A Phase II Study of Intrathecal Autologous Adipose-derived Mesenchymal Stromal Cells for Amyotrophic Lateral Sclerosis

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Investigational Agent: adipose-derived mesenchymal stromal cells (MSCs), FDA IND #14788, FDA Orphan Drug #14-4270

Document History	**Date**	**Major Changes**
Addendum	July 24, 2017	Addition of fluoroscopically-guided lumbar puncture
Addendum	January 24, 2018	Visit 4 Edinbourgh Cognitive ALS Screen (ECAS) was removed from the Schedule of Events
Addendum	March 6, 2018	Addition of Mayo Clinic Arizona and Mayo Clinic Florida as enrolling sites (biopsies and stem cell injections done in Rochester, all other visits done at enrolling site).
Addendum	November 15, 2019	Section 3.2: Updated exclusion criteria to include use of other investigational products and nutritional supplements; Added ALSSQOL-R questionnaire; Section 8: Expected adverse events added , stopping points added/clarified, definition of SAE clarified; Section 9: Added table of expected SAEs that do not have to be expeditedly reported; CTCAE version changed from v4 to v5; data collection method changed to RAVE.

Document History	Date	Major Changes
Addendum	January 14, 2020	Section 3.2: Addition of edaravone use to the exclusion criteria; Section 4.0 (Test Schedule): Moved ALSSQOL-R from Visit 15 to Visit 16 (final study visit); Section 6.2: Removed requirement for brain MRI
Addendum	February 26, 2020	Section 4.0 (Test Schedule): Moved the "research" blood collection from V1 to V2, and clarified that this and "research" CSF collection are optional. Section 6.2 (Study Procedures Delineated by Visit): added optional research blood and CSF collection to visits to align with test schedule.
Addendum	August 28, 2020	Title Page: Arizona Study Co-chair change. Section 3.2 (Exclusion Criteria): Changed exclusion of prior stem cell therapy to prior stem cell therapy for a neurological disease.
Modification	December 10, 2020	**Sections 2.1, 5.2, 7.2: Starting aaMSC dose changing from 1 x 10(8) to 5 x 10(7):** Due to increased recognition of back/leg pain after intrathecal autologous aaMSC treatment that is impacting study feasibility, the DSMB recommended developing mitigation strategies to lessen the impact of this Adverse Event. **Sections 5.2, 6.0, 6.2: Dose Modification Schema alteration to allow for dose escalation:** Due to the continued need to determine the most efficacious and best tolerated dose, the Dose Modification Schema has been modified to allow for increasing doses (7.5 x 10(7) and 1 x 10(8)), if minimal pain (0-1/10) exists at 6 weeks post preceding injection. **Sections 5.2, 6.2, 7.2: Dose Modification Schema alteration to drop threshold for dose reduction**: Due to increased recognition of back/leg pain after intrathecal autologous aaMSC treatment that is impacting study feasibility, we have dropped the threshold for dose reduction from pain 1-2/10 at 2 weeks prior to injection to pain1-2/10 at 6 weeks prior to injection. **Sections 2.1, 3.2: Addition of chronic back pain or lumbosacral spine surgery to the exclusion criteria**: It has become apparent that subjects with history of back pain or spine surgery have increased likelihood of developing post aaMSC injection back/leg pain. Therefore, we have made this an exclusion criterion.
Modification	January 11, 2022	**Sections 2.3.2, 6.2, 10.0, Table 4.0: Removal of VC at all visits except enrollment:** In the original clinical protocol, pulmonary spirometry to quantify vital capacity (VC) was included at all visits. In the setting of

		COVID-19 pandemic, COVID-19 testing is required prior to pulmonary spirometry, which requires subjects to come the day before spirometry. We have required that spirometry be performed at enrollment because a VC > 65% predicted for age/BMI is part of inclusion criteria. Due to the undue burden on subjects to travel an extra day, we have not required spirometry for other visits since the pandemic began. We thought this would be a temporary deviation, but it appears to be more permanent. We are going to remove VC as a measurement in all visits except the enrollment visit. Furthermore, VC will no longer be a trial endpoint. **Sections 6.2 and Table 4.0: Addition of window of timepoint to take vitals and pain assessment post-injection:** After the first two hours post-injection, a +/- 15 minutes window of time added to take vitals and pain assessment.
Modification	January 28, 2022	Increased enrollment to 69 patients.
Modification	June 9, 2022	Section 3.1 Inclusion criteria to include use of oral edaravone is acceptable if on stable dose for 30 days or starts 6 weeks after second MSC injection . Section 3.2 Exclusion criteria clarified that intravenous edaravone is excluded.
Modification	October 11, 2022	Enrollment completed. Addition of Section 8.2 Use of of RELYVRIO™ (sodium phenylbutyrate and taurursodiol; also known as AMX0035) during clinical trial. On 9/29/22, the U.S. FDA approved the use of RELYVRIO™ (sodium phenylbutyrate and taurursodiol; also known as AMX0035) in people with ALS. Relyvrio has modest efficacy with demonstrated slowing of disease as measured by the slope of the Revised ALS Functional Rating Scale. Patients with ALS, including subjects in the ALS-MSC Phase 2 clinical trial are eager to start this new FDA-approved medication. We have already received multiple requests from subjects in the trial to be able to take Relyvrio. It is an oral medication that can be taken by mouth or via feeding tube. Overall, the medication is well-tolerated with some gastrointestinal complaints (diarrhea, abdominal pain, and nausea being the most commonly seen). We do not anticipate any safety issues regarding the concomitant use of Relyvrio and participation in this clinical trial. Update to Table 4.0 Schedule of Assessments to accurately reflect that the HHD assessment is conducted at visits described in Section 6.2 Study Procedures Delineated by Visit.
Modification	May 2023	Addition of treatment extension of four additional intrathecal injection treatments for subjects that completed the trial and meet inclusion and exclusion criteria.

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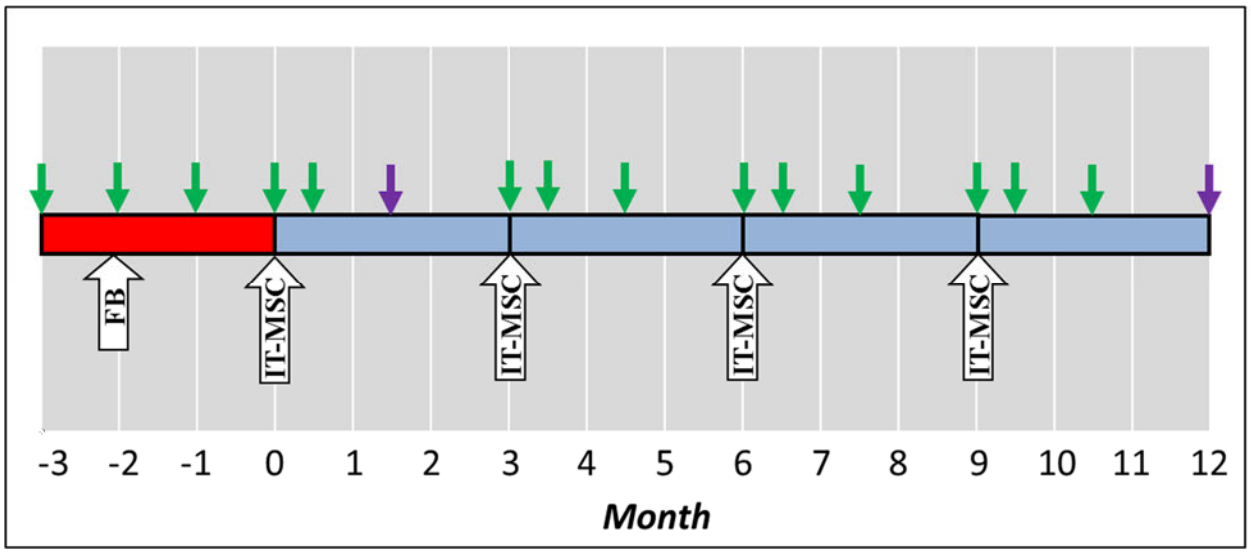
Protocol Schema:

Study Schema: FB: Fat Biopsy, IT-MSC: intrathecal MSC treatment; Arrows: clinic visits; Purple Arrows: clinic visit includes MRI lumbosacral spine



1.0 Background

1.1. Overall Approach of Phase II Clinical Trial: Amyotrophic lateral sclerosis (**ALS**) is a neurodegenerative disease of motor neurons that causes progressive, fatal paralysis. The incidence of ALS is 2-4 cases per 100,000 persons per year with an average life span of 2-3 years following diagnosis[1]. There are **no satisfactory treatments** to slow progression of the disease. In this project, we propose to complete a Phase II open-label multi-site clinical trial to further confirm safety and to explore the efficacy of intrathecal autologous adipose-derived mesenchymal stromal (stem) cell (aaMSC) therapy for ALS. In the context of this phase II clinical trial, our objectives are to:

1) Confirm the **safety** of repeated intrathecal delivery of aaMSCs in ALS
2) Investigate the **efficacy** of intrathecal aaMSCs in ALS using the Revised ALS Functional Rating Scale (ALSFRS-R) as the primary clinical endpoint.
3) Validate and verify proposed **biomarkers of ALS** by taking advantage of the study's comprehensive sampling of blood and CSF. Candidate biomarkers include markers of neurodegeneration (neurofilaments), and immune dysregulation (peripheral blood immunophenotyping (PB-IP) and CSF miRNA). This new data will provide insights into ALS biology and inform the design of a subsequent pivotal phase III clinical trial.
4) Be poised to elucidate the **mechanisms of action** for intrathecal aaMSC therapy by establishing **biomarkers of MSC potency** via *in vivo* (lumbosacral root MRI, CSF miRNA, PB-IP) and *ex vivo* (proteomics, extracellular vesicles, miRNA) analyses, and exploring how these biomarkers correlate with efficacy of intrathecal MSC therapy in ALS.

1.2. MSCs are a promising therapy for ALS: Multiple lines of evidence have suggested that MSCs may function as a paracrine neuroprotective agent in ALS and other neurodegenerative diseases, acting via neurotrophic growth factor secretion and immune modulation[2]. We have completed treatment in our

Phase I, 27-patient dose-escalation safety study using intrathecal aaMSC therapy in ALS. At this stage (August 2016), it is clear that there are no significant safety issues[3].

MSCs naturally reside in either the bone marrow or adipose tissue, whence they migrate to areas of injury, modulate inflammation and can differentiate into bone, cartilage, connective tissue, or fat[2]. In the context of ALS treatment, intrathecal aaMSCs are expected to be an excellent therapeutic for the following reasons:

1) **MSCs secrete growth factors.** MSCs secrete neurotrophic factors, such as GNDF, VEGF, and BDNF[4,5], all of which are known to be neuroprotective for motor neurons[6-9]. In addition to secreting neurotrophic factors themselves, MSCs have been shown to induce expression of neurotrophic factors in astrocytes[10]. Neurotrophic growth factors are critical for motor neuron health. While each of the above neurotrophic factors has been tested in ALS (systemically delivered), **they have never been tested when given together, as will be the case here.**
2) **MSCs modulate the immune system.** MSCs have a strong influence on the immune system and modulate microglia toward a protective M2 (versus a proinflammatory M1) phenotype[2,11]. Neuroinflammation has become a clear pharmaceutical target in treating ALS pathoprogression. While the precise mechanism that MSCs modulate the immune system is still being elucidated, it is likely that they work in a different way than the modalities of immunosuppression tried previously in ALS[12-16], thereby representing **a novel approach to ALS therapy**. Our repeated PB-IP and CSF miRNA analyses in this clinical trial will allow us to better **delineate how MSCs alter the immune system**. These results may be correlated with patient response and the potency of MSCs to modulate T cell proliferation *in vitro* aiming to evaluate MSC potency as an additional possible predictor of treatment response.
3) **Intrathecal injections bypass the blood brain barrier.** The blood-brain barrier has limited the ability to allow penetration of therapeutics (especially neurotrophic growth factors) in ALS. Intrathecal injections enable neurotrophic growth factors produced by MSCs to enter the CNS directly. Furthermore, sustained secretion of MSC-derived extracellular vesicles (MSC-EVs) may enable a continuous shuttle of factors supporting neuroregeneration or neuroprotection. Given that MSCs reside within the CNS after injection, we expect that MSCs may continue to **deliver neurotrophic growth factors for months**.
4) **MSCs are easy to harvest and expand *ex vivo* for therapeutic indications**. Adipose biopsies are easy to perform and are well tolerated by patients. MSCs derived from autologous sources eliminate issues of rejection and minimize risks of contamination.
5) **MSCs are safe and efficacious in animal models.** Animal studies of MSCs in neurodegenerative diseases, including ALS, have been promising. MSCs can be safely infused into the intrathecal space of animals and survive for up to 3-6 months after injection[17,18]. We completed our own pre-clinical studies of intrathecal administration of autologous MSCs with a favorable safety profile[19]. Animal models of neurological disease have revealed MSCs therapeutic potential. There are data showing MSC efficacy in animal models not only of ALS[2,20], but also in the other neurological diseases such as multiple sclerosis[21], Parkinson's disease[22], and stroke[23]. These animal data suggest that MSCs may be efficacious in many neurological diseases, and therefore **our data from this clinical trial will be useful in future human MSC trials for other neurological diseases**.
6) **MSCs are safe and possibly efficacious in human neurodegenerative diseases.** There is now growing evidence in human studies that MSCs can be safely administered intrathecally. Our Phase I dose-escalation safety trial using autologous adipose-derived intrathecal MSCs in ALS has had an excellent safety profile[3]. Our safety findings corroborate studies from other groups[24-27]. Of note, none of these studies has treated patients with more than two doses, which would likely be necessary in ALS. MSCs are also being used in other neurological diseases, and there are now some signs of success in stroke[28], multiple systems atrophy[29], and multiple sclerosis[30]. We expect that this treatment modality will continue to be pursued in other neurological diseases in the

future. **The Phase II clinical trial proposed here represents the first time that a large clinical trial will test the efficacy of intrathecal MSCs in a neurodegenerative disease. This study will also provide new data on the safety of repeated MSC injections over one year, while previous studies have not treated for over two months.**

7) **Precise mechanisms of action for MSCs are unclear.** Data regarding the mechanisms of action for MSC in the nervous system have exclusively come from animal models. This clinical trial provides a unique opportunity to better understand how aaMSCs affect the human nervous system. If clinical efficacy is observed in this clinical trial, we will have collected an enormous diverse dataset of ALS patients treated with intrathecal aaMSCs that will help us dissect **how intrathecal MSCs improve ALS**. This dataset includes:
   - **Clinical Data**: All patients will be classified in detail at the time of enrolment and longitudinally for >12 months that they are in the trial via our primary and secondary clinical endpoints.
   - **ALS Biomarkers:** As described above, there will be serial collection of blood and CSF throughout the clinical trial to assess **peripheral blood immune phenotyping, CSF/serum neurofilaments, and CSF miRNA.** We will have the ability to observe how these ALS biomarkers change in response to intrathecal MSC therapy. Additionally, stored blood samples may be used to determine ALS genotypes (SOD1, C9ORF72, etc.) in patients that may correlate with treatment response.
   - **MSC *in vivo* direct effects**: We will be able to correlate lumbosacral root nodularity with clinical response. Collected CSF will also be assayed for neurotrophic growth factor secretion from MSCs.
   - **MSC *ex vivo* assays:** MSCs will be stored from all patients in the study in order to correlate characteristics of each patient's MSC with a treatment response. If clinical efficacy is demonstrated with intrathecal MSCs in ALS we will perform detailed analyses of subjects' MSCs to better understand that response by correlating the following tests with clinical response to MSCs. First, we will assay the MSC culture supernatant and investigate **extracellular vesicles**[31], which may provide cellular signaling via **miRNA** and other factors. Second, we will quantify the ability of each subject's MSCs to **suppress the immune response**[32]. Third, the **proteome** and **mRNA profiles**[33] of MSCs will be compared between responders and non-responders to MSC treatment.
   - **Autopsies** will be requested from all patients in the clinical trial to directly investigate aaMSCs in the CNS and their consequences.

1.3. Results from our Phase I Dose-Escalation Study of Intrathecal aaMSCs in ALS. We have treated all patients in our 27-patient, Phase I dose-escalation safety trial using intrathecal aaMSCs in ALS. Patients have been treated with either 1 x 10^7, 5 x 10^7 (one or two monthly doses), or 1 x 10^8 (one or two monthly doses)[3]. Mean age at injection was 56 (range 36-75) and the male:female ratio was 15:12. The mean follow-up post-injection has been 345 days (range 27-759 days). There have been 16 deaths, all attributed to ALS, occurring with a mean time of 391 days post-injection (range 27-892 days). One autopsy has been completed and three are pending.

The **safety** profile thus far has been **excellent** and neither patients nor investigators have felt that treatment worsened the course of disease. Apart from single cases of self-limited headache and tinnitus, the most common side-effect has been temporary low back pain and position-dependent radicular pain, which has occurred exclusively in patients receiving 1 x 10^8 cells (8 of 10 patients). In all but one patient, this side effect has either resolved (n=5, pain duration 21-77 days), or was improving and tolerable on last follow-up. In one patient that was in the group receiving 1 x 10^8 cells x 2 injections, the pain after the first injection was severe enough to postpone the second injection for 2 months, at which point the pain

was 0/10. Following the second injection, the pain returned within one week and persisted at a moderate level 5/10 with the use of analgesics until his death 4 months following the second injection. In no patient were there bowel/bladder sphincter problems, continuous numbness/paresthesia, or noted

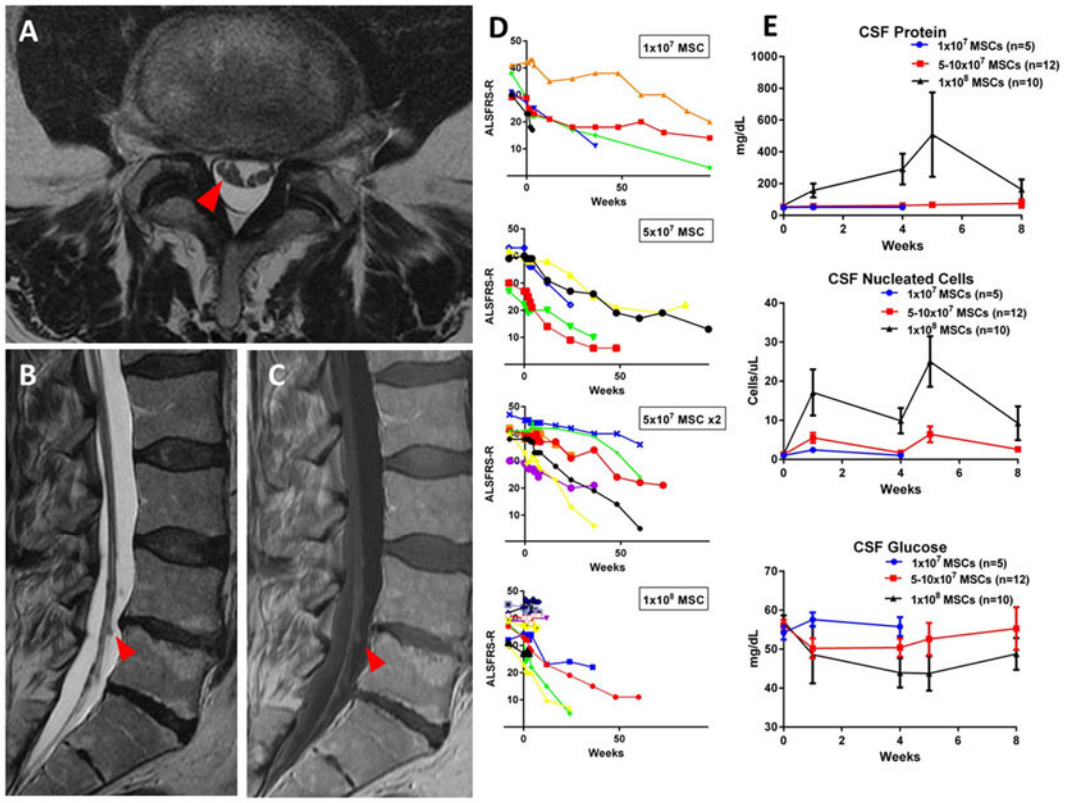


FIGURE 1: Results from Phase I dose-escalation safety trial of intrathecal aaMSCs in ALS. Nodularity of lumbosacral nerve roots (red arrows) was observed in patients 3 weeks following treatment with 5 x 10^7 aaMSCs (40%) and 1 x 10^8 aaMSCs (100%), as shown on MRI images (**A:** axial T2-weighted sequence, **B:** sagittal T2-w2eighted sequence and **C:** sagittal gadolinium-enhanced T1-weighted sequence). **D:** ALSFRS-R decreased over time in all patients. **E:** Elevations in CSF protein and nucleated cells were seen in a dose-dependent fashion.

unexpected progression of weakness. Signs of modest CSF inflammation were noted in patients receiving either 5 x 10^7 or 1 x 10^8 aaMSCs (Figure 1). Nodular thickening of lumbosacral nerve roots was observed on MRI in 1 patient receiving 1 x 10^7, 9 patients receiving 5 x 10^7 and 9 patients receiving 1 x 10^8 aaMSCs, which we hypothesize represents engraftment of the injected MSCs (Figure 1 – autopsies on 3 patients pending). While our Phase I study was not designed to provide efficacy data, 17 of the 27 patients treated thus far report that they have noted changes in their ALS impairments for up to one month following injection, including (with overlap) as improved bulbar function (n=8), increased limb strength (n=5), decreased fasciculations (n=4), decreased stiffness (n=4), and improved energy (n=4).

Our safety findings corroborate studies from other groups using intrathecal administration MSCs[24-27]. Again, it is notable that none of these studies has treated patients with more than two doses, which would likely be necessary in a chronic disease such as ALS.

1.4 Biomarker Discovery and Validation are critical in ALS: Clinical trials in ALS are hampered by the lack of biomarkers for diagnosis, or that track with disease severity and respond to therapies. Recent evidence suggests that neurofilaments and peripheral blood immune phenotyping (PB-IP) are excellent candidates for **biomarkers of ALS**. Currently there are no validated biomarkers in ALS for diagnosis, nor

that track with disease severity or rate of progression. Furthermore, there are no pharmacodynamics biomarkers that are altered by the one ALS treatment, riluzole.

Cerebrospinal Fluid (**CSF) neurofilament**, reflecting motor neuron axonal breakdown, has emerged as a promising surrogate of disease progression[34] and we will incorporate this biomarker into the present study. Measured by ELISA or chemiluminescent immunoassay, reactivity for the phosphorylated heavy subunit of neurofilament (pNF-H) and neurofilament light subunit (NF-L) is increased in CSF and plasma in ALS compared to healthy subjects and disease controls, and is stable over 6 months. This supports the hypothesis that elevated pNF-H and NF-L concentrations may be useful biomarkers in ALS[34-36]. pNF-H and NF-L levels in CSF are reported to correlate with rate of disease progression and survival in ALS, with elevated levels consistently shown to be associated with more rapidly progressive disease and shorter survival[35,36].

Published and unpublished data from longitudinal studies in patients with ALS have shown that pNF-H in CSF measured serially remain relatively stable over intervals of at least 6 months[35]. These observations are consistent with studies demonstrating that a single baseline measurement of pNF-H or NF-L may predict subsequent progression rate and survival. However, there are no data available regarding the half-life of CSF pNF-H or NF-L in chronic neurologic disorders that have persistent elevation of neurofilament levels (such as ALS). In the absence of effective neuroprotective/neuroregenerative therapy in ALS, there has been no means to readily investigate anticipated reductions in CSF or plasma pNF-H or NF-L levels in response to treatment. Evaluation of pNF-H and NF-L as putative pharmacodynamic biomarkers as such requires further study and is an appropriate aim in ALS therapeutic trials in which pNF-H and NF-L can be collected pre- and post-treatment.

Immune markers: Likewise, alterations of the **immune system** have been associated with ALS[37,38]. A recent study has demonstrated that drugs that modulate macrophage phenotype may positively impact ALS[39]. Our preliminary data using 10-color flow cytometry demonstrate differences in the **peripheral blood immune phenotyping (PB-IP)** between patients with ALS and healthy volunteers. We are in the process of correlating these findings with clinical data to determine how they may be used in clinical practice and clinical trials.

MicroRNAs (miRNAs) are non-coding RNA molecules that are involved in post-transcriptional regulation and have recently gained attention as biomarkers associated with ALS. Alterations of specific miRNAs have been reported in ALS patient samples of leukocytes[37,40,41], CSF[37,40], and CNS tissue[40,42,43]. Further studies will be necessary to validate these findings and interpret their meaning. Importantly, it will be necessary to establish if these miRNA change over time and whether they are correlated with disease progression and/or in response to therapies. We have established the ability to detect miRNA in patient CSF and will carry this further into this trial.

2.0 Goals

The Goal of the Proposed Study is to perform an open label, 69 subject, Phase II multi-site clinical trial to investigate the **safety** and **efficacy** of intrathecal treatment of aaMSCs in ALS. Patients will be treated with up to 1 x 10^8 aaMSCs every 3 months for a total of 4 intrathecal injections over 12 months. Reduced dose treatments will be allowed based on specific adverse events described below. Multiple **biomarkers** will be tracked throughout the clinical trial and correlated with response to treatment. Patients will be enrolled at Mayo Clinic Rochester, Arizona or Florida. <u>This study will initially have all fat biopsies and MSC administrations performed at Mayo Clinic in Rochester. Subsequently, these procedures will expand to the two other Mayo Clinic sites in Arizona and Florida as the manufacturing capabilities are finalized.</u>

2.1 Primary Endpoint (Safety): Our Phase I dose-escalation study of this treatment demonstrated safety at the maximum dose (1 x 10^8 aaMSCs x 2 monthly injections). 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 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.

Summary of Recommendations from November 19, 2020 DSMB Meeting:
At the November 19, 2020 DSMB meeting, it was recommended that the trial protocol be updated to address the increased recognition that subjects appear to be having increased back/leg pain following ad-MSC intrathecal injection and that this AE is threatening long-term feasibility of the study and the therapy. In response to this, the investigators have decided to change the starting dose from 1 x 10^8 aaMSCs to 5 x 10^7 aaMSCs. For those that tolerate this dose, there is opportunity to increase the dose by 2.5 x 10^7 aaMSCs at subsequent doses to maximum dose of 1 x 10^8 aaMSCs. For those that do not tolerate a given dose, there continues to be option to decrease subsequent doses (by 2.5 x 10^7 aaMSCs to a minimum dose of 1 x 10^7 aaMSCs) or skip dose if there is moderate pain. Furthermore, the DSMB recommended that subjects with preexisting chronic back pain and treatment (epidural injections or surgeries) should be excluded from study.

As part of this study, all future patients will start at 5 x 10^7 aaMSCs. Dose-Modification Rules (**Section 7.2**) are in place to allow for lower doses in patients that have a significant Expected Adverse Event of back and leg pain. The Dose-Modification Rules also allow for increasing doses for those who tolerate a given dose (to a maximum dose of 1 x 10^7 aaMSCs). This will provide further safety information that can be beneficial in future studies.

2.2 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 69 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[44] 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.

2.3 Exploratory Endpoints:

2.3.1 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.

2.3.2 Exploratory Clinical Endpoints:

1) **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.
2) **Modified Ashworth Spasticity Scale:** The muscle tone of bilateral elbows and ankles will be quantified using the Modified Ashworth Spasticity Scale[45]. Comparisons will be made between the 3-month lead-in and the 12-month observation period in study subjects.
3) **Edinbourgh Cognitive ALS Screen (ECAS):** This is a 136-point test of cognitive function designed for patients with ALS[46], 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.
4) **ALS Specific Quality of Life-Revised (ALSSQOL-R):** This is a 50 item instrument that measures quality of life (QOL) for subjects with ALS[47]. Comparisons will be made between the 3-month lead-in (V3) and the 12-month observation period (V16) 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.

3.0 Patient Eligibility

3.1 Inclusion Criteria

- All patients will have ALS diagnosed as possible, laboratory-supported probable, probable, or definite as defined by the World Federation of Neurology criteria for the diagnosis of ALS. [redacted]
- Examination and neurophysiological testing confirm a pure motor syndrome compatible with the diagnosis of ALS. All other possible causes of weakness have been excluded by extensive investigations.
- Age greater than 18 years, if female, must be post-menopausal, had a hysterectomy, or agree to two forms of birth control.
- Permanent resident or citizen of the United States.
- Geographic accessibility to the study site and willingness and ability to comply with follow-up.
- History of a chronic onset of a progressive motor weakness of less than two years duration.
- Subjects must be taking a stable dose of riluzole for at least 30 days prior to enrolment or not be on riluzole, and not have been on it for at least 30 days prior to enrolment (riluzole-naïve subjects are permitted in the study).
- Subjects must be taking a stable dose of oral Radicava® (edaravone) for at least 30 days prior to enrolment or not be on oral Radicava® (edaravone), and not have been on it for at least 30 days prior to enrolment (edaravone-naïve subjects are permitted in the study).
- Able to comply with protocol requirements, including MRI testing.
- Can provide written informed consent.

3.2 Exclusion Criteria

- Use of intravenous Radicava® (edaravone) within 30 days of screening or intent to use Intravenous Radicava® at any time during the course of the study including the follow up period.
- Any clinically significant medical condition (e.g., within six months of baseline, had myocardial infarction, angina pectoris, and/or congestive heart failure) that, in the opinion of the investigator, would compromise the safety of patient.
- Pulmonary Slow Vital Capacity (SVC) less than 65% of predicted for age, gender, and body type.
- Autoimmunity, including Crohn's disease or rheumatoid arthritis
- Current use of immunosuppressant medication or use of such medication within 4 weeks of Screening visit (Visit 1).
- Current use of any of the following investigational medicinal products or nutritional supplements being used or studied for the treatment of ALS:

○ Acetyl carnitine	○ Methylcobalamin (B12)
○ BASIS (nicotinamide riboside and pterostilbene)	○ N-acetyl-cysteine
○ Biotin	○
○ Calogen	○ Retigabine
○ Inosine	○ Rasagiline
○ Interleukin 2(Aldesleukin, Proleukin®)	○
○ L-Serine	

- Unwilling to forgo initiating the use of *any new* supplements during participation in the study.
- Malignancy 5 years prior to enrollment, including melanoma, with the exception of localized skin cancers (with no evidence of metastasis, significant invasion, or re-occurrence within three years of baseline).
- Active systemic or local infection near the lumbar puncture site.

- Inability to lie flat for the duration of intrathecal cell transplantation, or inability to tolerate study procedures for any other reason.
- Other active systemic disease as defined by laboratory abnormalities delineated in Appendix III.
- Unwillingness to discontinue herbal medications or other unapproved drugs
- Enrolled in an investigational drug trial within 30 days of baseline visit
- Prior stem cell therapy for a neurological disease
- Kokmen Short Test of Mental Status score <32
- Presence of a tracheostomy
- Ventilator dependent
- Pregnancy
- Men or women of childbearing potential who are unwilling to employ adequate contraception
- Chronic low back pain requiring invasive procedures (i.e. epidural injections or lumbar spine surgery)

4.0 Test Schedule

	Within 2 yrs of MSC	V1 -12 wks +/- 1 wk	V2 -8 wks +/- 1 wk	V3 -4 wks +/- 1 wk	Ph1 -2 wks +/-5 days	V4 Wk 0	V5 +2 wks +/- 5 days	V6 +6 wks +/- 5 days	Ph2 +10 wks +/- 5 days	V7 +12 wks +/- 5 days	V8 +14 wks +/- 5 days	V9 +18 wks +/- 5 days	Ph3 +22 wks +/- 5 days	V10 +24 wks +/- 5 days	V11 +26 wks +/- 5 days	V12 +30 wks +/- 5 days	Ph4 +34 wks +/- 5 days	V13 +36 wks +/- 5 days	V14 +38 wks +/- 5 days	V15 +42 wks +/- 5 days	V16 +48 wks +/- 5 days
Eligibility		X																			
Informed Consent		X																			
Demographic Info		X																			
Physical Exam		X	X	X		X	X	X		X	X	X		X	X	X		X	X	X	X
Neuro Exam[8]		X	X	X		X	X	X		X	X	X		X	X	X		X	X	X	X
Modified Ashworth Spasticity Scale		X				X	X			X	X			X	X			X	X		X
ALS History		X																			
Past Medical History		X																			
Medication Review		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs[5, 9]		X	X	X		X	X	X		X	X	X		X	X	X		X	X	X	X
Height/Weight[6]		X	X	X		X	X	X		X	X	X		X	X	X		X	X	X	X
ALSFRS-R		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-SSRS		X	X	X		X	X	X		X	X	X		X	X	X		X	X	X	X
ECAS		X																			X
ALSSQOL-R				X																	X
Pain Assessment[9]						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review Patient Perceived Changes							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review ADL[9]		X			X				X				X				X				
12 Lead ECG		X																			
Safety blood tests[1]		X				X	X			X	X			X	X			X	X		X
Optional research blood collection			X			X	X			X				X				X			X
Urinalysis[2]		X				X	X			X	X			X	X			X	X		X
Urine Pregnancy[2]		X				X				X				X				X			
Slow Vital Capacity		X																			
HHD		x	x	x		x	x	x		x	x	x		x	x	x		x	x	x	x
Fat biopsy[4]			X																		
CRTU Admission						X				X				X				X			
Lumbar Puncture						X		X		X		X		X		X		X		X	
MSC treatment[4]						X				X				X				X			
Safety CSF[3]						X		X		X		X		X		X		X		X	
Optional research CSF collection						X		X		X		X		X		X		X		X	
MRI lumbosacral spinal cord	X (not budgeted)							X													X
Patient Diary[7]						X	X			X	X			X	X			X	X		

Review of Adverse Events			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Test Schedule Footnotes

[1] CBC with differential, Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, Creatinine, Ca, AST, ALT, alkaline phosphatase) Mg, Phos, Uric Acid, Creatine Kinase, ESR, and PT/INR
[2] Urinalysis (dipstick) (Urine pregnancy test)
[3] CSF: protein, glucose, nucleated cells, RBCs, cytology, oligoclonal bands, IgG Index
[4] Visit 2 (fat biopsy) and Visits 4, 7, 10 and 13 (MSC treatments) must be done at Mayo Clinic in Rochester, Minnesota; all other visits will occur locally at the enrolling site.
[5] Vital signs include blood pressure, temperature, pulse and respiratory rate.[6] Height measured only at visit 1; weight measured at every visit.
[7] Subjects will be provided with a study diary at Visits 4, 7, 10 and 13 and will be instructed to complete the diary every day for two weeks following the aaMSC injection. Diaries will be reviewed with the subjects during Visits 5, 8, 11 and 14.
[8] Kokmen Short Test of Mental Status to be done only at screening visit (Visit 1).[9] Activities of Daily Living (ADL): reviewed at V1, and phone calls 1, 2, 3 and 4
[9] Vital signs and pain assessment will be taken post-transplant every 15 minutes for the first hour. Vital signs and pain assessment will be taken hourly for four hours (+/- 15minutes after the 2nd, 3rd, and 4th hour) and then every four hours +/-15 minutes until discharge which is 24 hours after injection

5.0 Protocol Treatment

5.1 Study Drug Description

The investigational product consists of autologous adipose-derived MSCs, suspended in 5-10 mL Lactated Ringer's. The MSC are provided in a sterile syringe labelled with appropriate patient and product identifiers ready for intrathecal injection. The MSCs uniformly express CD90, CD105, CD73 HLA Class I, and CD44. The MSC are negative for CD14, CD45 and HLA DR. Additionally the cells must have no clonal abnormalities detected by cytogenetic testing.

5.2 MSC Dose Regimen

Cells will be administered intrathecally at a single dose in a volume of 5-10 mL, all patients will receive 5 x 10^7 intrathecal aaMSCs at the first injection (Visit 4). Subsequent doses may be reduced or increased by 2.5 x 10^7, or skipped based on Dose Modification Rules (**Section 7.2**). Maximum dose is 1 x 10^8 aaMSCs, and minimum dose is 1 x 10^7 aaMSCs.

5.3 Preparation of Cellular Product

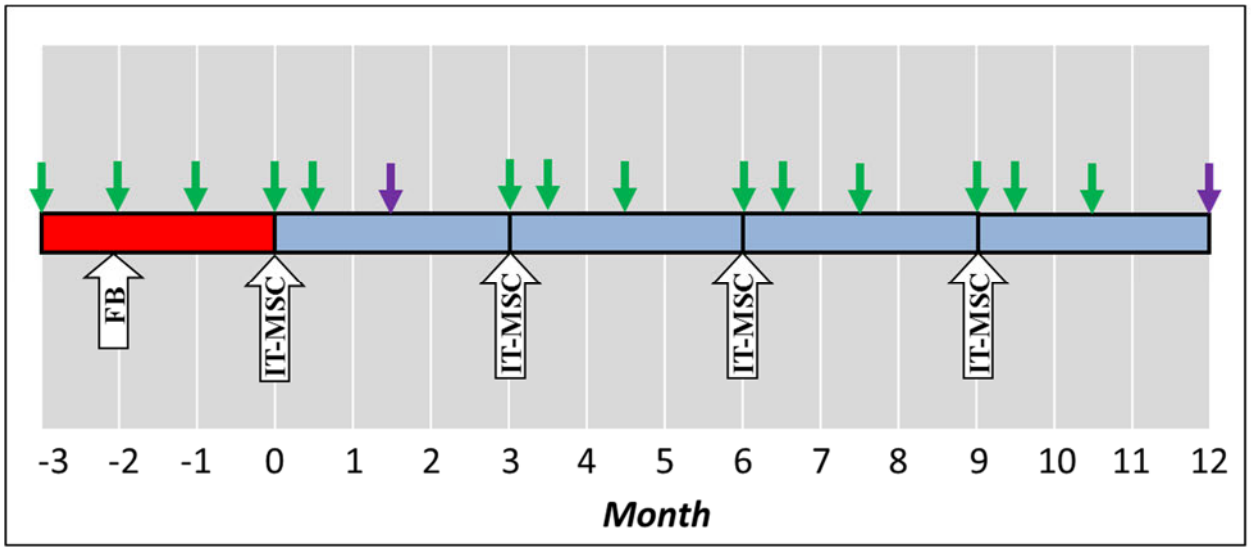
The adipose biopsy tissue will be delivered to and processed in the Mayo Clinic Rochester Human Cellular Therapy Lab. Cells will be expanded *ex vivo* in this cGMP facility using standard operating procedures. Cells will be cryopreserved during release criteria analysis. Cells will be tested for phenotype, mycoplasma, culture sterility, and cytogenetic analysis. Cells not meeting release criteria will not be administered to the patient. Upon meeting release criteria and upon appropriate patient scheduling, cells will be returned to culture and administered 3-5 days after culture. Additional release testing will occur, but cells will be administered without completion of final testing. In the event that the final number of MSCs is less than the scheduled amount (if scheduled amount is 2.5 x 10^7 – 1 x 10^8 aaMSC), MSCs will be delivered intrathecally to patient as long as the MSC number is > 2 x 10^7 (20-100 million MSCs).

In the event there is no cell growth from the tissue obtained from the first biopsy, one further attempt will be made from a second biopsy. If the second attempt fails to grow cells, no further attempts will be made, and the subject will not continue in the study. Thus far we have successfully generated cell growth from a single fat sample in all 28 ALS patients attempted. Thus we anticipate a very low failure rate for cellular manufacturing.

6.0 Study Procedures

This is an open-label Phase II study of intrathecal aaMSCs in patients with ALS. The goal is to treat 69 patients in this study. Patients will undergo a 3-month lead in period followed by 4 intrathecal injections spaced 3 months apart. The standard dose will be 5 x 10^7 aaMSCs, but dose modification may occur for a given patient as dictated by Expected Adverse Events and tolerability. Patients will undergo systematic safety studies (blood, CSF, MRI) and efficacy measures (ALSFRS-R, HHD) throughout the trial.

6.1 Study Schema



Study Schema: FB: Fat Biopsy, IT-MSC: intrathecal MSC treatment; Arrows: clinic visits; Purple Arrows: clinic visit includes MRI lumbosacral spine

6.2 Study Procedures Delineated by Visit

Subjects that are enrolled at Mayo Clinic Rochester will have all Visits in Rochester. Subjects that are enrolled in Mayo Clinic Arizona or Mayo Clinic Florida will have Visit 2 (fat biopsy) and Visits 4, 7, 10 and 13 (MSC treatments) in Rochester, but all other visits will occur locally at the enrolling site.

Screening Visit & Informed Consent (Visit 1 – V1; 12 weeks prior to 1st MSC treatment)
Informed Consent will be obtained during the screening visit by a neurologist with experience in the conduct of clinical trials for treatment of ALS. The draft informed consent document is attached (Appendix II). Patients will have access to the consent document at least 24 hours prior to meeting with the study neurologist.

As part of the informed consent, potential subjects will be asked to volunteer for two voluntary aspects of the study:

- Genetic screening for ALS-causing genes
- Autopsy at the time of death

After informed consent is obtained, screening tests will take place during which the patient will be assessed for study eligibility. This includes the following:

1) Review of inclusion and exclusion criteria
2) Screening blood collection in order to screen for systemic disease, including analysis for complete blood counts (with WBC differential), comprehensive metabolic panel (Na, K, Cl, HCO_3, BUN, creatinine, Ca, AST, ALT, alkaline phosphatase), Mg, Phosphorus, uric acid, creatine kinase, ESR, and PT/INR
3) 12 lead ECG
4) Urinalysis and urine pregnancy test
5) Slow vital capacity
6) Hand-held dynamometry (HHD)

7) ALSFRS-R
8) Review of concomitant medications and instructions regarding the use of these medications while enrolled in this study.
9) Edinbourgh Cognitive ALS Screen (ECAS)
10) Columbia Suicide Severity Rating Scale (C-SSRS)
11) Modified Ashworth Spasticity Scale
12) Review activities of daily living (ADL)

Within two years of MSC treatment, patients must undergo MRI of the lumbosacral spinal cord (lumbar level). If MRIs were performed at an outside institution, a study investigator will review these images for quality and pathology. If the MRIs have not been performed elsewhere (or are poor quality), these studies will be performed prior to MSC treatment. The MRIs will serve two functions: 1) helps ensure that there is no alternate explanation for patients' symptoms, 2) serves as a baseline safety study prior to MSC treatment.

Subcutaneous Fat Biopsy (V2; 8 weeks prior to 1st MSC treatment)

A subcutaneous fat biopsy will be taken from either the abdomen or thigh of the study subject. The actual site of the biopsy will be determined by the general surgeon or trained health professional, at the time of the procedure. The subject will be given a local anesthetic (usually 1% lidocaine) at the site of the biopsy. The fat biopsy will be done through a small (1-2 inch) incision where approximately 15 cc of subcutaneous fat is removed from under the skin. The biopsy site will be sutured with an intradermal absorbable suture that will not require suture removal. The suture will be reabsorbed in 1-3 weeks. This type of biopsy is usually well tolerated and offers minimal risk to the subject. These risks can include discomfort and bruising at the biopsy site. The subject will be given instructions on after care of the biopsy site following the procedure.

Lead-in Period (V1-3)
Patients will have monthly study visits prior to MSC infusion, for a total of a 3-month lead-in period. At each lead-in visit, patients will undergo the following:

1) Physical Examination
2) Neurological Examination
3) Vital Signs
4) Medication Review
5) ALSFRS-R
6) C-SSRS
7) HHD
8) Review Adverse Events **(V2 and V3 only)**
9) Review Activities of Daily Living (ADL) (**V1 only**)
10) ALSSQOL-R **(V3 only)**
11) Optional research blood collection **(V2 only;** only if patient has consented to this optional collection/testing**)**

Phone Calls
Two weeks prior to each intrathecal administration of aaMSCs, the study coordinator will contact the subject by phone, review adverse events, ADL, ALSFRS-R, pain assessment and review patient perceived changes.

Intrathecal administration of aaMSCs and acute monitoring (V4, V7, V10, V13; 12 weeks apart)
Study subjects will be admitted to Hospital the day of MSC infusion, and will stay there for at least 24 hours after treatment. During the day of hospital admission, the patients will undergo the following:

1) Physical Examination
2) Neurological Examination
3) Safety blood collection to serve as baseline prior to MSC injection, including analysis for complete blood counts (with WBC differential), comprehensive metabolic panel (Na, K, Cl, HCO3, BUN, creatinine, CA, AST, ALT, alkaline phosphatase), Mg, Phosphorus, uric acid, creatine kinase, ESR, and PT/INR
4) Optional research blood collection (to be collected at same time as safety bloods – if patient consents to optional collection/testing)
5) Urinalysis and Urine Pregnancy test
6) Modified Ashworth Spasticity Scale
7) Vital Signs
8) Medication Review
9) ALSFRS-R
10) C-SSRS
11) Pain assessment (prior to discharge from CRTU at each injection visit)
12) Review patient perceived changes **(starting at V5)**
13) HHD
14) Safety CSF collection
15) Optional research CSF collection (to be collected at same time as safety CSF – if patient consents to optional collection/testing)
16) Intrathecal administration of MSCs
17) Distribute Patient Diary (for review during V5, V8, V11 and V14)

Prior to the intrathecal injection, the patient will have a saline lock placed. This is a safety measure should the subject need IV fluids or medications should they experience a medical emergency during or after the injection.

MSCs will be delivered from the Human Cellular Therapy Lab in syringes at 4^{o}C and administered via lumbar puncture within 12 hours of preparation. A lumbar spinal needle will be placed in the subarachnoid space by a trained health practitioner (possibly under fluoroscopic guidance), and a baseline CSF sample will be collected (later analyzed for protein, glucose, nucleated cells, RBCs, cytology, oligoclonal bands, IgG Index). Subsequently, aaMSCs will be infused into the CSF in 5-10 mL of Lactated Ringers solution over 1-2 minutes, followed by 1mL Lactated Ringers flush by one of the study physicians. In the event that the final number of MSCs is less than the scheduled amount, MSCs will be delivered intrathecally to patient as long as the MSC number is $> 2 \times 10^7$ (20-100 million MSCs). After cell infusion and if the patient is tolerant, they will be rotated every 15 minutes in a Trendelenburg position (with help from nursing staff if necessary) for 2 hours to maximize even distribution of cells in the CSF.

During the patient's stay in the CRTU the subject will be observed by research nurses for any Adverse Events during and immediately following the intrathecal injection of the MSC's. The patient's vital signs (including pain assessment) will be monitored every 15 minutes for one hour, and then hourly for four hours (+/-15 minutes after the 2nd, 3rd, and 4th hour), and then every four hours (+/-15 minutes) until discharged from the CRU, which will be at least 24 hours after injection.

If after MSC injection, release testing reveals unexpected contamination, the following procedure will be implemented:

a) The Principal Investigator and study physicians and patient will be immediately notified by members of the Human Cell Therapy Laboratory.

b) An emergent infectious disease specialty consultation will be made to help determine appropriate further testing (CSF and/or blood cultures), antibiotic therapy directed to identified pathogen, and continued clinical follow-up. Continued active patient monitoring will comprise weekly patient contacts by study staff (clinic visits or phone calls) for four weeks (or until completion of antibiotics) to document symptoms.
c) An investigation will be conducted by the Human Cell Therapy laboratory to determine the root cause of contamination,
d) An action plan will be enacted for corrective/preventative actions in order to mitigate the likelihood for recurrence.

Early Follow-up Visits (V5, V8, V11, V14; two weeks after aaMSC treatment)
Two weeks following MSC therapy, patients will have a study visit and undergo the following:

1) Physical Examination
2) Neurological Examination
3) Modified Ashworth Spasticity Scale
4) Vital Signs
5) Medication Review
6) Adverse Events Review
7) ALSFRS-R
8) C-SSRS
9) Pain assessment
10) Review patient perceived changes
11) HHD
12) Safety Bloods
13) Optional research blood collection **(V5 only;** (to be collected at same time as safety bloods – if patient consents to optional collection/testing)**)**
14) Urinalysis
15) Review Patient Diary

Late Follow-up Visits (V6, V9, V12, V15; 6 weeks after aaMSC treatment)
Patients will have a visit 6 weeks after aaMSC treatment. At Late Follow-up visit, patients will undergo the following:

1) MRI of lumbosacral spinal cord for safety purposes **(V6 only)**
2) Physical Examination
3) Neurological Examination
4) Vital Signs
5) Medication Review
6) Adverse Events Review
7) ALSFRS-R
8) C-SSRS
9) Pain assessment (which will dictate subsequent aaMSC dose)
10) Review patient perceived changes
11) HHD
12) Lumbar Puncture for safety CSF collection (possibly under fluoroscopic guidance) for safety (protein, glucose, nucleated cells, RBCs, cytology, oligoclonal bands, IgG Index) and stored for research
13) Optional CSF collection (to be collected at same time as safety CSF collection – if patient consents to optional collection/testing)

Final Observation Visit (V16; 48 weeks after 1st aaMSC treatment; 6 weeks after last Late Follow-up Visit)

At the Final Observation Visit, patients will undergo the following:

1) Physical Examination
2) Neurological Examination
3) Vital Signs
4) Medication Review
5) Adverse Events Review
6) Safety blood collection
7) Optional research blood collection (to be collected at same time as safety bloods – if patient consents to optional collection/testing)
8) Urinalysis
9) ALSFRS-R
10) C-SSRS
11) Modified Ashworth Spasticity Scale
12) Pain assessment
13) Review patient perceived changes
14) ALSSQOL-R
15) HHD
16) MRI of lumbosacral spinal cord.
17) ECAS

6.3 Procedures for when patients are unable to attend Study Visits.

ALS is a progressive paralytic disorder and it is common that patients progress to where they are unable to travel to a clinic visit. When this occurs for study patients, subjects (and/or caregivers) will be contacted by phone to review adverse events and perform the ALSFRS-R.

6.4 Voluntary testing for patients

6.4.1 Genetic testing: Given the increasing number of genes that cause ALS, it has been suggested that patients voluntarily subject to genetic testing for clinical trials in ALS in order to have this information available as a post-hoc analysis. We will collect DNA for post-hoc genetic testing following informed consent.

6.4.2 Autopsy: A key question in stem cell therapy and ALS is: What is the fate of injected cells and what are their effects? All patients in this study will be asked to volunteer for eventual autopsy at the time of their death in order to address this question.

7.0 Dosage Modification Based on Adverse Events

7.1 Adverse Event Monitoring

An adverse event is defined as any noxious, pathologic or unintended change in anatomical, physiologic, or metabolic function as indicated by physical signs, symptoms, and/or laboratory changes occurring during this phase II clinical trial. This includes an exacerbation of a pre-existing condition.

Dose limiting toxicity (DLT) criteria: DLT will be defined as an adverse event attributed (definitely, probably, or possibly) to the study treatment AND meeting one of the following criteria:

Toxicity	***DLT Definition (CTCAE v5)***
Hematologic	≥ Grade 3 ANC for ≥ 7 days or PLT < 75,000 for ≥7 days
Infection	≥ Grade 3
Febrile Neutropenia	Defined as fever ≥ 38.5°C (38 > 1 hour) with grade ≥ 3 neutropenia
Dermatologic	≥ grade 2 erythema multiforme, ulceration, or urticaria that does not resolve to < grade 2 within two weeks
Pulmonary	Newly identified ≥ grade 2 bronchial obstruction, pneumonitis, or wheezing
Immune system	≥ grade 2 allergic reaction or autoimmunity
Other Non-hematologic	≥ grade 2 that does not resolve to < grade 2 within two weeks
Neurologic or psychiatric	≥ grade 3 that do not resolve within two weeks

Serious Adverse Events: Serious Adverse Events are defined as an adverse event meeting any one of the following criteria:

1. Fatal or life-threatening
2. Temporarily or permanently disabling
3. Requires or prolongs inpatient hospitalization
4. Requires intervention to prevent permanent impairment or damage
5. Is a cancer or overdose

In addition, specific neurological incidents that will be considered a Serious Adverse Event include the following:

- unexpected CNS infection or inflammation
- new onset seizures
- sudden unexplained deterioration of ALS
- new CNS MRI abnormalities with mass effect, or
- severe and long-lasting (> two weeks) headache with increased intracranial pressure.

Furthermore, experiences that in the investigator's opinion suggest a significant hazard, contraindication, side effect, or precaution that may be associated with the use of the intrathecal MSC therapy will be considered a Serious Adverse Event.

When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:

Definite - The adverse event *is clearly related* to the investigational agent(s).
Probable - The adverse event *is likely related* to the investigational agent(s).
Possible - The adverse event *may be related* to the investigational agent(s).
Unlikely - The adverse event *is doubtfully related* to the investigational agent(s).
Unrelated - The adverse event *is clearly NOT related* to the investigational agent(s)

Expected adverse events: In our Phase I dose-escalation safety trial of intrathecal aaMSCs for ALS, eight of the ten subjects receiving 1 x 10^8 MSC dosage developed low back and leg pain that began within a week of treatment, peaked in the first three weeks, and then resolved or became very mild (1/10 on visual analog scale). The pain exhibited variable intensity (2-8/10 on visual analog scale) and duration (range 42-77 days) among the subjects. In one subject, the pain led to postponement of the 2nd treatment by 2 months until pain had resolved. The pain recurred upon 2nd injection, again lasting two months[3] (Appendix I).

Additionally, previously reported MRI lumbosacral spine abnormalities will be considered expected adverse events. Elevated CSF protein and cell count will also be considered expected adverse events. These adverse events related to MRI and CSF will be logged as part of the patient's CRF.

In the study by Karussis et al.[48], which used intrathecal treatment of bone-marrow derived MSCs, no Serious Adverse events occurred; however, adverse events occurred (without mention of severity). In their 19 ALS patients, the following adverse events were noted: fever (11), headache (5), leg pain (2), and dyspnea (1). Based on the available data, fever, headache, and back/leg pain may be regarded as expected adverse events.

In summary, Expected Adverse Events for this study attributable to the treatment are:

- Back pain developing within 2 weeks of lumbar puncture, improving or resolving within 4 weeks of onset
- Headache developing within 2 weeks of lumbar puncture, improving or resolving within 4 weeks of onset
- Leg pain developing within 2 weeks of lumbar puncture, improving or resolving within 4 weeks of onset
- CSF pleocytosis (<50 cells/uL)
- CSF protein elevation
- Abnormal MRI spine with meningeal thickening, nerve root enlargement and nodularity, +/- gadolinium enhancement

7.2 Dose modifications and stopping rules based on adverse events

Adverse events, MRI lumbosacral spine abnormalities, and subjective clinical responses occurred in a dose-dependent fashion. Eight of ten patients receiving frequency 1 x 10^8 aaMSCs in our Phase I study developed low back pain and leg pain of variable intensity and duration, which was not observed at the 5 x 10^7 dosage. MRI lumbosacral abnormalities were seen in 9/12 receiving 5 x 10^7 and 9/10 receiving 1 x 10^8 aaMSCs. Subjective clinical response frequency was similar in the 5 x 10^7 and 1 x 10^8 dosages; however, the responses were more compelling in the higher dosage. The following dose modification rules were developed with the Phase I data in mind, and modified following the DSMB meeting of November 19, 2020 (Figure 2). The goal is to provide the maximum tolerated dose during the one-year study, starting at the middle dose in our Phase I trial (5 x 10^7). In summary, if patients tolerate a given dose, the next dose can be increased by 2.5 x 10^7 (to 7.5 x 10^7 or 1 x 10^8). Conversely, if a subject develops significant low back and leg pain, the offending dose can be lowered at the next injection (either to 7.5 x 10^7, 5 x 10^7, or 1 x 10^7, depending on what dose caused pain). These rules will provide dose finding information for subsequent studies.

7.2.1 If patients tolerate a given dose (defined by 0-1/10 pain at six weeks prior to the next scheduled injection), the next injection dose may be increased by 2.5 x 10^7, up to a maximum dose of 1 x 10^8 aaMSCs.

7.2.2 If patients develop an Expected Adverse Event (back and leg pain of any severity) that remains at 2-4/10 intensity (mild-to-moderate pain on visual analog scale) six weeks prior to the next scheduled injection, patients must receive the next lower dose (7.5 x 10^7, 5 x 10^7, or 1 x 10^7 aaMSC) in subsequent doses. If the patient is already receiving 1 x 10^7 aaMSCs, this dose may continue.

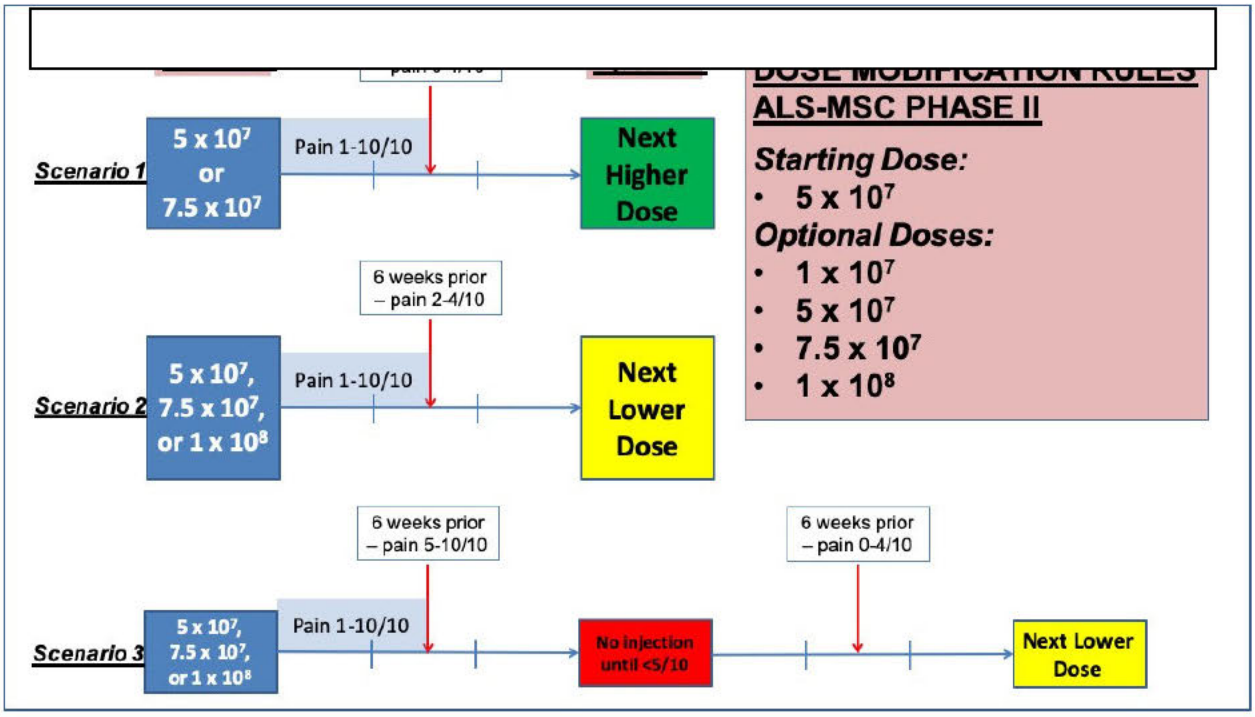
7.2.3 If patients develop an Expected Adverse Event (back and leg pain of any severity) that remains 5-10/10 intensity (moderate-to-severe pain on visual analog scale) at six weeks prior scheduled aaMSC treatment, treatments will be discontinued until pain reduces to <5/10. At that point, the patient will receive the next lower dose (7.5 x 10^7, 5 x 10^7, or 1 x 10^7 aaMSCs) at the next scheduled injection timepoint. If the patient is already receiving 1 x 10^7 aaMSCs, this dose may continue.

7.2.4 If patients develop a Dose-Limiting Toxicity (defined in 7.1), patients must receive the next lower dose (7.5 x 10^7, 5 x 10^7, or 1 x 10^7 aaMSC) in subsequent doses. If the patient is already receiving 1 x 10^7 aaMSCs, this dose may continue.

7.2.5 Once the dose has been lowered, it may not be increased at subsequent treatments.

7.2.6 If at any point a Serious Adverse Event occurs that is determined to be possible, probable, or definitely related to aaMSC, no further intrathecal aaMSC treatments will be given to that patient until thorough review by the principal investigator, Institutional Review Board (IRB) and FDA.

7.2.7 If at any point during the course of the trial, Serious Adverse Events occur in three subjects that are determined to be probable or definitely related to aaMSC, no further intrathecal aaMSC treatments will be given to any patient until thorough review by the principal investigator, DSMB, Institutional Review Board (IRB) and FDA.



8.0 Ancillary Treatment/Supportive Care

- Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

- Care for the patient's underlying ALS will continue at the Mayo (or equivalent) ALS Clinic. Any neurologic DLT will be treated using standard supportive care.

8.1 Use of oral Radicava (edaravone) during clinical trial

On 5/12/22, the U.S. FDA approved the use of oral Radicava (edaravone) in people with ALS. In response to this change, the Inclusion Criteria for this clinical trial were amended to allow subjects to be on a stable dose of oral edaravone at enrollment (see 3.1 Inclusion Criteria). For subjects already enrolled at the time of oral edaravone FDA approval, we will request that subjects do not start oral edaravone until 6 weeks after the 2nd MSC injection (Visit 7). If subjects choose to initiate oral edaravone prior to that time it will be treated as a protocol deviation, but subjects may stay in the clinical trial.

8.2 Use of of RELYVRIO™ (sodium phenylbutyrate and taurursodiol; also known as AMX0035) during clinical trial

On 9/29/22, the U.S. FDA approved the use of RELYVRIO™ (sodium phenylbutyrate and taurursodiol; also known as AMX0035) in people with ALS. In response to this change,

for subjects already enrolled at the time of Relyvrio FDA approval, we will request that subjects do not start Relyvrio until 6 weeks after the 2nd MSC injection (Visit 7). If subjects choose to initiate Relyvrio prior to that time it will be treated as a protocol deviation, but subjects may stay in the clinical trial. Inclusion criteria did not need to be amended because all subjects were enrolled at the time of the amendment regarding Relyvrio.

9.0 Adverse Event (AE) Reporting and Monitoring

Throughout the study the investigators will record all adverse events on the Adverse Event Case Report Forms (CRF), regardless of the severity or relationship to the study treatment.

Investigators will treat patients with adverse events appropriately and observe them at suitable intervals until the experiences resolve or stabilize. Adverse events may be discovered through observation of the patient, questioning of the patient, complaint by the patient, or by abnormal clinical laboratory values.

The subject will be observed and questioned regarding the occurrence of possible adverse events. The questioning should be conducted with due regard for objectivity and, in particular, the questioner should gather information regarding adverse events from questioning the patient, spontaneous report by the patient, laboratory reports (including ECG), and clinician's observations.

In addition, adverse experiences may also include laboratory values that become clinically significant out-of-range values (defined in Appendix IV). In the event of an out-of-range value, the laboratory test should be repeated until it returns to normal, or can be explained, and the patient's safety is not at risk.

Worsening of a pre-existing condition that is not consistent with the patient's usual clinical course will be reported as an adverse event.

9.1 Adverse Event Characteristics

CTCAE term (Adverse Event description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be used for Adverse Event reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site.

9.1.1 Attempts will be made to characterize all Adverse Events. Patients enrolled in the study will be followed per Schedule of Events, out to 3 months after the last injection therapy. Patients who are unwilling (due to study withdrawal) or unable (due to ALS debility) to attend clinic appointments will be contacted by phone at the scheduled intervals (if the subject gives consent) for inquiry into Adverse Events.

9.1.2 Adverse event monitoring and reporting is a routine part of every clinical trial. First, the event will be identified and graded using the CTCAE v5.0. Next, it will be determined if the adverse event is related to the medical treatment or procedure. With this information, it will be decided whether an adverse event should be reported to the IRB and FDA as an expedited report (see Section 9.2) or as part of the routinely reported clinical data. All Adverse Events reported via expedited

mechanisms will also be reported via the routine data reporting mechanisms defined by the protocol.

9.1.3 Assessment of Attribution

When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:

Definite - The adverse event *is clearly related* to the investigational agent(s).
Probable - The adverse event *is likely related* to the investigational agent(s).
Possible - The adverse event *may be related* to the investigational agent(s).

Unlikely - The adverse event *is doubtfully related* to the investigational agent(s).
Unrelated - The adverse event *is clearly NOT related* to the investigational agent(s)

9.2 Expedited Adverse Event Reporting Requirements

A report will be submitted to the FDA and IRB within7 working days in the following scenarios:

- Unexpected Adverse events that are CTCAE v5.0 Grade 3, 4, or 5, and attributed to the treatment (possible, probable, or definite), that occur within 30 days of the last treatment
- Any hospitalizations attributed to the treatment (possible, probable, or definite) that occur within 30 days of the last treatment
- Adverse events that are CTCAE v5.0 Grade 5 (death), and attributed to the treatment (possible, probable, or definite), regardless of timeframe
- Specific neurological adverse events: unexpected CNS infection or inflammation, new onset seizures, sudden unexplained deterioration of ALS, new CNS MRI abnormalities with mass effect (lumbosacral nerve roots excluded), or severe and long-lasting (> two weeks) headache with increased intracranial pressure, regardless of timeframe

9.21 Special Situations for Expedited Reporting

EXPECTED Serious Adverse Events: Protocol Specific Exceptions to Expedited Reporting

For this protocol only, the following Adverse Events/Grades are expected to occur within this ALS population and do not require *Expedited* Reporting, unless considered attributed to the treatment (possible, probable, or definite). These events must still be reported via Routine Reporting (see Section 10.3). *

System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will *not* be *expeditedly* reported.
Generalized disorders and administration site conditions	Gait disturbance	≤Grade 3
Gastrointestinal disorders	Dysphagia	≤Grade 3
Investigations	Vital Capacity Abnormal	≤Grade 3
	Weight loss	≤Grade 3
Injury, poisoning and procedural complications	Fall	≤Grade 3
Musculoskeletal and connective tissue disorders	Generalized muscle weakness	≤Grade 3
	Muscle Cramp	≤Grade 3
	Muscle weakness lower limb	≤Grade 3
	Muscle weakness trunk	≤Grade 3
	Muscle weakness upper limb	≤Grade 3
Nervous system disorders	Dysarthria	≤Grade 3
	Facial muscle weakness	≤Grade 3
	Muscle weakness left-sided	≤Grade 3
	Muscle weakness right-sided	≤Grade 3
	Spasticity	≤Grade 3
Respiratory, thoracic and mediastinal disorders	Dyspnea	≤Grade 4
	Hypoxia	≤Grade 3
	Laryngospasm	≤Grade 3

NOTE: Suspected/actual events covered in this exception include any event that is considered ALS disease progression.

9.3 Routine Adverse Event Reporting

The following Adverse Events will be reported to the FDA and IRB at the required intervals:

- Grade 1 and 2 Adverse Events deemed *possibly, probably, or definitely* related to the study treatment or procedure.
- Grade 3 and 4 Adverse Events regardless of attribution to the study treatment or procedure
- Grade 5 Adverse Events (Deaths)
  - Any death within 30 days of the patient's last study treatment regardless of attribution to the study treatment or procedure
  - Any death more than 30 days after the patient's last study treatment that is felt to be at least *possibly* related.

Adverse events required to be submitted via the expedited route (Section 9.2) will also be submitted via the routine route.

Mayo Clinic Rochester, Arizona and Florida should each follow their site-specific reporting guidelines for reporting all adverse events.

9.4 Data Safety and Monitoring Board

A Data Safety and Monitoring Board (DSMB) will be assembled for this phase II clinical trial, and will constitute a neurologist (with no involvement in this trial), a neurologist with experience in stem cell trials, and a stem cell specialist. The DSMB will have access to all records in the study at all times. The DSMB charter and plan are documented separately from this protocol.

9.5 Records and Data Collection Procedures

All data must be entered by Remote Date Entry (RDE) and completed by qualified and authorized personnel. All data on the eCRF must reflect the corresponding source document. [redacted]

10.0 Statistical Plan

Sample Size and Power Calculations

Preliminary slope estimates of ALSFRS-R, used as a primary endpoint in this trial, were obtained using PRO-ACT database[44], where we matched 5 controls to each case from our ongoing study on age, gender and riluzole use. All controls had at least 4 measurements within the first 12 months for slope estimates. The mean (standard deviation) for the slope using 12 month follow-up data were -1.010 (1.07). Based on these slope estimates using an alpha level of 0.10, we would need 57 patients per group to detect a 50% difference in slopes between active treatment group and PRO-ACT database cohort (-0.505 vs -1.010) for a 80% power assuming a 2-sided two sample t-test is appropriate. If the study meets its primary objective of demonstrating safety and indication of efficacy of intrathecal MSC, and if the secondary outcome data is also supportive then the slope estimates generated from this study would allow us to design a Phase III study with appropriate effect size. Sample size calculations were performed using nQuery Advisor version 7.0 software (Statistical Solutions, Boston, MA, USA).

Statistical Methods

Descriptive summaries as mean and SD for continuous variables, and frequencies and percentages for categorical variables will be reported for both primary safety and efficacy

endpoints as well as secondary endpoints. For an assessment of primary efficacy endpoint, we will regress each patient's ALSFRS-R score at each of the visits against time (months) to estimate a patient specific slope i.e. rate of decline in score per month. This will allow us to use all data points from a patient without a need for any imputation as long as a patient has a baseline and at least one post baseline measurement. This slope estimate will then be compared between two groups using two sample t-test or Wilcoxon rank sum test. If we identify imbalances among the groups at baseline or would consider accounting for any possible confounders then we will use regression based approach with slope as an endpoint adjusting for respective variables of interest. All tests will be two sided, and p values of less than 0.05 will be considered statistically significant. Given the smaller sample size and Phase IIB nature of the study, no adjustment for multiple comparisons will be made while analyzing several secondary endpoints. Analysis will be performed using intention to treat (ITT) data, which will include patients that were randomized and have received at least one intrathecal injection. ITT approach minimizes biases and allow for more accurate assessment of the safety or efficacy of the treatment. Both safety and efficacy assessments will be considered prior to making a decision on future Phase III study. Analysis of secondary endpoints such as ALSFRS-R (patient baseline as control), HHD and modified Ashworth Spasticity Scale will be assessed by comparing slope estimates between the lead-in period and the treatment period in a similar fashion as in the case of primary efficacy endpoint. A graphical analysis will also be done using scatter plots and/or boxplots by displaying the pattern of actual values as well as slopes between placebo and treatment groups.

11.0 Treatment Extension Protocol

Background: During the execution of this Phase 2 trial, there have been subjective positive responses from patients that encouraged the researchers to pursue an extension arm of this trial. Due to limited resources, we have elected to offer Treatment Extension to the following sub-cohort meeting the following Inclusion & Exclusion Criteria within the Phase 2 clinical trial:

Inclusion Criteria:

1) Completion of the Phase 2 clinical trial, including the final visit.
2) Improvement (or < 0.2 overall change) of the slope of the ALSFRS-R between the lead-in (V1-V4) and treatment periods (V4-V16)
3) If available, a decrease in the serum neurofilament light chain levels between the lead-in and treatment periods. Decrease in neurofilament light chain has been accepted as a surrogate biomarker of reduced neurodegeneration by the FDA for the submission of Tofersen (anti-sense oligonucleotide) for SOD1-associated ALS.

Exclusion Criteria

1) Any clinically significant medical condition that, in the opinion of the investigator, would compromise the safety of patient.

At the time of this protocol amendment submission, 6 patients meet these criteria (4 are yet to complete study).We will begin screening / inviting subjects that completed the study first and proceed to those that recently completed the study until the funding is exhausted.

Treatment Extension Protocol

The trial will offer the option for subjects who meet criteria as defined above to be retreated every 3 months for one year (4 injections total). See Schedule of Events for details. In-person visits will be performed for the consent visit and MSC injection visits. All other visits will be via phone or video. Dose-escalation rules will be used as per the main Phase 2 study (**Section 7.2**). Subjects will undergo repeat fat biopsy for manufacturing of MSCs.

Patients will have post-treatment video visit follow-ups at 2 weeks and 6 weeks (in-person), which may be converted to in-person at the discretion of the investigator. Safety monitoring and reporting will continue as in the main protocol (**Section 9.0**)

Informed Consent
A new modified Informed Consent will be obtained by one of the investigator-neurologists with experience in the conduct of clinical trials for treatment of ALS. The modified informed consent document is attached.

Subcutaneous Fat Biopsy & MSC manufacturing

A subcutaneous fat biopsy will be taken from either the abdomen or thigh of the study subject as per **Section 6.2** (Subcutaneous Fat Biopsy V1). MSC manufacturing will occur as per **Section 5.3** (Preparation of Cellular Product).

Intrathecal administration of autologous MSCs and acute monitoring
The protocol will follow the procedures detailed in **Section 6.2** (Intrathecal administration of aaMSCs and acute monitoring).

Schedule of Events

	V1 -8 to -2 wks	**Ph1 -2 wks +/-5 days**	**V2** Wk 0	**Virt1** 2 wks +/- 5 days	**Virt2** +6 wks +/- 5 days	**Ph2** +10 wks +/- 5 days	**V3** +12 wks +/- 5 days	**Virt3** +14 wks +/- 5 days	**Virt4** +18 wks +/- 5 days	**Ph3 +22 wks +/- 5 days**	**V4** +24 wks +/- 5 days	**Virt5** +26 wks +/- 5 days	**Virt6** +30 wks +/- 5 days	**Ph4 +34 wks +/- 5 days**	**V5** +36 wks +/- 5 days	**Virt7** +38 wks +/- 5 days	**Virt8** +42 wks +/- 5 days	**V6** +48 wks +/- 5 days
Eligibility	X																	
Informed Consent	X																	
Physical Exam	X		X				X				X				X			X
Neuro Exam	X		X				X				X				X			X
Medication Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs[4]	X		X				X				X				X			X
Height/Weight[5]	X		X				X				X				X			X
ALSFRS-R	X		X	X	X		X	X	X		X	X	X		X	X	X	X
C-SSRS[8]	X		X	X	X		X	X	X		X	X	X		X	X	X	X
Pain Assessment[9]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review Patient Perceived Changes				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review ADL[6]	X	X				X				X				X				
Safety blood tests[1]	X		X				X				X				X			X
Urinalysis[2]	X		X				X				X				X			X
Urine Pregnancy[2]	X		X				X				X				X			
Fat biopsy	X																	
CRTU Admission			X				X				X				X			
Lumbar Puncture			X				X				X				X			
MSC treatment[7]			X				X				X				X			
Safety CSF[3]			X				X				X				X			
MSC dose decision	X				X				X				X				X	
Review of Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Test Schedule Footnotes

[1] CBC with differential, Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, Creatinine, Ca, AST, ALT, alkaline phosphatase), Mg, Phos, Uric Acid, Creatine Kinase, ESR, and PT/INR, Neurofilament light chain
[2] Urinalysis (dipstick) (Urine pregnancy test)
[3] CSF: protein, glucose, nucleated cells, RBCs, cytology

[4] Vital signs include blood pressure, temperature, pulse and respiratory rate.
[5] Height measured only at visit 1 if able to stand; weight measured at every visit.
[6] Activities of Daily Living (ADL): reviewed at V1, and phone calls 1, 2, 3 and 4
[7] Vital signs and pain assessment will be taken post-transplant every 15 minutes for the first hour. Vital signs and pain assessment will be taken hourly for four hours (+/- 15minutes after the 2nd, 3rd, and 4th hour) and then every four hours +/-15 minutes until discharge which is 18-24 hours after injection
[8] If there is a positive response on the C-SSRS, within 4 hours an MD-investigator will discuss with subject whether further evaluation and treatment is indicated.

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Guidelines for Clinically Significant Laboratory Values, Vital Signs, and Clinical Assessments

AST and ALT (SGOT and SGPT)		>3 times ULN **OR** pretreatment >4 times ULN
Albumin		<0.9 LLN **OR** pretreatment value <0.75 LLN
Albumin/Globulin ratio		<0.75
Alkaline phosphatase		>3 times ULN
Anion Gap		>25 m/Eq/L
Beck's Depression Inventory (BDI)		Worsening of BDI score by 3 or more points
Bilirubin	**Direct**	>1.5 times ULN **OR** pretreatment >2 times ULN
	Total	>2 times ULN, **OR** pretreatment above >4 times ULN
Blood urea nitrogen (BUN)		>2 times ULN **OR** pretreatment >3 times ULN
BUN/Creatinine ratio		>35
C-reactive Peptide		>20 mg/L
Calcium		<0.8 times LLN **OR**>1.2 times ULN
Chest X-ray		Clinically significant change in chest x-ray as judged by the site principal investigator
Chloride		<0.9 LLN **OR** > 1.1 times ULN
Cholesterol		>300 mg/dl **OR** 2 times pretreatment value
Creatinine		>4.0 **OR** 2 times pretreatment value
EKG		Clinically significant change in EKG as judged by the site investigator
Erythrocytes		<0.75 times LLN **OR**< 0.75 times pretreatment value
Erythrocyte Sedimentation Rate		>60 mm/hour
Gamma Glutamyl transpeptidase (GGT)		1.25 times ULN
Glucose		< 60 or > 140 for two consecutive readings
Hematocrit		male <0.37, female <0.32 **OR** < 0.75 pretreatment
Hemoglobin		>3 g/dl decrease from pretreatment value **OR** male <115 g/L, female < 95g/L
INR		> 1.5
Lactic Dehydrogenase		>3 times pretreatment value
Leukocyte count (WBC)		<2.8 times 10^9 **OR** >16 times 10^9/L
	Blasts	immature forms >0
	Basophils (%)	>3% if 0-1% pretreatment **OR** >3% times pretreatment value if pretreatment >1%
	Eosinophils	>2 times ULN pretreatment, and >6% if pretreatment normal; **OR** if >6% pretreatment, >3 ULN pretreatment >10%
	Lymphocytes	< 10 %

Mammogram		Any clinically significant change in mammogram as judged by the site investigator
Neutrophils and Bands		< 15 %
Ophthalmological examination		Presence of papilledema **OR** loss of visual acuity of 2 or more lines on the standard visual acuity card.
Partial thromboplastin time		Subject's baseline > 1.33 ULN
Phosphate		< 0.75 times LLN **OR** > 1.25 times ULN
Platelet count		< 75 times 10^9 **OR** > 700 times 10^9
Potassium		< 0.9 times LLN **OR** >1.1 times ULN
Prostate Specific Antigen (PSA)		>2 times increase in PSA level
Protein, Total		<0.9 times LLN **OR** >1.1 times ULN
Prothrombin time		Subject's baseline > 1.33 times pretreatment
Serum Iron		< 20 mg/Dl **OR** > 200 mg/dL
SGOT and SGPT (AST and ALTT)		> 3 times ULN **OR** if pretreatment > ULN, >4 times pretreatment value
Sodium		< 0.95 times LLN **OR** > 1.05 times ULN
Spinal Fluid	Protein	> 150 mg/dL
	WBC	> 10 cells/uL
Triglycerides		>450 mg/kl **OR**> 2 times pretreatment
Uric Acid		> 1.5 times ULN or then > 2 times pretreatment value if pretreatment > ULN
Urinalysis	**urinary RBC**	->5 /HPF **OR** >4 times pretreatment value if pretreatment value >5/HPF
	urinary WBC	->5 /HPF **OR** >4 times pretreatment value if pretreatment value >5/HPF
Urine pH		< 4 or > 9
Urine specific gravity		< 1.001 or > 1.050
Vital signs	Systolic blood pressure	< 80 or > 200 mm/hg
	Diastolic blood pressure	< 50 or > 115 mm/hg
	Pulse	< 50 or > 120 beats/minute
	Temperature	< 97 or > 100 degrees F < 35 or > 38.3 degrees C
	Respirations	< 10 or > 40 breaths/minute

The ALS Specific Quality of Life-Revised (ALSSQOL-R) User's Guide

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(Separate PDF uploaded into IRBe.)