The 7m8.scGfABC1.GDNF vector achieved significant hGDNF secretion in rd10 and functional delay of retinal degeneration. **Conclusion:** Selectively targeting glia is critical to more precisely "dissect" the role of glia in the healthy and diseased retina. It represents an attractive approach for novel therapies (e.g. stem cell therapies focused on the dedifferentiation of glia into photoreceptors) requiring no off-target expression. Our study shows that the short GFAP promoter, gfaABC1D, in combination with 7m8 leads to strong, pan-retinal and selective Müller glia expression in degenerating and healthy retinas with a low ocular toxicity profile compared to the long parental promoter, and can be the new standard vector for MGC-targeted gene therapies.

1121. Human Undifferentiated Neural Progenitor Stem Cells (NPC) Alleviate Motor Dysfunction, Repair Morphological Damage and Extend Life Span in a Spastic Han-Wistar Model of Cerebellar Ataxia

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The spastic Han Wistar (sHW) rat represents a model for human cerebellar ataxia characterized by forelimb tremor, hind-leg rigidity, gait abnormality, weight loss, life span about 65 days caused by neurodegeneration of Purkinje cells and CA3 pyramidal neurons. To address these problems, a line of NPCs that are able to tune their proliferation and vector of differentiation in response to micro-environmental cues was manufactured, fully characterized and used in this study. Proteomic analysis of NPC detected secretion of Neuromodulin, 14-3-3 Epsilon Protein, Insulin-like Growth Factor II and other neuroprotective and anti-inflammatory factors that can be a part of mechanism of action of NPC. At 30 days of age, male sHW mutant rats underwent subcutaneous implantation of Alzet osmotic pump that infused cyclosporin (15 mg/kg/day). At 40 days, sHW rats received bilateral injections (500,000 cells in 5 μl media) of live NPCs (experimental) or dead NPCs (control) into interposed nucleus of the cerebellum. Motor activity scores and weights of the animals were recorded weekly. At day 45, the experimental animals began to exhibit improved motor activity and survived past 100 days of age. The motor scores were statistically similar to those of normal animals. Immunohistochemistry revealed few surviving NPCs in the cerebellum of 100 day old experimental animals. Stereological analysis demonstrated significant increase in the numbers of Purkinje neurons compared to controls. These data warrant a clinical trial of NPCs for the treatment of cerebellar ataxias that currently have no recourse

1122. CRISPR/Cas9 Mediated Genetic Correction of Hematopoietic Stem Cells from Patients with Friedreich's Ataxia

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Friedreich's ataxia (FRDA) is a multi-systemic autosomal recessive disorder that is predominantly caused by a homozygous GAA repeat expansion mutation within the first intron of the frataxin gene (*FXN*). This mutation leads to the transcriptional silencing of *FXN* through heterochromatin formation, and an accompanying reduction of frataxin, a mitochondrial protein involved in iron metabolism. FRDA is characterized by neurodegeneration leading to ataxia, areflexia, sensory loss, muscle weakness, and cardiomyopathy. Currently, there is no treatment for FRDA. In 2017, we showed that transplantation of mouse wild-type hematopoietic stem and progenitor cells (HSPCs) in the YG8R mouse model of FRDA, prevents the development of the locomotor deficits, and the neuronal degeneration in the dorsal root ganglia, as well as reduction of the oxidative stress in brain and muscle. We also showed that the mechanism of rescue was mediated by the transfer of frataxin from the HSPC-derived microglia/macrophages to neurons/myocytes. This study builds upon these previous findings through the manufacturing optimization of the future clinical product consisting of autologous gene-corrected HSPCs isolated from the peripheral blood of FRDA patients. Gene correction occurs through CRISPR/Cas9-mediated excision of the intronic repeat expansion mutation. Optimization was performed in lymphoblasts isolated from FRDA patients in which gene editing efficiency reached up to 60%. Corrected cells displayed frataxin expression levels comparable to their carrier parents' cell lines for both transcription and translation levels, and displayed better mitochondrial function. Then, we transitioned to developing the human product first in $CD34^+$ cells isolated from healthy donor peripheral blood; gene editing efficiency ranged from 33.6 to 49.8%. A decrease in proliferative capacity was transiently observed in the edited cells over the 48 hours post electroporation due to double stranded breaks exacerbating p53 expression. *In vitro* Colony Forming Unit assays and *in vivo* transplant into NOD scid gamma immunodeficient mice showed the gene-modified $CD34^+$ cells retained normal hematopoiesis. Gene editing in $CD34^+$ cells isolated from FRDA patients ranged from 12.1 to 55.9% and correlated with a therapeutically relevant increase in frataxin expression. No off-target crRNA indel events were found at computationally predicted sites. This editing methodology is now being tested on FRDA murine HSPCs ($Sca1^+$) which are transplanted into an FRDA mouse model to evaluate their therapeutic capacity *in vivo*. With this study, we are laying the foundation for a future clinical trial using autologous HSPC transplantation for FRDA.