Development of a Translational Novel Gene Therapy for Fabry Disease: AAV Encoding Engineered Alpha-Galactosidase A Transgene in a Fabry Murine Model and Nonhuman Primates

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Introduction

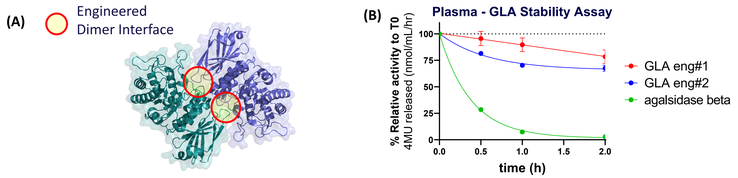
Fabry disease is a rare, X-linked lysosomal disorder caused by mutations in the GLA gene, which encodes a lysosomal hydrolase: alpha-galactosidase A (α-Gal A). Deficiency of α-Gal A results in the progressive lysosomal accumulation of globotriaosylceramide (Gb3) or related glycosphingolipids in a variety of tissues. The primary treatment options for patients with Fabry disease consist of 1) regular infusions of recombinant human α-Gal A, termed enzyme replacement therapy (ERT); and 2) the oral pharmacologic chaperone, migalastat. However, migalastat is only used in patients with certain amenable mutations and ERT requires bi-weekly injections and has limited tissue penetration and poor biodistribution. It's also worth noting that α-Gal A has low physical stability and a short circulating half-life at neutral pH of the blood, which may limit the bystander effect that is achievable with secretion-uptake of enzymes.

Aims

- To develop a translational gene therapy with the potential to achieve higher and steadier levels of α-Gal A in disease-relevant tissues and blood.
- To evaluate if a stabilized human α-Gal A produced in vivo through gene therapy would provide a larger window of time for the enzyme to be taken up into the target tissues.

Rational Design-Stabilized Human GLA

Engineered Human GLAs (hGLAs) Has Enhanced Stability



(A) Engineered GLA dimer with artificially introduced disulfide (S-S) bridges for enhanced stability (engineered dimer interface highlighted with circle)
(B) GLA stability assay (performed at neutral pH and 37°C in plasma).

- **Engineered hGLA constructs were stable over the course of 2 hours whereas agalsidase beta lost more than 50% of its activity within 30 min of incubation.**
- **Engineered GLAs showed increased stability at neutral pH and are expected to be more stable in vivo in circulation.**

Conflict of Interest Statement

JMW is a paid advisor to and holds equity in Scout Bio and Passage Bio; he also has sponsored research agreements with Amicus Therapeutics, Biogen, Elaaj Bio, FA212, Janssen, Passage Bio, Regeneron, and Scout Bio, which are licensees of Penn technology. JMW and JH are inventor on patents that have been licensed to various biopharmaceutical companies and for which he may receive payments. TW, PT, DE, SX, HE, JS, RG, JW and HO are employees of Amicus Therapeutics. This work was supported by Amicus Therapeutics.

Preclinical POC Study in Fabry Disease Mouse Model

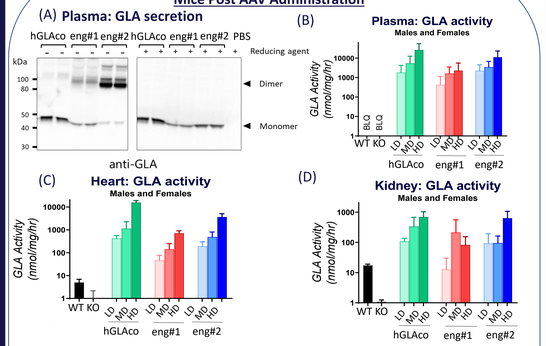
Animal Model:
- Fabry model *GLA* KO
- 4 Males and 4 Females/group
- 3.5-4.5 months at dosing
- 4 weeks study

AAV vector:
- Capsid: Proprietary pantropic
- Promoter: Ubiquitous

Transgene:
- Human GLA wildtype, codon optimized (hGLAco)
- 2 constructs with stabilized and codon optimized human hGLA, (hGLA eng#1, hGLA eng#2)

Dose / Route:
- Dose ranging study with low (LD, 2.5e12 GC/kg), mid (MD, 5e12 GC/kg) and high dose (HD, 2.5e13 GC/kg)
- Tail Vein IV

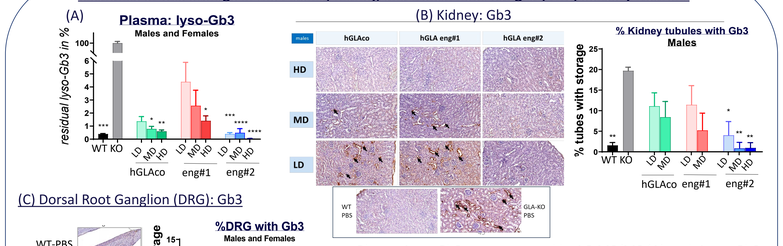
Increased Expression and Activity of Human α-Gal A in GLA KO Mice Post AAV Administration



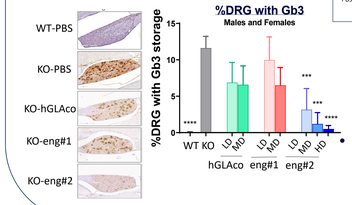
(A) Protein levels of human α-Gal A from AAV treated animals in plasma determined by WB. (B)-(D) GLA enzyme activity measured in Plasma, Heart and Kidney. N=8 (4M/4F) per group

- **Engineering of α-Gal A results in the formation of a stable secreted homodimer**
- **AAV treatment increased GLA enzyme activity in Plasma, Heart, and Kidney in a dose-dependent manner in *GLA* KO mice**

Clearance of Gb3 Storage in the Plasma, Kidney, and Dorsal Root Ganglion (DRG) Sensory Neurons



(C) Dorsal Root Ganglion (DRG): Gb3



Plasma Lyso-Gb3 was measured by LC-MS/MS (A) and tissue Gb3 was determined by immunohistochemistry and quantified by ImageJ in kidney (B) and DRG (C). *p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001, compared with KO-PBS, Dunn's multiple comparison's test

- **hGLA eng#2 stabilized construct showed significantly better efficacy indicated by greater reduction/prevention of Lyso-Gb3/Gb3 in plasma, kidney, DRG and heart (data not shown)**

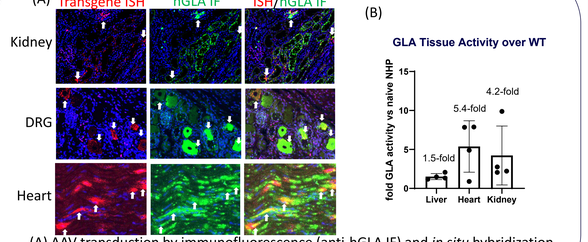
Pilot Study in Non-Human Primates (NHP)

Animal:
- *Cynomolgus Macaque*
- 2 Males/2 Females
- Pre-screened for NAb <1:5
- 2-3 years old
- 2 months study

AAV.hGLA eng#2

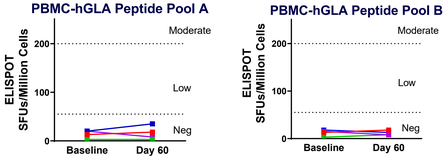
Dose / Route:
- 2.5e13 GC/kG
- Intravenous injection
- No immune suppression nor steroids

AAV Transduction, Transgene Expression and Increased GLA Activity



(A) AAV transduction by immunofluorescence (anti-hGLA IF) and *in situ* hybridization by RNAscope probes against the transgene sequence. Arrows to show colocalization of hGLA IF and transgene ISH; (B) GLA enzyme activity in tissues

(C) ELISpot-hGLA



Safety
(C) T cell responses to hGLA or capsid (data not shown) in lymphocytes from PBMC, liver, spleen or cardiac lymph node are all negative
At day 60, there was no significant finding on histopathology in any of the organs analyzed including the target organs heart, kidney, and dorsal root ganglia (data not shown)

- **AAV.hGLA eng#2 significantly increased GLA enzyme expression and activity and was well tolerated in non human primates.**

Conclusion

- Efficacy of AAV.hGLA eng#2 in Fabry mouse model is dose dependent with significantly greater lyso-Gb3 / Gb3 reduction compared with knockout across all Fabry disease relevant tissues (DRG, kidney, heart) at all tested doses. Demonstration of DRG correction in a disease model is unprecedented, to our knowledge.
- AAV.hGLA eng#2 administered IV in NHP (Cynomolgus macaques) led to high transgene expression that reached efficacious therapeutic levels obtained in the mouse model in key target organs.
- **The remarkable efficacy of AAV-hGLA eng#2 at low doses in Fabry mouse model and robust transgene expression at a moderate dose in NHP suggest a safe and translational approach for Fabry disease. AAV-hGLA eng#2 (AT-GTX-701) has been selected as candidate for IND-enabling studies.**

Acknowledgements

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