and
  - Reach exposures associated with efficacy in preclinical models (eg, weight loss, improvements in hepatic and plasma TG)
- The tolerability and PK profile of TLC-6740 support its ongoing evaluation in MAD cohorts in this study and in future studies among patients with obesity and obesity-associated disorders

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