Preclinical Development of SIG-007 for Treatment of Fabry Disease

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Abstract

Fabry disease is an X-linked lysosomal storage disorder (LSD) caused by the deficiency of α-galactosidase A (αGal-A) and accumulation of substrates including globotriaosylceramide (Gb3) in cells. This results in progressive, life-threatening, multi-organ pathology, including kidney failure, gastrointestinal symptoms, strokes, and heart disease at a young age. Current therapy includes enzyme replacement therapy, conventional medical treatment and adjunctive therapies, with more recently approved chaperone therapy. Fabry remains incurable with long-term complications and high patient burden. We hypothesized that sustained therapeutic effect could be achieved by administration of αGal-A-secreting allogeneic cells shielded within spheres designed to avoid immune rejection and foreign body reaction in the host organism. We developed 1.5 mm two-compartment spheres optimized to nurture and shield genetically modified cell lines. For Fabry disease, our product candidate SIG-007 is composed of: a) genetically modified human stable cell line to constitutively express and secrete human αGal-A (hGBA); b) inner compartment optimized for the cell line to thrive and produce hGBA; c) outer compartment which contains a novel small molecule conjugated form of alginate (Albromer™) which was designed to avoid immune rejection and foreign body reaction after administration. We administered different doses of SIG-007 into the intraperitoneal (IP) space of the Fabry mice. Ten days post-administration of SIG-007, we observed hGBA enzyme activity in mouse plasma, liver and spleen. Additionally, we detected statistically significant reduction of Gb3 in liver, spleen and plasma (up to 90% at highest dose of SIG-007) compared to the control (5 animals per group). We observed statistically significant reduction in lysosomal biomarker across all relevant tissues. These data confirm that SIG-007 is a potential alternative to established enzyme replacement therapy, an alternative that we believe will fundamentally change the approach to treating serious chronic diseases and, in doing so, transform the care for patients living with the burden of their disease.

Introduction

• Fabry disease is a progressive, X-linked inherited disorder of glycosphingolipid metabolism due to deficient or absent lysosomal αGal-A (GLA) activity.
• It results in progressive accumulation of Gb3 and related glycosphingolipids within lysosomes, in a variety of cell types, including capillary endothelial cells, renal, cardiac, and nerve cells.
• Comprehensive therapy includes enzyme replacement therapy (ERT), conventional medical treatment and adjunctive therapies.
• Current standard of care is not curative, long-term complications still occur, and the burden on patients is high. Therapeutic access to the brain tissue remains a significant challenge.

Methods

• Figure 2. hGBA Km was determined by adding the enzyme or equivalent dilution of conditioned cell media to varying amounts of 4-Methylumbelliferyl β-D-galactopyranoside substrate and incubated for 15 minutes at 37°C. The reaction was stopped with 200 μL of 0.5 M sodium hydroxide and 0.5 M glycine at pH 11.5. Fluorescence intensity was measured on a Biotek Synergy LX (Excitation: 360/40, Emission: 460/40).
• Figure 3. hGBA secreting cells were incubated in complete media for 48 hours. The concentration of active hGBA in conditioned media was determined using the 4MU enzymatic assay using recombinant GLA as the standard. Fabry patient fibroblasts were incubated with 500 ng of SIG-007 from the diluted conditioned media for 72 hours. Cell pellets were collected and thoroughly rinsed. The amount of intracellular GLA present in cell pellets was determined by LC/MS/MS.
• Figure 4. GLA KO mice (αGal-A-/-) contain a neo cassette replacing exon 3 and intron 3 of the galactosidase, alpha (Gal) gene, abolishing gene expression (Jackson Laboratory, B6.129-GalTα/-GALD). All mice were housed under pathogen-free conditions in an animal facility according to IACUC approved protocols. Procedures involving mice followed the guidelines established by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Empty spheres (control) or SIG-007 were placed IP in GLA KO mice. 14 days after implantation, mice were sacrificed and lyso-Gb3 levels were measured in plasma, urine, liver, kidney, spleen and heart using LC/MS/MS.
• Figure 5. SIG-007 was placed IP in GLA KO mice. hGBA activity was measured in liver, spleen and plasma of GLA KO mice using a fluorometric substrate assay.

Figure 1. SIG-007: Genetically Modified, αGal-A Expressing Cell Line, Shielded by a Two-Compartment Sphere

Figure 2. αGal-A Produced by Human Engineered Cells has Similar Activity as the Commercially Available Enzyme

Figure 3. Uptake of hGBA by Fabry Patient Fibroblast

Figure 4. SIG-007 Reduces Lyso-Gb3 Accumulation Across Multiple Relevant Tissues in GLA Knock-Out Mice

Figure 5. αGal-A Produced by SIG-007 has Enzymatic Activity in Liver, Spleen and Kidney of GLA Knock-Out Mice

Conclusions

• αGal-A produced by genetically modified human cells used for development of SIG-007 has similar biochemical properties as the commercially available, recombinant GLA
• Active αGal-A enzyme was detected in multiple tissues after intraperitoneal (IP) administration of SIG-007 to GLA KO mice
• A dose-responsive reduction in Lyso-Gb3 accumulation was observed in plasma, urine and across multiple tissues of GLA KO mice after IP administration of SIG-007
• SIG-007 is currently under further investigation for potential use in humans with the goal of transforming the standard of care for patients living with this serious chronic disease

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