Development of an AAV5 gene therapy for Fabry disease

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Forward-looking Statements

This presentation contains forward-looking statements. All statements other than statements of historical fact are forward-looking statements, which are often indicated by terms such as “anticipate,” “believe,” “could,” “estimate,” “expect,” “goal,” “intend,” “look forward to,” “may,” “plan,” “potential,” “predict,” “project,” “should,” “will,” “would” and similar expressions. Forward-looking statements are based on management’s beliefs and assumptions and on information available to management only as of the date of this press release. These forward-looking statements include, but are not limited to, statements regarding the development of our gene therapies, the success of our collaborations, and the risk of cessation, delay or lack of success of any of our ongoing or planned clinical studies and/or development of our product candidates. Our actual results could differ materially from those anticipated in these forward-looking statements for many reasons, including, without limitation, risks associated with collaboration arrangements, our and our collaborators’ clinical development activities, regulatory oversight, product commercialization and intellectual property claims, as well as the risks, uncertainties and other factors described under the heading "Risk Factors" in uniQure’s Quarterly Report on Form 10-Q filed on November 1, 2017. Given these risks, uncertainties and other factors, you should not place undue reliance on these forward-looking statements, and we assume no obligation to update these forward-looking statements, even if new information becomes available in the future.
- X-linked genetic disorder
- Deficiency of α-galactosidase A (α-Gal A or GLA)
- Females also suffer from Fabry and 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 may not be effective due to poor organ incorporation due to lack of cross correction (uptake into lysosomes)
- Furthermore, a significant number of patients develop antibodies to GLA


<in black: early symptoms
<in red: late symptoms

CNS: neuropathy strokes
Eyes: corneal opacities
Heart: dysfunction
Kidney: dysfunction leading to failure and dialysis
Gastro-Intestinal problems
Skin: Angiokeratoma hypohidrosis

What is cross correction and why is it important?

Disadvantages 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 MPS II, there are asymptomatic carriers due to sufficient cross correction
- Poor cross correction hamper clearance of all substrate in target organs
- Long-term ERT does not prevent disease progression

Adapted from Parenti et al. Int J Mol Med 2013
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 LysoGb3 accumulation

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

More effective (cross-correction) than ERT

Tajima et al. 2009 (PMID: 19853240)

modNAGA is active in the presence of GLA inhibitors

Patients with and without inhibitors


Expression of endogenous NAGA in classic Fabry patients

Non-immunogenic

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

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

Constitutive protein expression from target organs

**L1**

**C1**

NAGA

coNAGA

spNAGA

SV40pA

GLA signal peptide

ModNAGA

AAV5
Studies to show proof of concept of modNAGA *in vitro* and *in vivo*

*In vitro, cells*

- GLA activity
- M6P-receptor mediated uptake

*Wt mice*

- GLA activity
  - (plasma and target organs)

*Fabry mice*

- GLA activity
  - LysoGb3
  - (plasma and target organs)
Expression of modNAGA results in GLA activity in cells and cellular uptake is mediated via M6P-receptor

Conclusions:
- Expression and secretion of modNAGA, that exhibits GLA-activity
- M6P-receptor blockage results in increased GLA activity in the supernatant, suggesting M6P-receptor mediated uptake of NAGA.
Glycosylation pattern of *in vitro* expressed modNAGA indicates presence of high mannose oligosaccharides

**PNGaseF**
- **Cleaves high mannose, hybrid and complex oligosaccharides**

**EndoH:**
- **Is unable to cleave N-linked complex type glycans**

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**modNAGA**

<table>
<thead>
<tr>
<th>KDa</th>
<th>M</th>
<th>PNGase</th>
<th>endoH</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td></td>
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<tr>
<td>37</td>
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</table>

*In vitro expressed modNAGA: Contains both high mannose and complex N-linked glycans*
GLA activity and reduction of LysoGb3 in Fabry (GLA-KO) mice plasma following AAV5-modNAGA injection

Collaboration with Hans Aerts, Leiden and Carlie de Vries, Amsterdam
LysoGb3 reduction in target organs in GLA-KO mice upon AAV-injection

**Conclusion:** LysoGb3 reduction in all target organs upon AAV5-modNAGA injection in GLA-KO mice

**GLA-KO mice IV injected with 5\(^{13}\) gc/kg AAV5-modNAGA**

**lysoGb3 levels in organs**

**liver**

- Wt Vehicle
- Fabry Vehicle
- AAV5-C7-NAGA
- AAV5-C7-coNAGA
- AAV5-EF1a-NAGA
- AAV5-EF1a-coNAGA

**kidney**

- Wt Vehicle
- Fabry Vehicle
- AAV5-L1-NAGA
- AAV5-L1-coNAGA
- AAV5-C1-NAGA
- AAV5-C1-coNAGA

**heart**

- Wt Vehicle
- Fabry Vehicle
- AAV5-L1-NAGA
- AAV5-L1-coNAGA
- AAV5-C1-NAGA
- AAV5-C1-coNAGA

Collaboration with Hans Aerts, Leiden
ModNAGA poses a low predicted immunogenicity risk versus endogenous NAGA

**Immunogenicity evaluation of modNAGA**

<table>
<thead>
<tr>
<th>Step 1 – In silico</th>
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<tbody>
<tr>
<td>Algorithms to screen potential T-cell epitopes</td>
</tr>
<tr>
<td>Identify linear motifs (9-10 aa) that bind to HLA MHC class I or II molecules</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>ModNAGA</th>
<th>Moderate affinity</th>
<th>High affinity</th>
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</thead>
<tbody>
<tr>
<td>MHC I peptides</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MHC II peptides</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Step 2 – In vitro**

- 2 MHC class I peptides tested to common MHC class I (HLA) alleles:
  - A*01:01, A*02:01, A*03:01, A*11:01, A*24:02, A*29:02, B*07:02, B*08:01, B*14:02, B*15:01, B*27:05, B*35:01, B*40:01

- Quantitative and qualitative analysis:
  - Peptide binding properties demonstrate that the two peptides do not pose an increased immunogenicity risk compared to endogenous NAGA
Conclusions and future plans

Conclusions

- AAV5-NAGA results in therapeutic GLA activity and Lyso-Gb3 reduction in plasma and target organs at already 12wks
- Plasma GLA activity is not indicative for efficacy of therapy
- ModNAGA may be a more effective therapy (Tajima et al. 2009)
  - More stable
  - Can be used in GLA inhibitor patients
  - Better organ distribution
- Expressed modNAGA contains high mannose glycans and is taken up via M6P-receptor

Future plans

- Determine GLA activity and Lyso-Gb3 reduction in target organs of AAV-injected Fabry mice (> 6 mo)
- Determine AAV vector distribution and expression in target organs (>6 mo)
- Glycosylation characterization of in vivo expressed modNAGA
- Detect Zebrabodies in kidney cells and localization of active modNAGA in lysosomes
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GLA and NAGA protein expression

**GLA**

- **RNA expression (TPM)**
  - Brain
  - Endocrine tissues
  - Bone marrow & immune system
  - Muscle tissues
  - Lung
  - Liver & gallbladder
  - Pancreas
  - Gastrointestinal tract
  - Kidney & urinary bladder
  - Male tissues
  - Female tissues
  - Adipose & soft tissue
  - Skin

- **Protein expression (score)**
  - Central cortex
  - Adrenal gland
  - Lymph nodes
  - Liver
  - T cells

**NAGA**

- **RNA expression (TPM)**
  - Brain
  - Endocrine tissues
  - Bone marrow & immune system
  - Muscle tissues
  - Lung
  - Liver & gallbladder
  - Pancreas
  - Gastrointestinal tract
  - Kidney & urinary bladder
  - Male tissues
  - Female tissues
  - Adipose & soft tissue
  - Skin

- **Protein expression (score)**
  - Central cortex
  - Adrenal gland
  - Lymph nodes
  - Liver
  - T cells
GLA-activity in plasma of wild type mice injected with AAV5-mod-NAGA

Wild type mice IV injected with $5 \times 10^5$ gc/kg AAV5-modNAGA

Vector DNA

GLA-activity

liver

plasma

<table>
<thead>
<tr>
<th>Genome copies /μg DNA</th>
<th>7 wks post IV</th>
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<tr>
<td></td>
<td>$10^1$</td>
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<table>
<thead>
<tr>
<th>Vehicle</th>
<th>AAV5-L1-NAGA</th>
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<tbody>
<tr>
<td>GLA-activity (nmol/h/mL)</td>
<td>2, 4 and 7 wks post IV</td>
</tr>
<tr>
<td>Vehicle</td>
<td>AAV5-L1-coNAGA</td>
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<tr>
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<td>AAV5-L1-spNAGA</td>
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<tr>
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<td>AAV5-C1-NAGA</td>
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</table>
Conclusions: All AAV5-modNAGA vectors increase GLA-activity in the liver by at least 10 times above wild type AAV5-EF1a-modNAGA might increase GLA-activity in target organs.
GLA activity in liver of Fabry (GLA-KO) mice upon AAV5-modNAGA injection

GLA-KO mice IV injected with 5e13 gc/kg AAV5-modNAGA

Conclusion: Increased GLA activity in the liver of AAV-modNAGA injected GLA-KO mice
Vector DNA and NAGA mRNA expression in target organs of Fabry (GLA-KO) mice upon AAV5-modNAGA injection