Liver-based expression of the human alpha-galactosidase A gene (GLA) in a murine Fabry model results in continuous high levels of enzyme activity and effective substrate reduction.

“Preclinical data supporting a gene therapy for Fabry Disease”

Thomas Wechsler, Ph.D
May 10th, 2017
In 2017, Sangamo is focused on enrolling four clinical trials including the first ever human *in vivo* genome editing studies.

**Four lead clinical development programs**

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*In Vivo* Gene Therapy  *In Vivo* Genome Editing
Fabry Disease is a lysosomal storage disorder

- Fabry disease is an X-linked monogenic disease caused by mutations in GLA gene encoding the enzyme alpha-galactosidase A (α-Gal A)

- α-Gal A plays a role in degradation of globotriaosylceramide (Gb3) and Lyso-Gb3

- The liver is a significant source of Gb3/Lyso-Gb3 storage material accumulating in lysosomes of endothelial cells, kidney and heart

- Depending on residual enzyme activity there are two major phenotypes:

  *Type I - Classic Fabry: <1% of α-Gal A activity; 1 : 40,000*
  *Type II - Later-onset Fabry: >1% of α-Gal A activity; ~ 1 : 5,000*
Fabry Disease is a progressive systemic disease

Skin: Angiokeratoma Hypohidrosis

Heart: Early dysfunction to cardiac death

Kidney: Progressive renal dysfunction leading to failure and dialysis

Nervous System: Neuropathy Stroke (~7%)

Standard of care is enzyme replacement therapy (ERT):
- Recombinant α-Gal A is produced in CHO cells
- Biweekly infusion (2-4 hr) of α-Gal A
- Annual cost: ~ $250,000 per patient

Unmeet needs:
- Cardiac Gb3 clearance
- Podocyte Gb3 clearance
- Neuropathic pain
- Long-term stroke risk

Other therapeutic strategies:
- HSC gene therapy
- Pharmacological chaperone therapy
- Substrate reduction therapy
Fabry Disease is a progressive systemic disease

Skin:
- Angiokeratoma
- Hypohidrosis

Heart:
- Early dysfunction to cardiac death

Kidney:
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Nervous System:
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- HSC gene therapy
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- Substrate reduction therapy
Fabry Disease is a progressive systemic disease

Skin: Neuropathy

Heart: Early dysfunction to cardiac death

CNS: Stroke (~7%)

Kidney: Progressive renal dysfunction leading to failure and dialysis

Standard of care is enzyme replacement therapy (ERT):
- Recombinant $\alpha$-Gal A is produced in CHO cells
- Biweekly infusion (2-4 hr) of $\alpha$-Gal A
- Annual cost: ~ $250,000 per patient

Unmeet needs:
- Cardiac Gb3 clearance
- Podocyte Gb3 clearance
- Neuropathic pain
- Long-term stroke risk

Other therapeutic strategies:
- HSC gene therapy
- Pharmacological chaperone therapy
- Substrate reduction therapy
Advantages of liver-directed gene therapy

Liver-based AAV gene therapy has the following potential advantages:

- **Convenience**  
  Single administration versus biweekly infusions

- **Efficacy**  
  Constant supply of therapeutic enzyme (versus peak/trough) may lead to better efficacy in target tissues compared to ERT

- **Tolerance**  
  Liver expression may lead to tolerization towards transgene
Two approaches of single administration treatments for Fabry Disease

**cDNA gene therapy**

- AAV vector
- Therapeutic gene

Liver Cell

- Nucleus
- Promoter
- Therapeutic Gene
- Liver Cell DNA

Episomal therapeutic transgene with liver specific promoter

Liver produces and secretes therapeutic enzyme with glycosylation suitable for tissue uptake and lysosomal delivery

**In vivo genome editing**

- AAV vectors
- Zinc Finger Nuclease (ZFN) 1 and 2

Therapeutic gene

Strong albumin Promoter

Albumin locus

Therapeutic gene

Homology arm

Integrated therapeutic transgene using albumin promoter
Two approaches of single administration treatments for Fabry Disease

**cDNA gene therapy**

- AAV vector
- Therapeutic gene

- Single treatment
- Can provide long lasting benefit
- Single vector
- Episomal therapeutic transgene with liver specific promoter
- Treatment strategy is in clinic for Hemophilia

**In vivo genome editing**

- AAV vectors
- Zinc Finger Nuclease (ZFN) 1 and 2
- Therapeutic gene

- Single treatment
- Can provide life-long benefit
- Three vectors
- Integrated therapeutic transgene using albumin promoter
- Treatment strategy is entering clinic for both MPS I and MPS II

Liver produces and secretes therapeutic enzyme with glycosylation suitable for tissue uptake and lysosomal delivery

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cDNA gene therapy strategy for Fabry Disease

**cDNA gene therapy**

- AAV vector
- Therapeutic gene
- Liver Cell
- Nucleus
- Promoter
- Therapeutic Gene
- Liver Cell DNA
- Episomal therapeutic transgene with liver specific promoter

**Proof of concept study design**

Single injection of cDNA vectors into GLAKO mice

- cDNA dose: $2 \times 10^{12}$ Vg/kg
- Weekly plasma collection and α-Gal A activity measurement
- Takedowns at 2 months post-AAV injection for tissue analysis

**GLAKO mouse accumulates Gb3 and lyso-Gb3 in tissues**

- GLAKO mouse: increased Gb3 and lyso-Gb3 in tissues compared to WT (25 weeks)
  - Liver, kidney, heart, plasma

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AAV-mediated delivery of optimized GLA cDNA in GLAKO mice

α-Gal A activity in plasma

α-Gal A activity in tissues (2 months)

Original GLA cDNA construct at low dose (2e12 vg/kg)
Optimized GLA cDNA construct at low dose (2e12 vg/kg)

α-Gal A overproduced in liver is safe, secreted at high levels and taken up by other tissues in active form
Analysis of Gb3 and Lyso-Gb3 substrate clearance in target tissues

Gb3 in tissues

Lyso-Gb3 in tissues

α-Gal A produced in liver leads to significant Gb3 and Lyso-Gb3 reduction in plasma, liver and heart
Analysis of Gb3 and Lyso-Gb3 substrate clearance in target tissues

Gb3 in tissues

Lyso-Gb3 in tissues

α-Gal A produced in liver leads to significant Gb3 and Lyso Gb3 reduction in target tissues heart and kidney.
Evaluation of long-term $\alpha$-Gal A liver expression and efficacy

$\alpha$-Gal A activity in plasma

$\alpha$-Gal A overexpression from the liver is stable up to ~6 months and is well tolerated
**Proof of concept study design**

Single injection of ZFNs and hGLA transgene into GLAKO mice

- Total dose: 6e13 Vg/kg
- Regular plasma collection and α-Gal A activity measurement
- Takedowns at 2 months post-AAV injection for tissue analysis

**In vivo genome editing**

Integration of therapeutic transgene at the albumin locus leads to potentially life-long stable expression

- AAV vectors
- Zinc Finger Nuclease (ZFN) 1 and 2
- Therapeutic gene
- Strong albumin Promoter
- Albumin locus
- Therapeutic gene
- Homology arm
ZFN-mediated integration of hGLA cDNA at the albumin locus

Liver

Albumin locus

Albumin promoter

Exon1

Exon2

hGLA cDNA

ZFN pair

Liver

Integrated hGLA transgene

Exon1

Exon2

hGLA cDNA

SA

SD

Liver

ALB-hGLA fusion protein

Liver

Secreted hGLA protein

Plasma & other tissues

hGLA

Pre Pro

SP

hGLA

Signal peptide

SP

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In vivo genome editing leads to high $\alpha$-Gal A expression in plasma and uptake in secondary tissues

$\alpha$-Gal A activity in plasma

![Graph showing $\alpha$-Gal A activity in plasma over time for SP1, SP2, and wild type, with GLAKO as a comparison.](image)

- **SP2**: High levels of $\alpha$-Gal A activity in plasma.
- **SP1**: Moderate levels of $\alpha$-Gal A activity in plasma.
- **Wild type** and **GLAKO**: Lower levels of $\alpha$-Gal A activity in plasma.

Average:
- **Plasma**: 300x wild type
- **Plasma 21x wild type**

$\alpha$-Gal A activity in tissues (2 months)

![Bar graph showing $\alpha$-Gal A activity in liver, heart, kidney, and spleen 2 months after AAV transduction.](image)

- **Liver**: High levels of $\alpha$-Gal A activity.
- **Heart**: 31x wild type
- **Kidney**: 2.3x wild type
- **Spleen**: Lower levels of $\alpha$-Gal A activity.

**Average**:
- **Heart**: 31x wild type
- **Kidney**: 2.3x wild type

$\alpha$-Gal A produced from the albumin locus is secreted at high levels and taken up by other tissues in active form.
α-Gal A produced from the albumin locus has the appropriate glycosylation mediating cellular and lysosomal uptake

Modified after Lee et al. (2009)
α-Gal A secreted by liver and taken up by heart and kidney is able to clear nearly all of the substrate in these tissues within 2 months.
Conclusions

Transducing GLAKO (Fabry) mice with either hGLA cDNA vectors or in vivo genome editing vectors led to:

- Expression of very high levels of α-Gal A in the liver
- Secretion of functional α-Gal A into the bloodstream resulting in stable plasma α-Gal A activity levels many-fold above wild type
- Uptake of α-Gal A by secondary tissues, leading to α-Gal A activity exceeding wild type levels in the heart and spleen at the used doses
- Clearance of most or all of globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) substrates in plasma, liver, heart and kidney 2 months after cDNA gene therapy or genome editing

These data support the development of Sangamo Therapeutics’ liver-based AAV approaches as potential therapies for the treatment of Fabry disease
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