AAV-mediated expression of human α-galactosidase A gene in hepatocytes of a murine Fabry model results in continuous therapeutic levels of enzyme activity and effective substrate reduction

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Abstract

An AAV-mediated liver-targeted gene therapy was evaluated in a mouse model (GLAKO) that lacks α-GalA activity and accumulates high levels of Gb3/lyso-Gb3 in plasma and tissues. This strategy employs an episomal AAV vector encoding human GLA cDNA (HGLA) driven by a liver-specific promoter.

Administration of six single doses of increasing amounts of AAV HGLA cDNA resulted in supraphysiological expression of plasma α-GalA (up to 50 fold of WT) by day 14, was well tolerated, and was stable for 6 months post-injection. Dose-dependent increases in α-GalA activity were achieved in liver, heart, kidney and spleen with a corresponding reduction of Gb3/lyso-Gb3. An improved cDNA vector administered to GLAKO mice in a 2 month follow up study produced stable plasma α-GalA levels up to 200 fold greater than WT. α-GalA activity in heart and kidney averaged 20- and 3-fold over wild type levels, respectively, and Gb3/lyso-Gb3 in these tissues were near normal levels.

Production of glycosylated human α-Gal A in livers of treated GLAKO mice

Liver homogenates of GLAKO mice treated with AAV GLA cDNA contain high amounts of glycosylated human α-GalA.

- Homogenates were normalized for protein content.
- Aliquots were subjected to PNGaseF- or EndoH-mediated deglycosylation.
- The liver-derived α-Gal A had a similar glycosylation pattern to recombinant enzyme produced in CHO cells (far right panel).

Supra-physiological α-Gal A activity in treated GLAKO mice

Liver-produced α-Gal A is secreted into the bloodstream and taken up by secondary tissues.

- GLA cDNA gene therapy in GLAKO mice produced high, dose-dependent enzyme activity that was sustained for 6 months.
- Plasma α-Gal A activity scaled with AAV dose, up to 50-fold wild type.
- α-Gal A activity exceeded wild type levels in liver, spleen and heart for the higher dose groups.

Conclusions

AAV-mediated delivery of HGLA cDNA constructs in GLAKO mice led to:

- Expression of high levels of glycosylated α-Gal A in the liver
- Stable, high plasma α-Gal A activity up to 200x wild type for up to 6 months
- Tissue uptake of α-Gal A leading to activity exceeding wild type in heart, kidney and spleen
- Reduction of substrates Gb3 and lyso-Gb3 in tissues within 2 months of vector administration

These data support the development of Sangamo’s liver-targeted AAV GLA cDNA approach as a potential single-administration therapy for the treatment of Fabry disease. This AAV HGLA gene therapy is currently undergoing pharmacology and toxicology studies to support IND submission in 2018.

Conflict of Interest Statement

NMH, SSM, SS, KM, TM and MCH are full-time employees of Sangamo Therapeutics, Inc.