**Tracking and Hacking Autologous Adipose-Derived Mesenchymal Stromal Cells to Improve ALS Treatment**

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**Statement of Purpose:** Autologous adipose-derived mesenchymal stromal cells (adMSCs) have the potential to promote neuroprotection and regeneration in ALS patients. Recently, our group completed a Phase I clinical trial in which ALS patients tolerated an intrathecal infusion of $10^8$ adMSCs without significant complications [1]. Patients reported improved limb strength and function, though the study was not designed to test the therapeutic efficacy of the adMSCs. Our group also identified two limitations of using adMSCs to treat ALS that need to be addressed: 1) the inability to track the adMSCs in vivo, and 2) a lack of knowledge on how adMSCs may provide a therapeutic benefit to ALS patients. To tackle the first limitation, we aim to develop adMSCs that express the human sodium-iodide symporter (NIS), which will enable cell tracking in vivo using radioisotope imaging and in studies using NIS as a target for immunohistochemical localization and identification of long term cell fate. To address the second limitation, we aim to quantify and compare secreted factors (e.g., miRNA, growth factors) present in the cerebral spinal fluid (CSF) of control individuals and of ALS patients before and after treatment with unmodified adMSCs (without NIS expression). We also want to characterize phenotypic changes that adMSCs undergo in the intrathecal space. We aim to identify factors that could provide a diagnostic read out of adMSC activity and correlate with patient outcomes. In addition, if one factor proved significantly therapeutic, our group could transition that molecule into a form suitable for enhanced overexpression by the adMSCs.

**Methods:** We will transfect adMSCs with infectious lentiviral vectors containing the NIS gene and assess NIS expression via an iodine incorporation assay. We then will optimize imaging NIS-expressing cells via single-photon emission computed tomography (SPECT) within an imaging phantom prior to injection of the NIS-adMSCs into a rodent model for in vivo SPECT imaging. Subsequent histological analyses will inform our characterization of adMSC migration and positional preferences within the intrathecal space. The CSF samples required for our studies will be collected from patients enrolled in our Phase II clinical trial (a follow up to our Phase I trial) being conducted at Mayo Clinic. The CSF samples will undergo genomic and proteomic analyses (e.g., RNA-seq, microarrays, ELISA) to detect and quantify cellular factors present in the CSF. Thus, these samples will provide snapshots of the CSF milieu present in non-treated individuals and in ALS patients before and after receiving infusions of adMSCs.

**Results:** We constructed six varieties of infectious lentiviral vectors that can transfect adMSCs and induce expression of NIS and insulin-like growth factor-1 (IGF1; see Fig. 1 for example). We also have preliminary in vitro evidence demonstrating decreased adMSC survival and proliferation in an intrathecal-like environment (Fig. 2). In addition, we have collected and preserved CSF samples from ALS patients who have undergone multiple adMSC injections.

**Conclusions:** Our lentiviral vectors will allow us to test: 1) whether NIS can be adequately expressed in adMSCs to enable in vivo tracking, and 2) whether a growth factor or other molecule can be co-expressed with NIS to provide enhanced therapeutic benefit. Also, our in vitro work and CSF samples have paved the way for additional preliminary experiments, establishing a work flow and identifying cellular factors that will be guide analysis of CSF samples from our Phase II clinical trial participants.

**References:**