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

Robert Desnick[1], Silvere Pagant[1], **Marshall Huston[2]**, Susan St. Martin[2], Scott Sproul[2], Michael C. Holmes[2], Thomas Wechsler[2] and Makiko Yasuda[1]

[1]Icahn School of Medicine at Mount Sinai, New York NY [2]Sangamo Therapeutics, Richmond CA

Abstract

The effectiveness of a liver-targeted zinc-finger nuclease (ZFN)-mediated genome editing strategy that permanently integrates a therapeutic human *GLA (hGLA)* gene in the *Albumin* locus in hepatocytes was evaluated in a knock-out mouse model for Fabry disease (GLAKO mice). This approach ensures long-term expression of the transgene by exploiting the high level transcriptional activity of the native *Albumin* enhancer/promoter in stably modified hepatocytes and utilizes an endogenous promoter, obviating this requirement in the AAV payload.

GLAKO mice received a single injection of AAV encoding a h*GLA* cDNA donor in the presence of *Albumin*-targeted ZFNs under the control of a liver-specific promoter.

Co-administration of these three AAV vectors achieved up to 250x wild type α-GalA activity in plasma, which was sustained for the 2 month study, and supraphysiological activities in liver, heart, kidney and spleen. Gb3 and lyso-Gb3 concentrations in these tissues decreased to normal levels.

To estimate the fraction of hepatocytes that had been successfully modified by our genome editing strategy, liver expression of the Albumin-h*GLA* donor fusion mRNA was measured via in situ hybridization assay at two months post-transduction. The percentage of liver cells expressing the Alb-h*GLA* mRNA ranged from 5.1% (low dose of ZFNs and h*GLA* donor) to 14.6% (high dose).

These studies provide "proof-of-concept" for ZFN-mediated genome editing of hepatocytes to express high levels of human α-GalA, leading to high enzyme activity in plasma and target tissues.

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.

In vivo genome editing of the GLAKO mouse model of Fabry disease

AAV2/8 ZFN + Donor Genome Editing

Treatment of GLAKO Mouse Model

Single IV injection
8-12 weeks old
n=4-5 per group

Timeline & Readouts

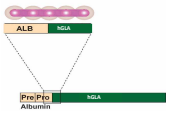
- Weekly plasma collection
- Takedowns at 2 months post-AAV injection
- Analyze
  I) Human α-Gal A protein expression
  II) α-Gal A enzyme activity
  III) Gb3 biomarker reduction

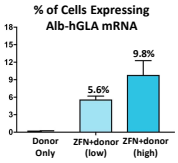
In vivo genome editing results in high levels of hepatocyte modification

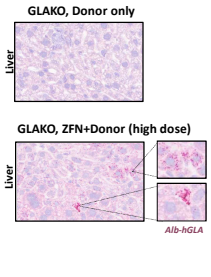
Liver tissue from GLAKO mice transduced with hGLA donors was used for **in situ RNA hybridization** assay.

Individual mice were screened for the presence of Alb-hGLA fusion mRNA, which only occurs when mouse Albumin mRNA is spliced onto the hGLA donor following successful integration.

- Mice given ZFNs and hGLA donor expressed the fusion mRNA in an average of 9.77% (high dose) or 5.55% (low dose) of their liver cells.
- One mouse given hGLA donor but no ZFNs had 0.03% fusion mRNA expression (<LOQ).



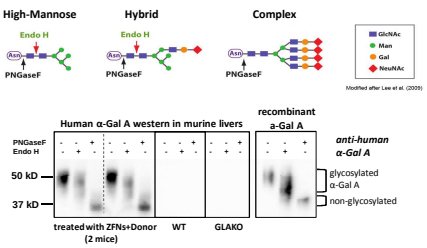




Production of glycosylated human α-Gal A in livers of treated GLAKO mice

Liver homogenates of GLAKO mice treated with ZFN-mediated genome editing contain high amounts of glycosylated human α-GalA.

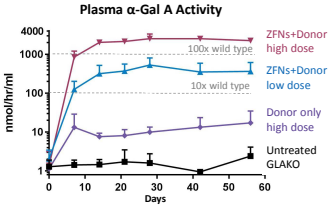
- Homogenates were normalized for protein content
- Aliquots were subjected to PNGaseF- or Endo H-mediated deglycosylation
- **The liver-derived α-Gal A had a similar glycosylation pattern to recombinant enzyme produced in CHO cells (far right panel)**

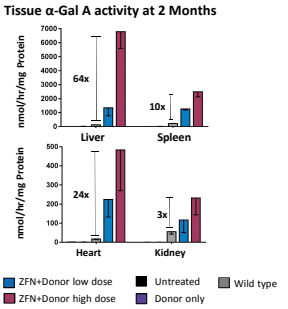


Conflict of Interest Statement
MWH, SSM, SS, MCH and TW are full time employees of Sangamo Therapeutics, Inc.

Supra-physiological α-Gal A activity in plasma and tissues of treated mice

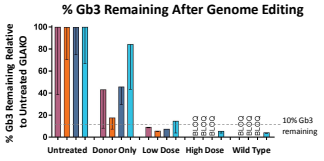
- GLAKO mice were treated with a single dose of ZFNs+hGLA Donor and followed for 2 months
- **Stable plasma activity up to 250-fold wild type** was achieved by Day 14
- α-Gal A activity reached **over 20-fold wild type in heart** and **over 3-fold wild type in kidney of treated GLAKO mice**

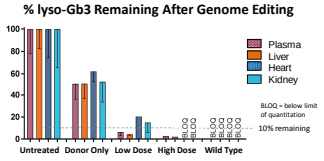




Liver-secreted α-Gal A clears substrates from target tissues

- **Gb3 and lyso-Gb3 are low or undetectable in plasma, liver, heart and kidney** 2 months post-genome editing

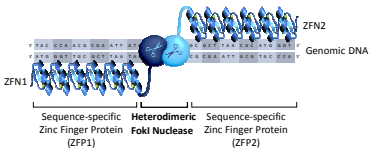




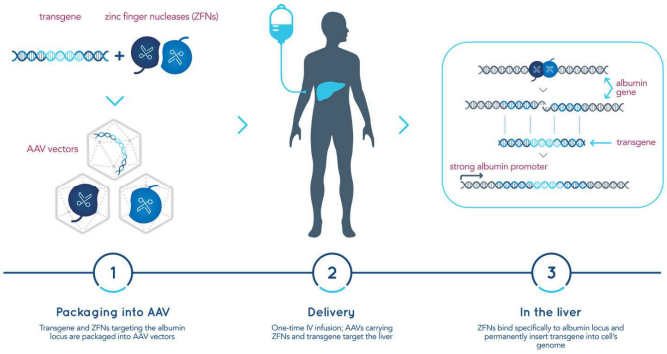
In Vivo Genome Editing Therapeutic Platform

Liver-based AAV genome editing has potential therapeutic advantages

- **Convenience**
  Single administration versus biweekly ERT infusions
- **Efficacy**
  Constant supply of therapeutic enzyme may lead to better efficacy in target tissues compared to ERT
- **Tolerance**
  Liver expression may lead to tolerization to transgene



ZFN-mediated integration of a donor gene at the Albumin locus for production of high, sustained levels of therapeutic enzyme in liver



Conclusions

AAV-mediated delivery of ZFNs + hGLA Donor sequences in GLAKO mice led to:

- Expression of high levels of glycosylated α-Gal A in the liver
- Stable plasma α-Gal A activity at levels many-fold above wild type
- Uptake of α-Gal A by secondary tissues, leading to α-Gal A activity exceeding wild type in the heart, kidney and spleen
- Highly reduced levels of Fabry substrates Gb3 and lyso-Gb3 in tissues 2 months post vector administration

These data support the development of Sangamo's liver-targeted AAV genome editing approach as a platform for potential single-administration therapy for the metabolic diseases, including Fabry disease.