Development of a novel gene therapy for Pompe disease: Engineered acid alpha-glucosidase transgene for improved expression and muscle targeting

Steven Tuske[1], Ting Yu[2], Juliette Hordeaux[2], Su Xu[1], Rebecca Soska[1], Anju Nair[1], Pai-Chi Tsai[1], Richie Khanna[1], Rick Hamler[1], Anthony Perry[1], Nastry Brignol[1], Suresh Venkateswaran[1], Finn Hung[1], Jessie Feng[1], Russell Gotschall[1], Tobias Willer[1], Ce Feng Liu[1], Renee Krampetz[1], Quyen Hoang[3], James M Wilson[2] and Hung Do[1]

[1]Amicus Therapeutics Inc. 1 Cedar Brook Drive, Cranbury, NJ 08512 USA,
[2]Gene Therapy Program, Department of Medicine, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA 19104, USA,
[3]School of Medicine, Indiana University, 635 Barnhill Drive Medical Science, Indianapolis, IN 46202

Abstract

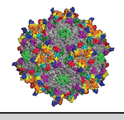
Pompe disease (PD) is a rare autosomal recessive lysosomal disorder caused by a deficiency in acid α-glucosidase activity. PD is characterized by the accumulation of glycogen in lysosomes leading to dysregulation of normal cellular function and tissue damage in the heart, muscles, and motor neurons. The current standard of care, enzyme replacement therapy (ERT), has limitations for improving muscle function and cannot cross the blood-brain-barrier leading to progressive neurologic deterioration in long-term survivors of classic infantile PD. The most severe form results in death within 2-3 years if untreated. ERT using recombinant human GAA (rhGAA) delivered every other week via intravenous infusion is the only approved treatment available for PD. For ERT to be effective, rhGAA must be internalized in target muscle cells and delivered to lysosomes at clinically relevant doses. The vast majority of ERTs, including rhGAA, utilize the cation-independent mannose 6-phosphate receptor (CIMPR) pathway for cellular uptake. However, only a small proportion of the total rhGAA contains *bis*-phosphorylated high mannose glycans that have high binding affinity for CIMPR because it is poorly phosphorylated in cells. rhGAA production cell lines and manufacturing processes therefore have to be optimized to produce appreciable amounts of rhGAA with mannose 6-phosphate (M6P) for CIMPR binding and cellular uptake.

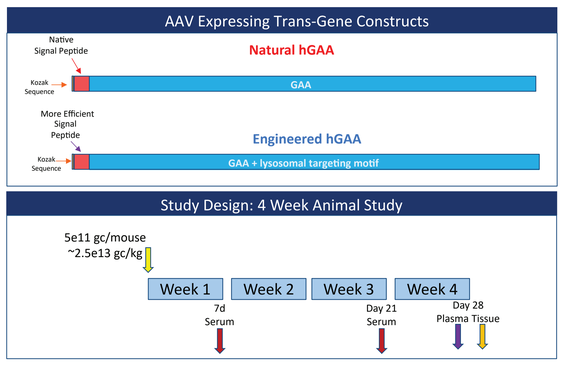
AAV-based gene therapy is a promising approach to achieving long-term expression of the enzyme in target organs. However, since carbohydrate processing cannot be controlled in transduced cells, inefficient GAA phosphorylation may pose substantial challenges to cellular uptake. Many animal studies therefore utilize high gene therapy doses to produce sufficient amounts of M6P-containing hGAA to establish proof of concept. However, producing comparable amounts of hGAA in higher organisms would likely require substantial gene therapy doses that have been associated with significant safety signals in both non-human primates and humans. Using such high doses would also exacerbate the already challenging manufacturing processes for PD gene therapies. It is therefore beneficial to develop gene therapies that produce high levels of hGAA that can be efficiently targeted to muscles using moderate doses.

We have engineered the human GAA transgene to replace the native signal sequence with a more efficient signal sequence to enable higher protein expression and secretion of hGAA (5- to 10-fold higher than native signal sequence). Further, the engineered transgene was designed to produce a hGAA containing a targeting motif that enables high-affinity binding to a cell-surface receptor for efficient uptake and delivery to lysosomes. Once in cells, our hGAA product is indistinguishable from natural hGAA (by western blotting) indicating that the targeting motif is properly removed and the enzyme is normally processed in lysosomes. We believe that the combination of greater expression and secretion of hGAA with efficient muscle targeting may ultimately translate into a more effective gene therapy for PD. Studies are currently underway in *Gaa* KO mice using AAV-mediated gene delivery to assess the effectiveness of our engineered hGAA relative to native hGAA. Preliminary data will be presented from the animal studies as available.

AAV Gene Therapy Initial High-Dose Preclinical POC Study

- AAV Transgene:
  - Natural-hGAA (AAV.hGAA nat)
  - Engineered-hGAA (AAV.hGAA eng)
- Dose\Route:
  - 5e11 gc/mouse (~2.5e13 gc/kg)
  - Tail Vein IV
- Animal Model:
  - *Pompe Model Gaa -/-* B6:129-GAAtm1Rabn/J (aka 6neo)
  - *Wild-type Gaa+/+* (Pompe model litter-mates)
- Gender:
  - Male ■
  - Female ●
- Age: 4-6 weeks at AAV dosing





Methods

- **GAA Activity**

  Plasma was mixed with 5.6 mM 4-MU-α-glucopyranoside pH 4.0 and incubated for three hours at 37 °C. The reaction was stopped with 0.4 M sodium carbonate, pH 11.5. Relative fluorescence units, RFUs were measured using a Victor³ fluorimeter, ex 355 nm and emission at 460 nm. Activity in units of nmol/mL/hr was calculated by interpolation from a standard curve of 4-MU. Activity in individual tissue samples were further normalized based on total protein content in the homogenate

- **GAA Signature Peptide by LC/MS**

  Plasma was precipitated in 100% methanol and centrifuged. Supernatants were discarded. The pellet was spiked with a stable isotope-labeled peptide unique to hGAA as an internal standard and resuspended with trypsin and incubated at 37 °C for one hour. The digestion was stopped with 10% formic acid. Tryptic peptides were separated by C-18 reverse phase chromatography and Identified and quantified by ESI-mass spectroscopy. The total GAA concentration in plasma was calculated from the signature peptide concentration.

- **Cell surface Receptor Binding assay**

  A 96-well plate was coated with receptor, washed, and blocked with BSA. 28 day plasma from AAV treated mice was serially diluted to give a series of decreasing concentrations and incubated with coupled receptor. After incubation the plate was washed to remove any unbound hGAA and 4-MU-α-glucopyranoside added for one hour at 37 °C. The reaction was stopped with 1.0 M glycine, pH 10.5 and RFUs were read by a Spectramax fluorimeter; ex 370, emission 460. RFU's for each sample were converted to activity (nmol/mL/hr) by interpolation from a standard curve of 4-MU. Nonlinear regression was done using GraphPad Prism.

- **Histology**

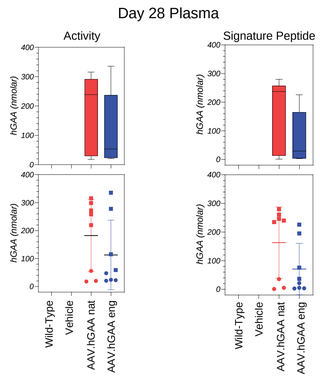
  Tissues were formalin fixed and paraffin embedded. Muscle slides were stained with PAS; CNS slides with luxol fast blue/Periodic Acid-Schiff (PAS). A board certified veterinary pathologist (JH) blindly reviewed histological slides. A semi-quantitative estimation of the total percentage of cells with glycogen storage and cytoplasmic vacuolization was done on scanned slides. A score from 0 to 4 was attributed as described in table to the right.

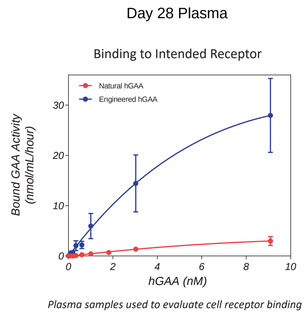
	Storage / Vacuolization
0	0
1	1 to 9 %
2	10 to 49%
3	50 to 74%
4	75 to 100%

- **Immuno-histochemistry (IHC)**

  We studied transgene expression and cellular localization from slides immunostained using an anti-human GAA antibody (Sigma HPA029126).

GAA Plasma Activity, Concentration and Cell-Surface Receptor Binding

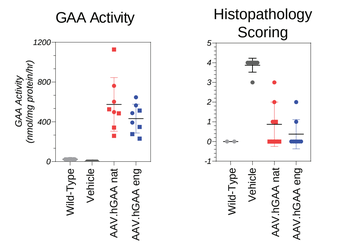


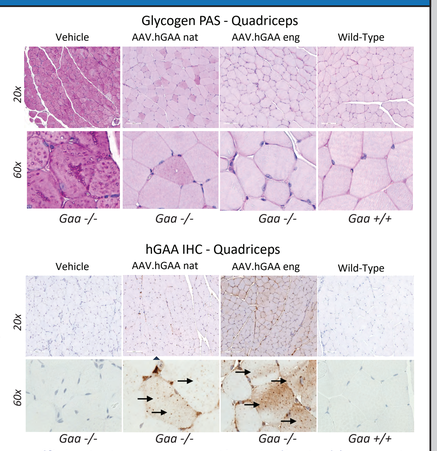


Plasma samples used to evaluate cell receptor binding

- *High levels of engineered and natural hGAA were measured in plasma at day 28*
- *Engineered hGAA was able to efficiently bind the intended receptor to enable cellular uptake*

Quadriceps

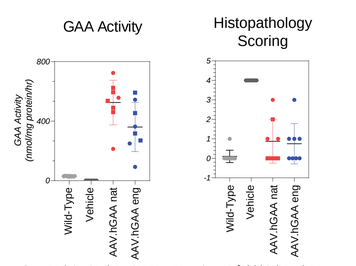


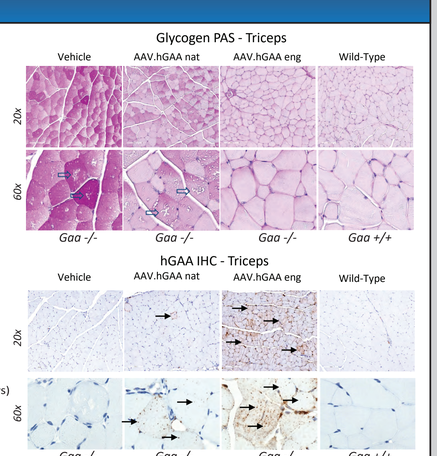


- GAA activity in the quadriceps was ~20 fold higher than wild-type levels for both engineered hGAA and natural hGAA
- Glycogen reduction was more uniform for engineered hGAA by PAS staining
- IHC illustrated greater cellular uptake (solid arrows) of engineered hGAA compared to natural hGAA

At the cellular level, engineered hGAA was more efficient at cross-correction, indicated by greater cellular uptake and uniform glycogen reduction.

Triceps

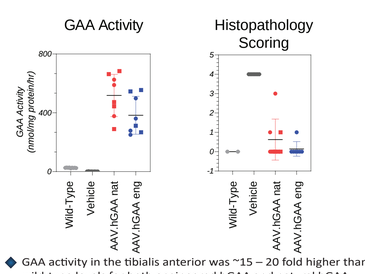


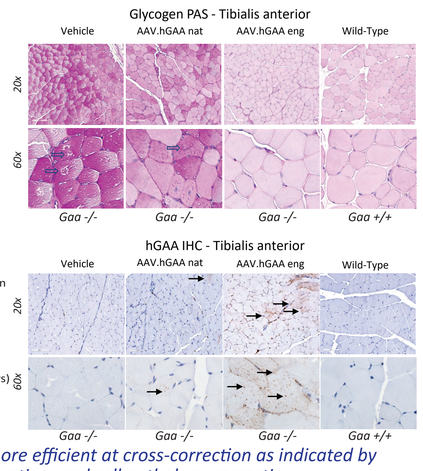


- GAA activity in the triceps was ~10 – 15 fold higher than wild-type levels for both engineered hGAA and natural hGAA
- PAS staining showed more complete and uniform glycogen reduction and clearance of autophagic vacuoles for engineered hGAA
- PAS staining showed incomplete glycogen reduction and less clearance of autophagic vacuoles for natural hGAA (open arrows)
- IHC illustrated greater cellular uptake (solid arrows) of engineered hGAA compared to natural hGAA

At the cellular level, engineered hGAA was more efficient at cross-correction as indicated by greater cellular uptake, uniform glycogen reduction, and cell pathology correction.

Tibialis anterior

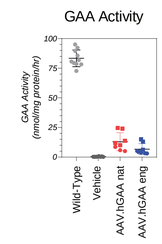


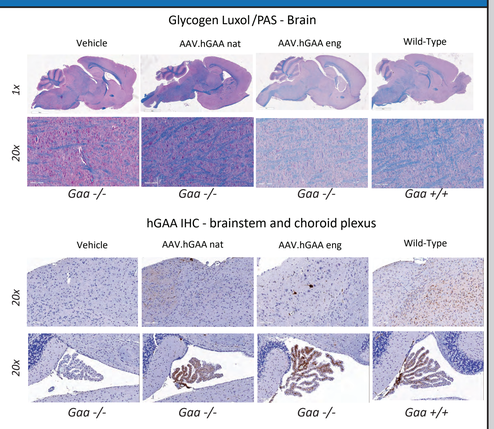


- GAA activity in the tibialis anterior was ~15 – 20 fold higher than wild-type levels for both engineered hGAA and natural hGAA
- PAS staining showed more uniform and complete glycogen reduction and clearance of autophagic vacuoles for engineered hGAA
- PAS staining showed incomplete glycogen reduction and less clearance of autophagic vacuoles for natural hGAA (open arrows)
- IHC illustrated greater cellular uptake (solid arrows) of engineered hGAA compared to natural hGAA

At the cellular level, engineered hGAA was more efficient at cross-correction as indicated by greater cellular uptake, uniform glycogen reduction, and cell pathology correction.

Brain

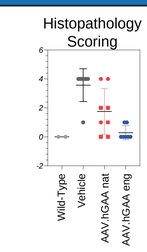


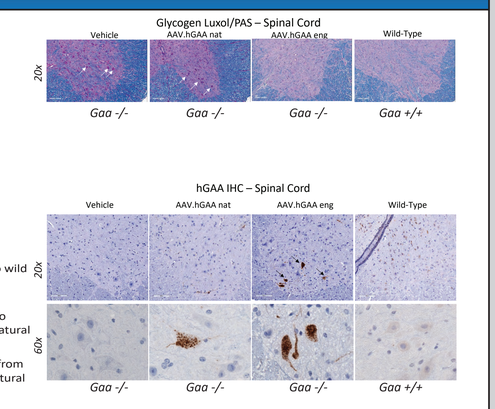


- GAA activity in the Brain was ~5 fold lower than wild-type mouse GAA activity levels
- Little/no glycogen clearance with natural hGAA
- Glycogen close to wild-type levels for engineered hGAA, even though activity was only 20% of wild-type levels
- IHC indicated direct transduction of cells of the brainstem and choroid plexus

Engineered hGAA likely produced by the choroid plexus and secreted into the cerebrospinal fluid was able to cross-correct the brain at low levels due to efficient cellular uptake while natural hGAA was not able to cross-correct at similar levels.

Spinal Cord





- PAS staining showed that glycogen levels were close to wild type in ventral horn, including motor neurons, for engineered hGAA
- PAS staining showed that glycogen levels were closer to vehicle in ventral horn, including motor neurons, for natural hGAA (white arrows)
- IHC demonstrated a stronger signal in motor neurons from animals receiving engineered hGAA compared with natural hGAA (black arrows)

Engineered hGAA was able to reduce glycogen efficiently in the spinal cord while little glycogen reduction was observed for natural hGAA.

Conclusions

- AAV gene therapy expressing the engineered hGAA led to more uniform cellular uptake, glycogen reduction, and resolved cellular dysfunction via efficient cross-correction
- AAV gene therapy expressing the engineered hGAA demonstrated efficient cross-correction with resolution of cellular pathology in the CNS at low expression levels. At similar expression levels, natural hGAA did not demonstrate such effects.

Next Steps

- Dose ranging studies to further explore efficacy and characterization of the high-affinity lysosomal-targeting receptor motif of Engineered hGAA for in vivo efficacy.
- Additional gene therapy experiments are planned to assess potential cross-correction benefits in the CNS and spinal cord.

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Conflicts of Interest

J.M. Wilson is an advisor to, holds equity in, and has a sponsored research agreement with Scout Bio and Passage Bio; he also has a sponsored research agreement with Ultragenyx, Biogen, Janssen, Precision Biosciences, Moderna Therapeutics, and Amicus Therapeutics who are licensees of Penn technology. JMW is an inventor on patents that have been licensed to various biopharmaceutical companies.

Ting Yu: No COI

Juliette Hordeaux is an inventor on provisional patent applications and patents related to AAV gene therapy.