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 Desnick¹, Silvere Pagant¹, Marshall Huston², Susan St. Martin², Scott Sproul³, Michael C. Holmes³, Thomas Wechsler² and Makiko Yasuda¹

¹Icahn School of Medicine at Mount Sinai, New York NY ²Sangamo 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 (NGLA) 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 promoter, allowing efficient expression of the transgene in stably modified hepatocytes and resulting in a prolonged promoter platform, obviating the requirement for the AAV payload. GLAKO mice received a single injection of AAV encoding a NGLA-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 25x wild type α-Gal A 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-NGLA donor fusion mRNA was measured via in situ hybridization assay at two months post-transduction. The percentage of liver cells expressing the AAV-NGLA mRNA ranged from 5.1% (low dose of ZFNs and NGLA donor) to 14.9% (high dose). These results provide ‘proof-of-concept’ for ZFN-mediated genome editing of hepatocytes to express high levels of human α-Gal A, 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

AAV/ZFN + Donor Genome Editing

<table>
<thead>
<tr>
<th>Treatment of GLAKO Mouse Model</th>
<th>Timeline &amp; Readouts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single IV injection 8-12 weeks old, n=4-5 per group</td>
<td>• Weekly plasma collection</td>
</tr>
<tr>
<td>ALK-HD (low dose)</td>
<td>• Takeda and at 2 months post-AIV injection</td>
</tr>
<tr>
<td></td>
<td>• Analyte</td>
</tr>
<tr>
<td></td>
<td>(i) Human α-Gal A protein expression</td>
</tr>
<tr>
<td></td>
<td>(ii) α-Gal A enzyme activity</td>
</tr>
<tr>
<td></td>
<td>(iii) GB3 biotin level reduction</td>
</tr>
</tbody>
</table>

% of Cells Expressing Abb-NGLA mRNA

GLAKO, Donor only

GLAKO, ZFN-Donor (high dose)

% GB3 Remaining After Genome Editing

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

Liver SECRETED α-Gal A clears substrates from target tissues

Liver-based AAV genome editing has potential therapeutic advantages

1. Convenience
   Single administration versus biweekly ERT infusions

2. Efficacy
   Constant supply of therapeutic enzyme may lead to better efficacy in target tissues compared to ERT

3. Tolerance
   Liver expression may lead to tolerization to transgene

In Vivo Genome Editing Therapeutic Platform

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-Gal A.

- 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 (left panel)

AAV-mediated delivery of ZFNs + GLA 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 heart, kidney and spleen
- Highly reduced levels of Fabry substrates GB3 and lyso-GB3 in tissues 2 months post-vector administration

Conclusions

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.