Development of a Novel Human Engineered Enzyme (ACN00177) for the Treatment of Homocystinuria

Christopher Daige[1], Jason Wiggins[1], Silvia Ferrati[1], Leslie Priddy[1], Jess Van Cleef[1], Danlee Enzler[1], James Campbell[1], Giulia Agnello[1], and Scott Rowlinson[1]
[1] Aeglea BioTherapeutics, Austin, TX

Introduction

Homocystinurina (HCU) is a family of inherited disorders of methionine metabolism caused by mutations in Cystathionine-β-Synthase (CBS), MMAB, MTHFR, MTR and MTRR genes leading to elevated plasma and tissue homocysteine and homocystine levels. High levels of homocysteine and homocystine affect multiple organ systems leading to:

- Early mortality
- Vascular: thrombosis, stroke
- Developmental: delay, cognitive impairment
- Bone: osteoporosis, skeletal deformities
- Ocular: dislocation of the lenses, nearsightedness
- Hepatic: fatty liver

Unmet need is high due to inadequate control of plasma homocysteine levels in many patients AND difficulties in complying with current disease management approaches (dietary protein [methionine] restriction, vitamins, and betaine). Betaine supplementation results in hypermethioninemia and additional side effects such as cerebral edema[1].

Therapeutic Approach

Our research approach identifies innovative therapeutics by creating novel enzyme activities with clinical value using human enzyme scaffolds. Engineered Cystathionine γ-Lyase (CGL) enzyme was mutated to change its native substrate specificity from cystathionine to both homocysteine AND homocysteine. Degrading both homocysteine and homocystine enables rapid and sustained depletion of the disease inducing metabolites. The activity of the clinical candidate molecule ACN00177 was tested as well as the polyhistidine-tagged version of ACN00177 (ACN00177-HIS). Other enzyme therapeutics in development only report degrading homocysteine and not homocystine[2,3].

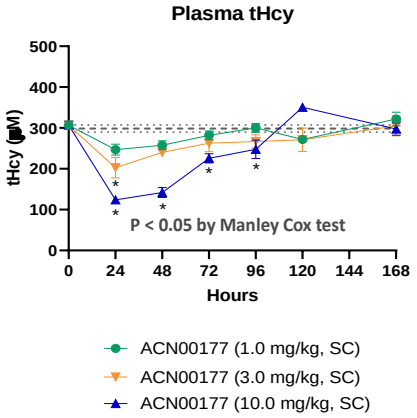
Methods

The CBS deficient mouse (CBS -/-) is a clinically relevant model for Classical Homocystinuria. In vivo pharmacodynamics (PD) of ACN00177 was assessed in the CBS -/- model by evaluating total homocysteine (tHCy) lowering. In vivo efficacy evaluation was also assessed in the CBS -/- model by evaluating multiple clinically relevant and pathology endpoints associated with tHcy. SD Rats and Cynomolgus monkeys were used for non-GLP and GLP pre-CTA enabling toxicology assessments.

Results

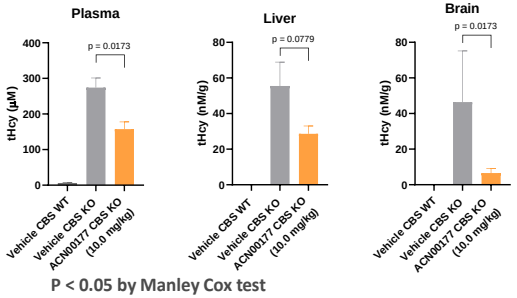
Single dose *in vivo* pharmacodynamics (PD) with ACN00177

- CBS -/- mice were dosed twice per week (BIW) starting at D10 with 10 mg/kg ACN00177 for 5 weeks to ensure survival. ADA development that impacts the PD response cannot be ruled out at this latter timepoint
- Animals underwent a two-week washout of drug followed by a single subcutaneous (SC) injection of ACN00177 at 1, 3, or 10 mg/kg and PD assessed (tHcy) over 1 week



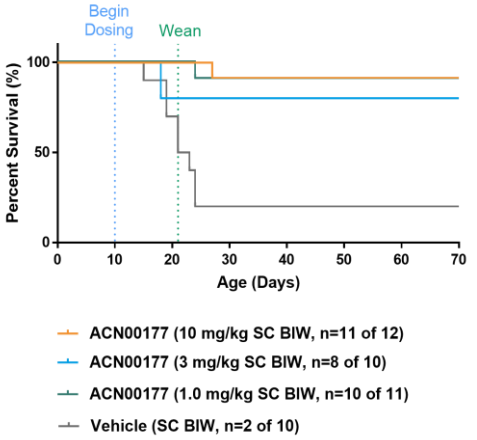
Multi-dose *in vivo* pharmacodynamics (PD) with ACN00177 in plasma, liver and brain

- CBS -/- or WT mice were dosed BIW starting at D10 or 11 with vehicle or 10 mg/kg ACN00177 for 3 total doses
- Animals were sacrificed and plasma, as well as liver and brain homogenates assessed for tHcy levels



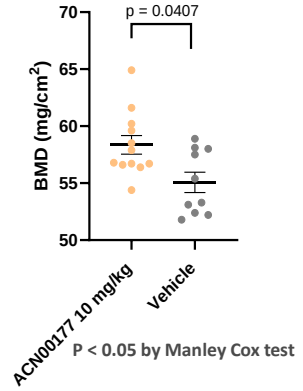
In vivo efficacy at multiple SC dose levels of ACN00177

- CBS -/- mice were dosed SC BIW starting at D10 through Day 70 to assess survival benefits with ACN00177



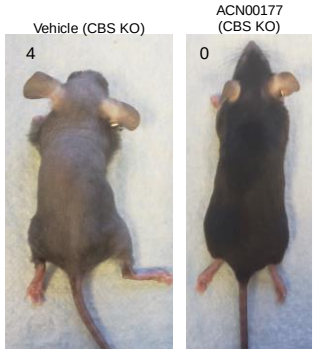
ACN00177 effects on the development of osteoporosis

- CBS -/- mice were dosed SC BIW with ACN00177-HIS starting at D10 through Day 169 were evaluated for bone mineral density (BMD) by dual-energy X-ray absorptiometry



ACN00177 effects on the development of Alopecia

- CBS -/- mice dosed SC BIW with ACN00177-HIS starting at D10 through Day 170 were evaluated for progressive alopecia



Animals evaluated on a scale from 0 to 4, 0 being no apparent alopecia to 4 being alopecia progressing from the head to the flank of the animal

CTA enabling Toxicology in rats and monkeys

- Sprague Dawley rats dosed once weekly with 6, 12, or 40 mg/kg IV and 12 or 40 mg/kg SC with ACN00177 did not show any adverse findings and the no observed adverse effect level (NOAEL) was determined to be 40 mg/kg (6.67 mg/kg Human equivalent dose)
- Cynomolgus Monkeys dosed once weekly with 3, 6, or 20 mg/kg IV and 6 or 20 mg/kg SC with ACN00177 did not show any adverse findings and the NOAEL was determined to be 20 mg/kg (6.67 mg/kg Human equivalent dose)

Conclusions

- Engineering of the CGL enzyme scaffold has generated a new clinical candidate molecule (ACN00177) with unique specificity for degrading both homocysteine and homocystine
- ACN00177 decreases total combined plasma homocysteine and homocystine levels in the CBS -/- mouse model and improves pathologies associated with the model
- ACN00177 was shown to be non-toxic at all dose levels tested in clinical trial application (CTA) enabling studies in rats and monkeys
- Given the severity of the disease and limitations of current disease management approaches, ACN00177 is being advanced into clinical studies

Disclosures:
Employees of Aeglea BioTherapeutics, Inc have and equity interest in the company

References:
1. US FDA; Center for Drug Evaluation and Research; Revised Label Cystadane (Betaine anhydrous for oral solution).
2. PMID: 29398487 3. PMID: 28821635