CONFIDENTIAL

Regenerative Medicine Minnesota
Progress Report
Due: 5/31/2017

Grant Title: **A Phase IIA study using intrathecal treatment of autologous adipose-derived mesenchymal stem cells for amyotrophic lateral sclerosis**

Grant Number: RMM 11215 CT002

Principal Investigator: Nathan Staff, PhD, MD

Project Timeline: 5/1/2016 - 4/30/2018

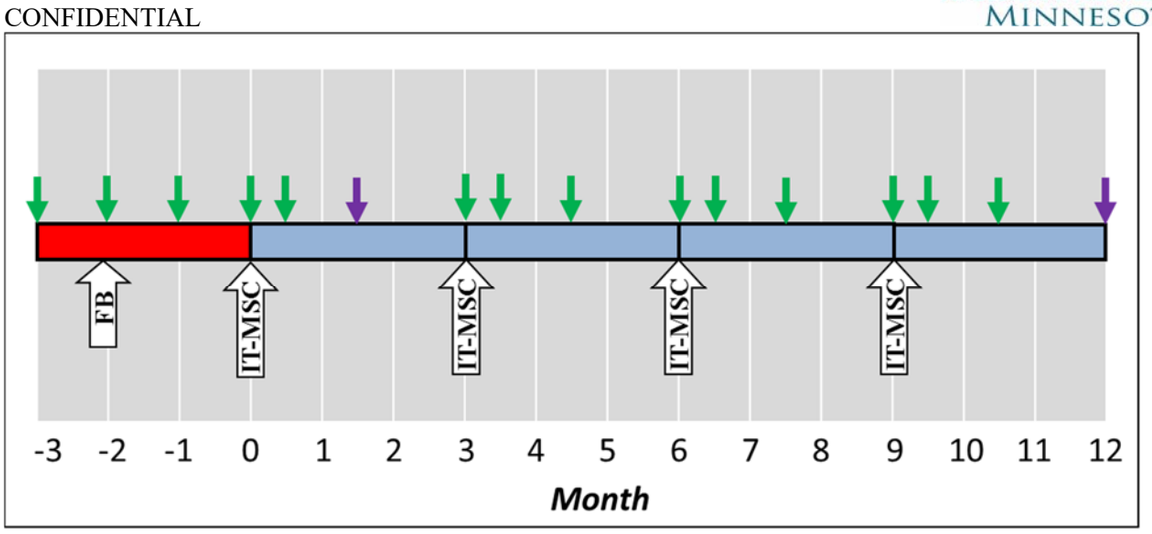
Progress to Date:
In the 1st budget year of this Regenerative Medicine Minnesota (RMM) Clinical Trial Award, we have made great strides towards opening our Phase II clinical trial using intrathecal autologous adipose-derived MSCs in ALS. In this Progress Summary, I will detail three main areas of progress: 1) Completion and Publication of Phase I ALS-MSC trial (supporting the Phase II trial), 2) FDA-approval of the finalized Phase II ALS-MSC clinical trial protocol, and 3) Mayo Clinic IRB-approval of the Phase II ALS-MSC clinical trial protocol. These three major advances position us to open the trial for enrollment in summer 2017.

1) Completion & Publication of Phase I ALS-MSC Safety Trial: November 2016.

In May 2016, we completed a minimum of six-month follow-up for all of the 27 subjects enrolled in our Phase I Dose-Escalation Safety trial of intrathecal autologous adipose-derived MSCs (ClinicalTrials.gov #NCT01609283). This study provided acceptable safety data at all studied doses. We published the results of this trial in the journal Neurology (Staff NP et al., Neurology 87(21):2230-2234. http://www.neurology.org/content/87/21/2230.full). While this Phase I study was not financially supported by RMM, its completion was critical in order to move forward with the RMM-supported Phase II ALS-MSC trial.

2) FDA-Approval of Phase II clinical trial of ALS-MSC: November 2016.

During 2016, as we discussed our Phase I results with the ALS community, it became apparent that we should modify the clinical trial design for our upcoming Phase II study. In our data (as well as others' data in related MSC trials) there appeared to be "responders" versus "non-responders". We proposed that having the largest number of patients receiving treatment would increase our likelihood of finding more "responders". This would allow for detailed biomarker/clinical phenotyping of these patients in order to try to understand this phenomenon better. Our original Phase II clinical trial design included three groups: a single MSC treatment arm, a multiple MSC treatment arm, and a placebo arm. In order to increase the number of "responders", we changed the protocol to have all subjects receive multiple MSC treatments, using historical cohort (https://nctu.partners.org/ProACT) as a control. The following figure and language are from the finalized Phase II clinical protocol, which describe the changes (*please let me know if you would like the entire clinical protocol*):



Clinical Trial Schema: Green arrows denote clinical visits and Purple arrows denote clinical visits with MRI of lumbosacral plexus; FB: fat biopsy, IT-MSC: intrathecal MSC treatment

It is likely that any stem cell therapy will require multiple treatments and this Phase II proposal extends the treatment period to 4 injections every 3 months in order to establish safety with this extended regimen. The primary safety endpoint will be the safety of repeated intrathecal autologous adipose-derived MSCs (aaMSCs). The overall number and frequency of adverse events and unexpected severe adverse events during the 12-month treatment period among study subjects will be documented. Number and frequency of adverse events will be recorded from the time of enrollment until the end of the follow-up period or, in the case of early withdrawal, to the time of study withdrawal. Neurological examination, MRI lumbosacral spinal cord, blood and urine samples will be analyzed at specified times throughout the study for safety. At the time of each intrathecal injection, CSF will be sampled for routine safety parameters.

As part of this study, all patients will start at the maximum tolerated dose from our Phase I dose-escalation trial (1 x 10^8 aaMSCs). Dose-Modification Rules are in place to allow for lower doses in patients that have a significant Expected Adverse Event of back and leg pain. This will provide further safety information that can be beneficial in future studies.

Secondary Endpoints (Efficacy): In order to investigate efficacy in this study, we will use both conventional ALS clinical endpoints and perform a "responder" analysis that can be correlated with the extensive proposed biomarker studies.

All 60 patients enrolled in this Phase II study will have a 3 month lead-in observation period to determine baseline slope of progression, and subsequently will receive 4 intrathecal aaMSC treatments every 3 months. Throughout the study, we will collect measures of function with the ALS Functional Rating Scale – Revised (ALSFRS-R), which is the current standard clinical endpoint in ALS clinical trials. **Primary statistical analyses for secondary efficacy endpoints will be:**

1) Average slope of ALSFRS-R progression in the study group will be compared with a cohort of subjects from the PRO-ACT ALS clinical trial database that will be matched for age, gender, and riluzole use (5 PRO-ACT subjects for every study subject).
2) Average slope of ALSFRS-R progression in the 3 month lead-in period will be compared to the average slope of ALSFRS-R progression in the 12 month treatment period for study subjects.

Exploratory Endpoints:

Responder Analysis of Biomarkers: At the maximum dose in our Phase I study, 60% of patients reported subjective improvements in speech, dexterity, and spasticity domains. Other patients; however, reported no changes in their ALS symptoms. It is critical to understand this "responder effect" and whether it is a treatment effect or whether it is

placebo-related. **We define "responder" as those who exhibit a >25% improvement in the slope of ALSFRS-R between the lead-in and treatment periods.** The following **biomarkers** will then be compared in the "responders" and "non-responders" (no improvement or worsening in ALFRS-R slope):

1) **Peripheral blood immunophenotyping**: we have evidence that a proportion of patients with ALS have a unique peripheral blood immune system signature. Furthermore, we have observed specific changes in peripheral blood immune system in patients receiving intrathecal aaMSCs in our Phase I study.
2) **Neurofilaments**: CSF and serum neurofilaments have emerged as a promising marker of neurodegeneration in ALS. Samples will be collected throughout the study to be used for markers of "responders" and progression in ALS.
3) **CSF microRNA**: Several lines of evidence suggest that miRNA may serve as biomarkers of ALS. Additionally, aaMSC potency may relate to miRNA secretion from extracellular vesicles. We will evaluate miRNA from CSF in study patients.
4) **aaMSC Biomarkers**: If there are clear responders to aaMSC therapy, stored MSCs will be evaluated *ex vivo* (via proteomics, extracellular vesicles, miRNA and immune suppression assays) to help determine whether response may reflect the aaMSC or the patient response to aaMSC therapy.

Exploratory Clinical Endpoints:

1) **Change in slope of the Slow Vital Capacity (SVC)**: Comparisons will be made between the treatment period in study subjects and PRO-ACT controls. Comparisons will also be made between the 3-month lead-in and the 12-month observation period in study subjects.
2) **Change in the slope of hand-held dynamometry (HHD):** We will assess global strength using a composite of 9 standard motions, performed bilaterally (shoulder flexion, elbow flexion/extension, wrist extension, finger spread, hip flexion, knee flexion/extension, and ankle dorsiflexion). Comparisons will be made between the treatment period in study subjects and PRO-ACT controls. Comparisons will also be made between the 3-month lead-in and the 12-month observation period in study subjects.
3) **Modified Ashworth Spasticity Scale:** The muscle tone of bilateral elbows and ankles will be quantified using the Modified Ashworth Spasticity Scale. Comparisons will be made between the 3-month lead-in and the 12-month observation period in study subjects.
4) **Edinbourgh Cognitive ALS Screen (ECAS):** This is a 136-point test of cognitive function designed for patients with ALS, which assesses executive, language, memory, visuospatial functions. Comparisons will be made between the 3-month lead-in and the 12-month observation period in study subjects.

The data gathered in this Phase II study will be used to decide whether to proceed with a pivotal Phase III study. The current sample size is powered to detect a major change (50%) in ALSFRS-R slope. A key goal is to identify correlations of "responders" and biomarkers in order to enrich their enrollment for a Phase III study.

3) Mayo Clinic IRB-Approval of Phase II clinical trial of ALS-MSC: November 2016.

The Mayo Clinic IRB was reviewing the Phase II protocol at the same time as the FDA and approved this in November 2016. We anticipate beginning enrollment in this study in summer 2017.

Please list any of the following that have resulted from the Minnesota Regenerative Medicine grant funding:
Publications and/or manuscripts submitted for publication: none
Disclosures/patents: none
Grant applications and/or awards: none

Budget Update:
Due to the delays in getting IRB/FDA approval, Year 1 budget funds are being spent in order to ramp-up the MSC manufacturing capacity in the Mayo Clinic IMPACT laboratory, under the direction of Al Dietz and Dennis Gastineau (previous known as Human Cell Therapy Laboratory). In order to meet the cGMP cell manufacturing requirements, many reagents/supplies must be purchased in bulk to ensure consistent product.

Year 2 budget will be used for MSC manufacturing and delivery of aaMSCs to 6 patients with ALS at Mayo Clinic Rochester. Furthermore, in this funding cycle, we will be applying for additional funding from: 1) FDA R01 Orphan Drug Mechanism (we received favorable score on previous funding cycle), 2) ALS Association, and 3) Mayo Clinic Center for Regenerative Medicine, which was the primary funding source for the Phase I study.

Reporting to all Minnesotans:

ALS is a fatal neurodegenerative disease that causes progressive paralysis and death in 2-5 years. There is currently no curative treatment for this devastating condition. While it is a rare disease (~120 individuals diagnosed in Minnesota yearly), treatments for ALS may be translated to other more common neurodegenerative diseases such as Alzheimer disease and Parkinson disease. The funding from RMM to initiate this Phase II study of mesenchymal stem cell therapy for ALS is critical to advance the ability to move regenerative treatments into everyday clinical practice.