Preclinical Development of SIG-005 for Treatment of MPS I

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Abstract

Mucopolysaccharidosis type I (MPS I) is a severe, genetic, multisystem disorder caused by a deficiency of the lysosomal enzyme α-L-iduronidase (IDUA), leading to GAG accumulation in tissues resulting in a complex array of clinical symptoms. Approved strategies include bone marrow transplant and enzyme replacement therapy with chaperones and gene therapies under investigation. MPS I remains incurable with long-term complications and high patient burden. We hypothesized that sustained therapeutic effect could be achieved by administration of IDUA-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. Our product candidate SIG-005 is composed of: a) genetically modified human stable cell line to constitutively express and secrete human IDUA (hIDUA); b) inner compartment optimized for the cell line to thrive and produce hIDUA; 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 various doses of SIG-005 into the peritoneal cavity of MPS I H mice, which display similar biochemical and clinical features as the severe phenotype of the human disease (Hurler Syndrome). Plasma was tested for a trisaccharide and total GAG levels at 5, 10 and 14 days post-administration. Total GAG analysis was performed in liver, spleen and kidney post-termination at 10 days. We observed a statistically significant GAG reduction in both plasma (at 10 and 14 days) and tissues (at 10 days) of more than 90%. These data confirm that SIG-005 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.

Methods

• SIG-005 Km was determined by adding iduronidase or the equivalent dilution of conditioned cell media to varying amounts of 4-Methylumbelliferone-α-L-iduronide substrate and incubated for 15 minutes at 37°C. The reaction was stopped and fluorescence intensity was measured on a Synergy LX (Excitation: 360/40, Emission: 460/40). MPS I patient fibroblasts were incubated with untreated media for 72 hours. Cell pellets were collected and thoroughly rinsed. The fluorescence intensity was measured on a Synergy LX (Excitation: 360/40, Emission: 460/40). MPS I patient fibroblasts were incubated with untreated media for 72 hours. Cell pellets were collected and thoroughly rinsed. The fluorescence intensity was measured on a Synergy LX (Excitation: 360/40, Emission: 460/40).

• hIDUA secreting cells were incubated in complete media for 48 hours. The concentration of active hIDUA in conditioned media was determined using an AMU enzymatic assay using recombinant IDUA as the standard. MPS I patient fibroblasts were incubated with hIDUA from the diluted conditioned media for 72 hours. Cell pellets were collected and thoroughly rinsed. The hIDUA concentration was determined by LC-MS/MS method.

• MPS I H mice (Idua−/−) were purchased from Jackson Laboratory. All mice were housed under pathogen-free conditions in an animal facility according to AAALAC approved protocols. Procedures involving mice followed the guidelines established by the Association for Accreditation of Laboratory Animal Care (AAALAC). SIG-005 spheres were implanted IP in MPS I H mice and plasma biomarker reduction was measured using LC-MS/MS method. (WT, wild-type mice)

• Empty spheres (Control) or SIG-005 (hIDUA) were implanted IP in MPS I H mice. 14 days after implantation, mice were sacrificed and GAG levels were measured in liver, spleen and kidney using LC-MS/MS. Liver hIDUA activity was measured using a fluorometric substrate assay.

Results

Figure 2. hIDUA Produced by Genetically Modified Human Cells has Similar Activity as the Commercially Available Enzyme

Figure 3. hIDUA Produced by SIG-005 is Active and Able to Access Lysosomes of Fibroblasts from MPS I Patients

Figure 4. SIG-005 Reduces the Trisaccharide Biomarker in Plasma Over Time In Vivo

Figure 5. SIG-005 Produces hIDUA in Dose-Responsive Manner, Reduces GAG (Heparin Sulfate) Accumulation in MPS I-H Mice

Conclusions

• hIDUA produced by genetically modified human cells used for development of SIG-005 has similar Km profile and activity as the commercially available recombinant enzyme

• SIG-005 produces hIDUA in a dose-dependent manner

• Even the lowest dose of SIG-005 is able to reduce GAG accumulation across relevant tissues of MPS I-H mice

• SIG-005 has the potential to replace ERT and reduce the burden of MPS I patients

• SIG-005 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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