Development of an AAV5 gene therapy for Fabry disease

uniQure

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Fabry disease: a Lysosomal Storage Disease (LSD)

- X-linked genetic disorder
- Deficiency of α-galactosidase A (α-Gal A or GLA)
- Females also suffer from Fabry, but severity depends on X-inactivation despite having GLA activity in the plasma
- Systemic accumulation of substrate; Gb3 and LysoGb3 in plasma, tissues and organs
- Bi-weekly ERT infusions have limited effectiveness due to lack of cross-correction
- Furthermore, a significant number of patients develop antibodies to GLA


in black: early symptoms
in red: late symptoms
Limitations of ERT:

- Poor cross-correction of GLA
  - Heterozygous females are also symptomatic
  - Thus, unaffected cells produce GLA but uptake into lysosomes via the Mannose 6-phosphate receptor is poor
  - In other LSD’s, such as MPS II, the enzyme effectively cross-corrects and hence carriers are asymptomatic
- Poor cross-correction hampers clearance of substrates in the target organs in particular the kidney and the heart
- Long-term ERT could slow disease progression, but effects may be limited
uniQure’s approach: modified NAGA

Novel Approach
- Expression of modified NAGA (modNAGA) using AAV5 vector (constant supply)
- ModNAGA has GLA activity and is able to reduce (Lyso-)Gb3 accumulation

- More stable in blood and at low pH
- More efficient uptake
- Better distribution

- Expression of endogenous NAGA in classic Fabry patients

- More effective (cross-correction) than ERT

Tajima et al. 2009 (PMID: 19853240)


Licensed from Prof. Sakuraba, Tokyo
Two approaches: liver specific or constitutive promoter

Liver produces and secretes protein, which can be taken up into target organs

Constitutive protein expression from target organs

L1

C1

modNAGA

ModNAGA

AAV5

NAGA

coNAGA

SV40pA

or

or
Studies to show proof of concept of (AAV5-)modNAGA - *in vitro* and *in vivo* -

*In vitro*, Fabry fibroblasts
- GLA activity
- LysoGb3
- M6P-receptor mediated uptake

**Wt mice**
- GLA activity
- (plasma and target organs)

**Fabry mice**
- GLA activity
- LysoGb3
- (plasma and target organs)

**NHP**
- GLA-activity
- (plasma)
modNAGA cross-corrects Fabry patient-derived fibroblasts through M6P-dependent uptake

Conclusion: *In vitro* produced modNAGA is taken up via M6P-receptors and reduces lysoGb3 levels in Fabry patient derived fibroblasts.
AAV5-modNAGA increases plasma GLA activity and reduces LysoGb3 in Fabry (GLA-KO) mice

Collaboration with Hans Aerts, Leiden and Carlie de Vries, Amsterdam

Conclusion: Increased GLA-activity and approximately 50% reduction of LysoGb3 in plasma
Increased GLA activity and reduction of LysoGb3 in Fabry (GLA-KO) mice liver following AAV5-modNAGA injection

Conclusion: More than 20 times increased GLA-activity and roughly 80% reduction of LysoGb3 in the liver
AAV5-modNAGA reduces LysoGb3 in target organs in Fabry (GLA-KO) mice

GLA KO mice (n=7)
5e13 gc/kg (i.v.)
AAV5-modNAGA

Conclusion: Continuous (30 wks) LysoGb3 reduction in target organs kidney and heart and potentially for the brain.
Reduction of Gb3 in the kidneys of Fabry (GLA-KO) mice

- Immunohistochemistry
  - Anti-Gb3 staining (red); Nucleus (blue)
  - Fabry mice show high Gb3 accumulation in the medullary area

Conclusion: Moderate reduction of Gb3 in cortical and medullary structures of the kidney
Wild type cynomolgus monkey
9±13 gc/kg (n=2, i.v.)
AAV5-modNAGA

Preliminary conclusion: Expression from both AAV5-modNAGA vectors in the liver and GLA-activity levels in plasma follow results of GLA-KO mice
ModNAGA has a low immunogenicity risk

Collaboration with Abzena and Pro-immune

ModNAGA has a low immunogenicity risk

**Immunogenicity evaluation of modNAGA**

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Conclusions

- AAV5-modNAGA results in GLA activity in plasma of NHP
- AAV5-modNAGA results in increased GLA activity and Lyso-Gb3 reduction in plasma and target organs for at least 30 weeks in GLA-KO mice
- Plasma GLA activity is not indicative for efficacy of therapy
- Expressed modNAGA contains high mannose glycans and is taken up via M6P-receptor
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