Title: Lucerastat, an Iminosugar for Substrate Reduction Therapy in Fabry Disease: Preclinical Evidence

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Background: Fabry disease (FD) is a lysosomal storage disorder caused by mutations in the *GLA* gene coding for α-galactosidase A (α-GalA). These mutations lead to the accumulation of α-GalA substrates, including globotriaosylceramide (Gb3). As a consequence of lipid storage, Fabry patients can suffer from neuropathic pain, impaired kidney function and cardiomyopathy. Existing treatments for FD either require bi-weekly intravenous infusions of replacement enzyme, or are effective in a limited number of patients with specific "amenable" mutations. Substrate reduction therapy with lucerastat, an orally-available small molecule inhibitor of glucosylceramide synthase (GCS)[1] is an alternative mechanism to reduce Gb3 accumulation, that would be suitable for all FD patients.

Methods: Fabry patient-derived fibroblasts with the genotypes R301G (residual ⟨-GalA activity; 20%) R220X (<3%) and W162X (<1%) were obtained from the Coriell Institute and cultured for 9 days in the presence of 9 concentrations in duplicate of either lucerastat, migalastat or agalsidase alfa. Lysosomes were stained using LysoTracker® Red DND-99 and area was quantified. Sphingolipids were extracted with methanol and quantified with LC-MS/MS.
Fabry mice (*Gla*$^{-/0}$ *and Gla*$^{-/-}$, n = 5 or 6 for each gender) were treated from 5 weeks of age with lucerastat (1200 mg/kg/day food admix) or normal food for 20 weeks. Mice were sacrificed and sphingolipids were quantified in various organs.

Results: In Fabry patient-derived fibroblasts, lucerastat dose-dependently inhibited GCS, reducing glucosylceramide and increasing sphingomyelin, while ceramide remained unchanged. The downstream consequence of GCS inhibition was reduction of Gb3 and lysosome staining, including in cells from patients with no residual α-GalA activity.
In Fabry mice, lucerastat treatment reduced lipid storage in two major organs affected by FD: mean Gb3 in the kidneys (-33%, $p < 0.001$) and α-galactose- terminated glycosphingolipids in the dorsal root ganglia (-48%, $p < 0.05$). In the liver of the Fabry mice, mean glucosylceramide (GlcCer (24:0)) was reduced (-59%, $p < 0.001$) in addition to Gb3 (24:1) (-37%, $p < 0.05$), demonstrating substrate reduction through GCS inhibition.

Conclusion: Lucerastat, a GCS inhibitor, reduces Gb3 in the absence of residual ⟨-GalA activity both *in vitro* and *in vivo*. Lucerastat has potential to provide an oral substrate reduction therapy for all Fabry patients independent of genotype. A 12-week exploratory clinical study with lucerastat in Fabry patients has been completed, and a pivotal clinical efficacy study in Fabry patients is being designed.

Support:

References: 1. Guérard (2017) Orphanet J. Rare Dis.