Towards AAV5-mediated Gene Therapy for Hemophilia A with a Factor IX Variant that functions independently of FVIII

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Hemophilia A; a bleeding disorder due to lack of FVIII

- X-linked bleeding disorder

- Deficiency in coagulation factor FVIII that serves as a cofactor for factor IX for activation of the coagulation cascade

- Spontaneous bleeds

- Classified into severe, moderate and mild (<1%, 1-5% and >5-40% of FVIII activity)

- ~66,000 patients with severe HemA in US and Europe

- Treatment: FVIII concentrates

- 30% of patients develop inhibitors
Why not express FVIII in the liver?

- Endogenous FVIII synthesis in endothelial cells and not hepatocytes
- Expression in the liver may not be sustainable
uniQure’s approach: FIX variant

Novel Approach

- Expression of a FIX variant with FVIII-independent FX activity using AAV5 vector

- Hepatocyte friendly
  - Non immunogenic

- Correction of hemophilia phenotype

- Long-term expression

- Efficacious in patients with and without inhibitors

- Safety
  - Non thrombogenic
  - Normal activation
  - Low predicted immunogenicity risk
FIX-FIAV activates FX in the absence of FVIII

**FIX-FIAV:**
L6F, V181I, K265A and I383V

**Not hyperactive and normally activated**

Adapted from Kristensen L. H. et al Biochem J. 2016
AMT-180: AAV5-Q1-FIX-FIAV is physiologically activated

The inactive FIX-FIAV zymogen is expressed - Activation is required

- FX activation in the absence of FVIII
Studies to show proof of concept of FIX-FIAV *in vitro* and *in vivo*

*In vitro, cells*
- FIX protein
- FVIII-independent activity

*Wt mice*
- FIX protein

*HemA mice*
- FIX protein
- FVIII-independent activity

*Cynomolgus Macaques*
- FIX protein
- Safety / tolerability of the AAV product
- FIX-FIAV (5 µg/ml) shows 32% and 29% of FVIII-independent activity by APTT and thrombin generation relative to a FVIII standard
- FIX-FIAV thrombin generation curve overlaps with the normal curve
FIX-FIAV is not hyperactive and requires physiological activation (same as FIX)

**TF/FVIIa activation of FIX variants**

- **Fixa/min (nM)**
- **FIX (nM)**
  - FIX - WT
  - FIX - FIAV

**Western blot**

- **FIXa**
- **FIX-WT**
- **FIX-FIAV**

55kDa = FIX

45kDa = FIXa

- **kcat**
  - FIX - WT: 0.01103
  - FIX - FIAV: 0.01027

- **Best-fit values**
  - Et: 50.00
  - Km: 548.4
  - Vmax: 0.5516

- **t=0**
- **t=60**
FVIII-independent activity upon AAV injection in hemophilic mice

**Clotting (APTT) assay**

+ Calcium generation FXa

Actuator + phospholipids

FIX-FIAV in sample

FVIII deficient plasma

magnet

clotting time

**FVIII-independent activity**

Relative to a serial dilution of a FVIII standard

**STD FVIII**

\[ y = -6.935 \ln(x) + 67,408 \]

\[ R^2 = 0.9851 \]

**n=10, male**

FVIII KO mice

IV dose 5×13 gc/kg

**FVIII-independent activity vs FIX protein**

**Week 5**

- FIX-FIAV shows FVIII independent activity in hemophilic mice
- Measured in APTT assay
Fix-FIAV shows a therapeutic meaningful FVIII-independent activity in hemophilic plasma

- Normalisation of the FVIII-independent activity to 100% of FIX protein
- ~24% of FVIII-independent activity in hemophilic mice

Summary efficacy AMT-180

- Recombinant FIX-FIAV
  ✓ 29% FVIII-like activity in thrombin generation assay
  ✓ 32% FVIII like-activity in clotting assay
- AMT-180 in hemophilic mice
  ✓ 24% FVIII-like activity in clotting assay
- AMT-180 expected to show clinical meaningful efficacy (per 100% protein)
FIX-FIAV expression in NHPs expected to translate to therapeutically relevant FVIII independent activity in humans

male Cynomolgus macaque

n=2
IV, $9 \times 10^6$ gc/kg
adapted delivery

1 vehicle treated NHP

1) AAV5-LP1-FIAV
2) AAV5-Q1-FIAV

Q1 = a proprietary liver specific promoter

8-folds increased protein expression using Q1
Safety assessments: non thrombogenic & low predicted immunogenicity risk

**Thrombogenicity**

- No elevation of coagulation activation markers: TAT + D-dimer levels in AAV-injected mice and NHPs
- Histopathological examination of the NHP organs did not show signs of thrombus formation

**Immunogenicity**

- In silico assessment of potential T-cell epitopes
- 9-10 aa peptides that bind to HLA MHC Class II or I molecules
- 4 moderate affinity peptides found for MHC Class I and no peptides for MHC Class II
- Quantitative and Qualitative analysis of MHC Class I peptide binding properties predict a non significant risk compared to FIX-wt
Conclusions

• AMT-180 is expected to prevent bleeds; sufficient thrombin generation & clot formation
• Hepatocyte friendly
• Safe; non thrombogenic (normal activation & regulation) & low predicted immunogenicity risk
• Effective for HemA patients with and without inhibitors

Ongoing & Future plans

• Full biochemical characterization of recombinant FIX-FIAV protein
• Thrombin generation & clotting activity of AAV-injected NHP plasma samples (with or without addition of FVIII antibodies)
• GLP tox study in NHPs ongoing
• IND enabling
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FVIII-independent activity in AAV-FIX-FIAV transduced cells

- AAV transduction of Huh-7 cells
- Dose-dependent FIX-FIAV protein expression (ELISA) and FVIII-independent activity as measured by APTT clotting assay relative to a FVIII standard
Some mice were excluded for analyses based on bioanalyses data
Dose = 5°13 gc/kg
Dose-dependent increase in FVIII independent activity upon increased FIX-FIAV expression in hemophilic mice

Day 0
IV tail vein injection

Weeks 1, 2, 5 and 8
Collect blood

Week 8
Sacrifice

Phenotypic correction assay

n=11
Dosis= high:1.44 X 10^14, mid: 5 X 10^13, low: 1 X 10^13 gc/kg

1) Vehicle (n=6)
2) AAV-FIX-wt (high)
3) AAV-FIX-FIAV (high)
4) AAV-FIX-FIAV (mid)
5) AAV-FIX-FIAV (low)
6) AAV-FVIII (high)

Week 8 FVIII mimetic activity

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Note: The image contains a bar graph showing Week 8 FVIII mimetic activity across different groups.