# AAV-mediated expression of human α-galactosidase A gene in hepatocytes of a murine Fabry model results in continuous therapeutic levels of enzyme activity and effective substrate reduction

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## Abstract

An AAV-mediated liver-targeted gene therapy was evaluated in a mouse model (GLAKO) that lacks α-GalA activity and accumulates high levels of Gb3/lyso-Gb3 in plasma and tissues. This strategy employs an episomal AAV vector encoding human *GLA* cDNA (h*GLA*) driven by a liver-specific promoter.

Administration of six single doses of increasing amounts of AAV h*GLA* cDNA resulted in supraphysiological expression of plasma α-GalA (up to 50 fold of WT) by day 14, was well tolerated, and was stable for 6 months post-injection. Dose-dependent increases in α-GalA activities were achieved in liver, heart, kidney and spleen with a corresponding reduction of Gb3/lyso-Gb3.

An improved cDNA vector administered to GLAKO mice in a 2 month follow up study produced stable plasma α-GalA levels up to 200 fold greater than WT. α-GalA activity in heart and kidney averaged 20- and 3- fold over wild type levels, respectively, and Gb3/lyso-Gb3 in these tissues were near normal levels.

Appropriate glycosylation of the α-GalA enzyme produced from liver cells was confirmed by *in vitro* experiments to ensure efficient mannose-6-phosphate mediated lysosomal uptake in target tissues.

These studies provide "proof-of-concept" for AAV-mediated targeting of hepatocytes to express therapeutic levels of human α-GalA. The concomitant marked reduction in the accumulated Gb3/lyso-Gb3 in key tissues further support this liver-based AAV h*GLA* cDNA approach as a potential therapy for FD patients.

**About Fabry Disease (FD)**

This X-linked lysosomal storage disease is caused by mutations in the *GLA* gene encoding α-galactosidase A (α-GalA). FD is characterized by progressive systemic accumulation of the enzyme's substrates, globotriaosylceramide (Gb3) and lyso-Gb3, leading to renal, cardiac and/or cerebrovascular disease and culminating in premature demise.

FD is most commonly treated by enzyme replacement therapy (ERT). However, due to short enzyme half-life, ERT necessitates a lifetime of biweekly infusions and may not clear all substrate from secondary organs. A more effective and long-lasting treatment would benefit FD patients.

## AAV cDNA gene therapy of Fabry mouse model GLAKO

**AAV2/6 cDNA Gene Therapy**

hGLA cDNA with liver-specific promoter

**Treatment of GLAKO Mouse Model**

8-13 weeks old
n=4-7 per group

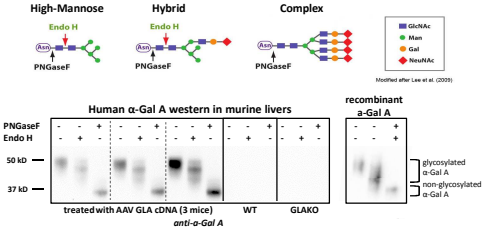
**Timeline & Readouts**

- Weekly plasma collection
- Takedowns at 2 or 6 months post-AAV injection
- Analyze
  I) Human α-Gal A protein expression
  II) α-Gal A enzyme activity
  III) Gb3 biomarker reduction

## Production of glycosylated human α-Gal A in livers of treated GLAKO mice

**Liver homogenates of GLAKO mice treated with AAV GLA cDNA contain high amounts of glycosylated human α-GalA.**

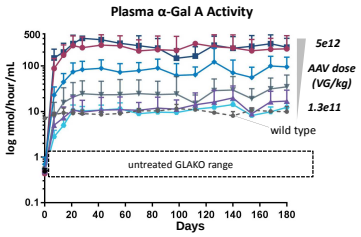
- Homogenates were normalized for protein content
- Aliquots were subjected to PNGaseF- or Endo H-mediated deglycosylation
- **The liver-derived α-Gal A had a similar glycosylation pattern to recombinant enzyme produced in CHO cells (far right panel)**

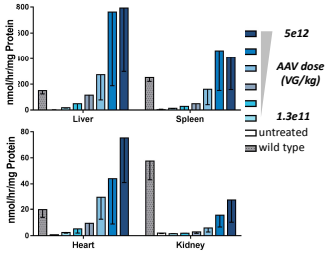



## Supra-physiological α-Gal A activity in treated GLAKO mice

### Liver-produced α-Gal A is secreted into the bloodstream and taken up by secondary tissues

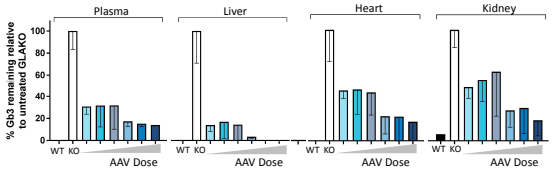
- GLA cDNA gene therapy in GLAKO mice produced high, dose-dependent enzyme activity that was **sustained for 6 months**
- **Plasma α-Gal A activity scaled with AAV dose, up to 50-fold wild type**
- **α-Gal A activity exceeded wild type levels in liver, spleen and heart for the higher dose groups**



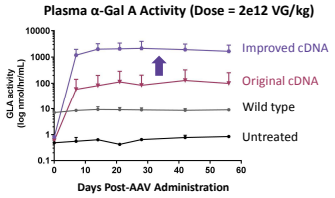

### High levels of α-Gal A activity caused a corresponding decrease of Fabry substrate Gb3 in tissues

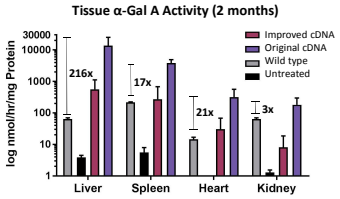
- At higher cDNA doses, **less than 20% of Gb3 remains in heart and kidney** of treated GLAKO mice after 6 months.



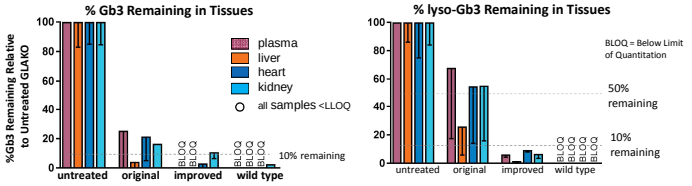

## An improved AAV GLA cDNA construct produces α-Gal A activity up to 200x wild type in plasma and up 20x wild type in target tissues of GLAKO mice

- High, stable plasma α-Gal A activity for two months post-AAV administration
- Activity 3-fold to 20-fold over wild type found in heart, kidney, and spleen



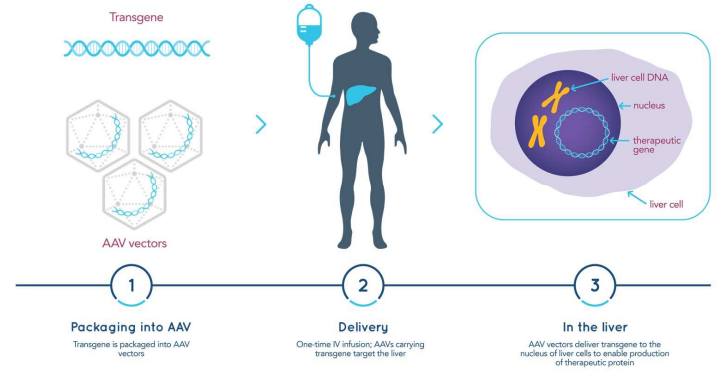




- GLAKO mice treated with improved GLA cDNA construct had **low or undetectable amounts of Fabry substrates Gb3 and lyso-Gb3** in the measured tissues at two months post-vector administration



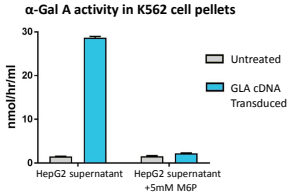

## Liver-targeted AAV gene therapy

### Episomal therapeutic transgene with liver-specific promoter




### cDNA-treated human hepatocyte cell line secretes high levels of α-Gal A which can be taken up by other cell lines via the M6P receptor

- Hepatocyte cell line HepG2 was transduced with AAV GLA cDNA
- α-GalA-enriched supernatant was harvested after 5 days
- K562 cells cultured for 24 hours with the enriched supernatant had high levels of α-Gal A activity
- K562 cells cultured with the enriched supernatant and an excess of Mannose-6 Phosphate (M6P) did not have increased α-Gal A activity
- **This suggests that α-Gal A uptake from supernatant is M6P receptor-mediated**




## Conclusions

AAV-mediated delivery of hGLA cDNA constructs in GLAKO mice led to:

- Expression of high levels of glycosylated α-Gal A in the liver
- Stable, high plasma α-Gal A activity up to 200x wild type for up to 6 months
- Tissue uptake of α-Gal A leading to activity exceeding wild type in heart, kidney and spleen
- Reduction of substrates Gb3 and lyso-Gb3 in tissues within 2 months of vector administration

**These data support the development of Sangamo's liver-targeted AAV GLA cDNA approach as a potential single-administration therapy for the treatment of Fabry disease. This AAV hGLA gene therapy is currently undergoing pharmacology and toxicology studies to support IND submission in 2018.**

**Conflict of Interest Statement**
MWH, SSM, SS, KM, TW and MCH are full time employees of Sangamo Therapeutics, Inc.