TISCHMS-MSCNP-002
CLINICAL STUDY PROTOCOL

NCT03355365

CLINICAL PROTOCOL: Summary Information

TITLE	Autologous, Bone Marrow-Derived Mesenchymal Stem Cell-Derived Neural Progenitor Cells (MSC-NP), Expanded Ex Vivo; Administered Intrathecally
SPONSOR	Tisch MS Research Center of New York (Tisch MSRCNY) 521 West 57th St, 4th floor, New York, NY 10019 (212) 265-8070
PROTOCOL NUMBER	TISCHMS-MSCNP-002
VERSION NUMBER AND DATE	V8 08/26/2021
PHASE OF CLINICAL INVESTIGATION	Phase II
IND NUMBER	13889
PRINCIPAL INVESTIGATOR	Saud A. Sadiq, MD, FAAN Director and Chief Research Scientist Tisch MS Research Center of New York
NUMBER OF SITES	1
STUDY DESIGN	Phase 2, Double-Blind, Placebo-Controlled, randomized, Parallel Study of Intrathecal autologous MSC-NP Cells in Patients With MS
PRIMARY OBJECTIVE	To determine efficacy of multiple (6) intrathecal administrations of autologous MSC-NPs compared to placebo through assessment of disability outcomes
SECONDARY OBJECTIVES	To determine continued safety and tolerability of multiple intrathecal injections of MSC-NPs in patients with MS
NUMBER OF SUBJECTS	50
SUBJECT SELECTION CRITERIA	Inclusion Criteria (summary): SPMS or PPMS patients who have significant disability (EDSS between 3.0 and 6.5) that was not acquired within the last 12 months. Exclusion Criteria (summary): EDSS > 6.5, or anyone with co-morbidities (past or present) that would put them at a higher risk for safety complications.
INVESTIGATIONAL NEW DRUG / INTENDED USE	Bone Marrow-Derived Mesenchymal Stem Cell-Neural Progenitor Cells (MSC-NP) for use as treatment for multiple sclerosis
CONTROL GROUP OR OTHER STUDY ARMS (if applicable)	Placebo (LP procedure)
DURATION OF SUBJECT	Subjects will participate in the study for 3 years

PARTICIPATION AND DURATION OF STUDY	• Pretreatment screening and bone marrow aspiration: potentially up to 4 months • Treatment phase: 2 years, total 12 treatments (6 MSC-NP injections, 6 placebo lumbar punctures) every 2 months • Follow-up: 1 year
PRIMARY ENDPOINT	EDSS Plus (measured at baseline, month 6, month 13, month 20, month 27, and month 36)
SECONDARY ENDPOINTS	MSFC EDSS T25FW (timed 25 foot walk) 9HPT (9 hole peg test) 12-item MS Walking Scale urodynamic studies 6 Minute Walk Test Time to Progression (measured at month 13, month 27, and month 36) Progression on MRI (measured by percent change from baseline in total volume of T2 Lesions and percent change in total brain volume from baseline at month 13, month 27, and month 36)
SAFETY EVALUATIONS	Incidence of serious adverse events Incidence of minor adverse events (headache pain scale, fever) Brain, cervical, and thoracic MRI

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TMSRCNY Study Coordinators: Michaela Malin, BA
Morgan Roche, BA

Study Locations: Tisch MS Research Center of New York
(Treatment and Assessment site)

Clinical Protocol

1. Introduction:

1.1 Background:

Multiple sclerosis (MS) is an autoimmune-mediated chronic demyelinating disorder, and is the leading cause of disability in young adults after trauma. At present, there is no treatment that reverses the effects of established disease. Over the last 15 years, the outlook for newly diagnosed patients with MS has improved considerably because of therapeutic advances that ameliorate the aberrant immune responses seen in these patients. However, there are extremely limited treatments available for progressive MS, where progressive disease worsening and disability are associated with neurodegenerative mechanisms.

In attempting to develop a stem cell-based reparative therapy for MS we are investigating the use of autologous **MSC-derived neural progenitors (MSC-NPs)** administered in multiple intrathecal doses. The objective of this novel cellular therapy is to promote CNS repair and regeneration in patients with progressive MS, with the ultimate goal of reversal of disability.

1.2 Rationale:

MSC-NPs represent a neural subpopulation of MSCs from bone marrow with reduced pluripotency and minimized risk of ectopic differentiation, thus are likely to be more suitable for CNS delivery [1, 2]. Importantly, characterization of MSC-NPs demonstrated their immunoregulatory and trophic properties, and MSC-NPs derived from MS and non-MS patients alike were therapeutically viable [1]. In *in vitro* studies, the release of trophic growth factors, including hepatocyte growth factor (HGF), promoted the differentiation of myelin-forming oligodendrocytes, suggesting a possible mechanism of repair for MS. MSC-NPs also secrete abundant immunoregulatory cytokines, including TGF-beta and IL-6,that may suppress immune responses related to MS.

In preclinical studies, intrathecal (IT) MSC-NP treatment in EAE, the mouse model of MS, resulted in significantly improved neurological function [2]. Importantly, MSC-NPs were injected into mice with established chronic EAE [2], since progressive MS patients in need of regenerative therapies have long passed the phase of disease onset and are well into the neurodegenerative phase of the disease. These experiments were designed to mimic most closely the clinical trial design in humans, and included the following parameters: 1) Cells were injected intrathecally in mice rather than intravenously in order to best target damaged CNS tissue, 2) Cells were injected during the chronic phase of

EAE rather than the inflammatory phase, 3) Dose range from $2x10^4$ to $1x10^6$ cells was tested to inform dosing in human trials, and 4) Single vs. multiple dosing was tested to inform dosing regimen in human trials. We found that three injections of MSC-NPs spaced one week apart resulted in decreased cumulative EAE disease score in mice compared to single injections. Histopathological analysis on animals carried out 8 weeks after the third injection showed evidence of MSC-NP engraftment in the CNS. Neurological recovery was associated with increased spinal cord myelination, decreased immune infiltration in the CNS, and increased recruitment of endogenous progenitor cells. The multiple injection dosing regimen was well tolerated in mice.

In a pilot feasibility study, 6 MS patients with advanced disease were treated with 2-5 injections of escalating doses of autologous MSC-NPs administered intrathecally [3]. Patients were followed for an average of 7.4 years after initial injection. There were no safety concerns noted and no serious adverse events supporting both short-term and long-term safety of this experimental approach. Importantly, the data showed that multiple (up to 5) treatments were well tolerated. Incidentally, four of the six patients showed a measurable clinical improvement following MSC-NP treatment, providing further justification for phase I/II clinical trials.

Based on these pre-clinical and early clinical data supporting safety and efficacy, we are conducting an FDA-approved Phase I clinical trial to test safety and tolerability of 3 separate doses of up to 10 million MSC-NPs administered intrathecally with doses spaced 3 months apart [4]. Twenty subjects with progressive MS were enrolled. Inclusion criteria included those with established disability (EDSS 3.5 to 8.0) and stable disability (no progression 6-12 months prior to enrollment). All 20 patients received the 3 MSC-NP treatments spaced 3 months apart with 3 month follow-up assessments. The dosing regimen was found to be well tolerated, with only minor adverse events including transient headache (lasting only up to 48 hours post-treatment) and less commonly a transient fever (less than 100^oF; always over within 24 hours). There were no safety concerns associated with the treatment. Efficacy trends show that 15 of the 20 patients have improved in at least one of the following categories: ≥ 0.5 improvement in EDSS; at least 20% improvement in 25 feet timed walk, greater than 20% positive change in none hole PEG test, moderate to marked improvement in muscle strength, or symptomatic and/or urodynamic improvement in bladder function. These results are remarkable because such reversal of disability in long standing SPMS and PPMS has not previously been reported.

Based on our phase I trial results that establish safety and tolerability and suggest efficacy, we have designed a phase II study with the primary goal of establishing efficacy. We propose to use the intrathecal (IT) route of injection of administration. This is because the IT route in our phase I study has proved safe, tolerable and given positive results and it has not been established that these

cells given by other routes such as IV is effective. In our clinical studies to date, doses of up to 10 million MSC-NPs are well tolerated [3, 4], therefore we propose to continue the same dosage for this phase II study.

The dosing regimen of the Phase II trial will consist of 6 separate intrathecal doses, spaced 2 months apart. This extended dosing is designed to achieve optimal results based on the dosing experience of the phase I trial and the 6-patient pilot study. The outcomes of the 6-patient pilot study suggested that multiple dosing of up to 5 separate doses was safe and well-tolerated [3]. This pilot study informed dosing for the Phase I clinical trial, which consisted of 3 doses spaced 3 months apart. The three doses were extremely well tolerated. Notably, we observed transient improvements in some patients that were not sustained after the third dose, suggesting that the peak response had not yet been reached. We propose doubling the amount of treatments and increasing the frequency to every 2 months as a way of reaching a plateau of response in patients. The peak of response needs to be established in the Phase II study.

2. Clinical Study Objectives:

2.1 Primary objective:

- To determine efficacy of multiple (6) intrathecal administrations of autologous MSC-NPs compared to placebo through assessment of disability outcomes.

2.2 Secondary objectives:

- To determine continued safety and tolerability of multiple intrathecal injections of MSC-NPs in patients with MS.

3. Study Design:

The study is a Phase II, double-blind, placebo-controlled, randomized, parallel study design with a compassionate crossover element. The IT-MSC-NP treatments and all clinical assessments will take place at a single center (Tisch MSRCNY). Both the patient and the examining neurologist will be blinded to the treatment. The treating clinician (Dr. Sadiq, principal investigator) and the cell manufacturing coordinator (Dr, Harris, co-PI) will not be blinded. Study subjects will be assigned to blocks stratified by baseline EDSS score (3.0-4.0, 4.5-5.5, 6.0, and 6.5) and disease subtype (SPMS or PPMS). The total number of subjects in each block is shown below. Subjects in each block will be randomized into placebo or treatment group in order of date of consent based on randomization scheme designated by Dr Linda

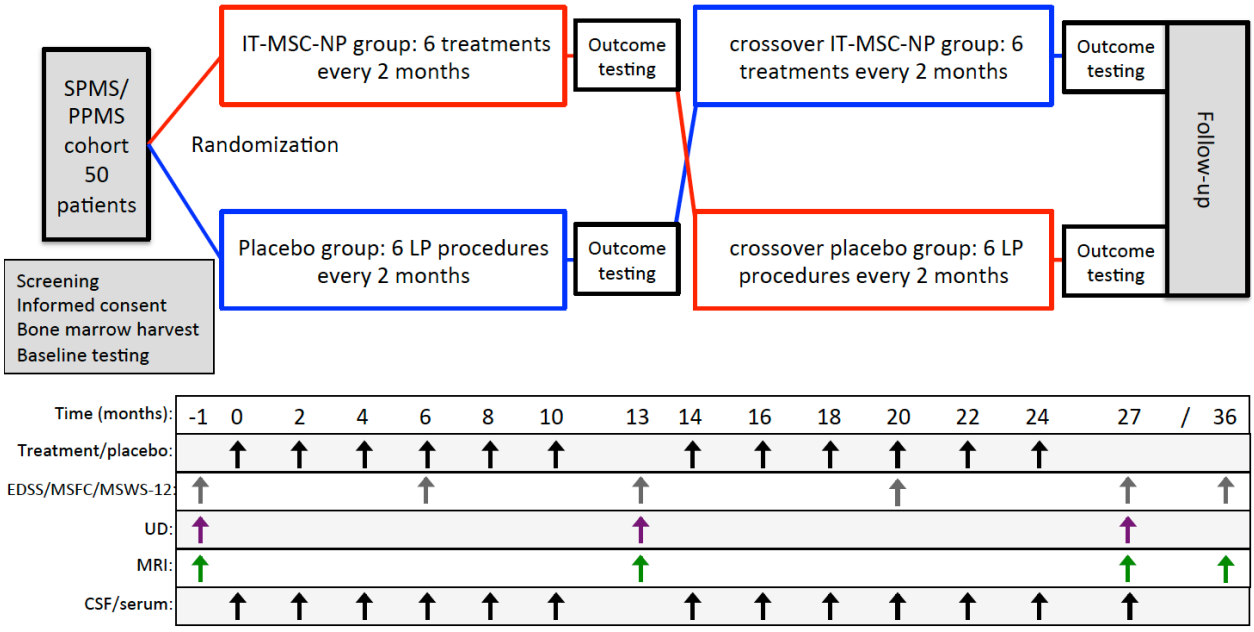
Gerber (Professor of Healthcare Policy & Research and Professor of Epidemiology in Medicine at Weill Cornell Medicine). In the second year, treated subjects will cross over to the placebo group and placebo subjects will cross over to the treated group. Study subjects are randomized in an equal fashion to study treatment and placebo at initial randomization.

Stratified block randomization scheme for Phase II trial:

EDSS	SPMS	PPMS
3.0-4.0	10	4
4.5-5.5	10	2
6.0	10	2
6.5	10	2
Total # of patients:	**40**	**10**

The total study duration will be 3 years upon enrollment. Each study subject will be required to attend up to 18 study visits, including 1 screening visit, 1 bone marrow visit, 1 baseline visit, followed by study visits every 2 months during the treatment period of two years (12 treatment/LP procedure visits and 2 outcome visits), and an additional follow-up visit at the end of year 3.

3.1 Study design schematic



3.2 Allocation to treatment

A total of 50 study subjects will be enrolled according to inclusion/exclusion criteria. Subjects will be randomly assigned to placebo or treatment group according to the stratified block randomization scheme described above in section 3. A master randomization assignment list will be generated and kept in a locked secure location accessible only by PI (Sadiq) and co-PI (Harris), who are the only two un-blinded investigators in the study. Dr. Harris will remain un-blinded in order to coordinate autologous cell manufacturing for subjects in the treatment group. The PI will be responsible for maintaining the blind.

3.3 Breaking the blind

In the event of any serious adverse effects that are deemed to be a direct result of the treatment, the examining neurologist or DSMB may request for the patient to be un-blinded.

4. Subject Selection:

Fifty subjects for this study will be selected based upon their clinical disease status. Only patients with primary progressive or secondary progressive MS who have significant disability (EDSS between 3.0 and 6.5) that was not acquired within the last 12 months will be eligible. Patients should have a relatively stable disease state (duration of MS $\leq$20 years at time of screening), which will allow better discernment between natural disease progression and treatment-related events. Disease stability will be determined by less than a 1.0-point change in EDSS in the last 12 months, and lack of gadolinium-enhancing lesions on an MRI and by a stable MRI disease burden (number and size of T2 lesions) in the last six months.

Enrollment criteria will also include specific clinical phenotypes which showed preliminary evidence of improvement during Phase I testing. Patients with symptomatic evidence of bladder dysfunction will be enrolled, and urodynamics testing will be conducted before and after treatment.

Exclusion criteria includes patients who have taken systemic chemotherapeutic or anti-mitotic agents within three months of the study start date due to possibility of interference with the bone marrow procedure and the potential negative impact on MSC-NP viability. Because of the experimental nature of this treatment, we are excluding patients who may be at greater risk for complications. Patients with central nervous system infections and blood diseases are at particular risk due to the route of administration of cells and the invasive nature of bone marrow aspiration. Patients who are anticipated to have difficultly accessing the intrathecal space related to scoliosis, obesity, or any other relevant factors determined by the PI. Participation in the trial will be terminated if a subject meets any of the exclusion criteria during the

course of the study. Detailed description of inclusion/exclusion criteria for this study will be provided in the clinical protocol.

Based on the close collaboration between Tisch MSRCNY and IMSMP, we do not anticipate difficulty in patient recruitment. Enrollment criteria include patients either within the geographical area or who are able to arrange reliable travel during the study period. To be enrolled, patients must consent to participate in all study visits, which include MRI. Enrollment into the study is defined as providing informed consent for study participation.

4.1 Subject inclusion criteria

To be eligible for enrollment in this study, a subject must meet the following inclusion criteria.

- Diagnosis of MS as defined by the McDonald criteria
- Diagnosis of primary progressive or secondary progressive MS
- Between the ages of 18-65 years
- Significant disability shown by an Expanded Disability Status Score (EDSS, [5]) ≥3.0 and ≤6.5 that was not acquired within the last 12 months
- Stable disease state as evidenced by a lack of gadolinium-enhancing lesions on an MRI and by a stable MRI disease burden (number of T2 lesions and size of lesions) in the last six months and no significant change in EDSS (1 point or more) in the last 12 months
- Must agree to undergo four MRIs: at the time of enrollment, after year 1, after year 2, and after year 3
- Patients either within the geographical area or who are able to arrange reliable travel during the study period

4.2 Subject exclusion criteria

Any of the following criteria exclude a subject from this study.

- EDSS >6.5
- Duration of disease >20 years at time of screening (duration of disease will be determined by onset of symptoms when symptom onset is clearly defined. In cases where symptom onset is difficult to determine, duration of disease will be based on date of diagnosis).
- Change of disease modifying agent <12 months prior to beginning treatment. Additionally, no changes in disease modifying agent will be made during the course of the study.
- Change in MS symptom management treatments, including Ampyra (dalfampridine) <6 months prior to beginning treatment. Additionally, no changes in MS symptom management treatments will be made during the course of the study, unless there has been clinical improvement, in which case, a patient may discontinue a medication

- Start of any new orthotic device or durable medical equipment <6 months prior to beginning treatment or during the course of the study (patients may discontinue use of these devices during the course of the study if they show clinical improvement).
- All patients who have ever been on Lemtrada (alemtuzumab)
- All patients who have had any prior stem cell treatments in the past five years
- All patients who have had any prior HSCT treatments
- Pregnant or nursing mothers or any woman intending to become pregnant in the next three years
- All patients will have screening blood tests done. Only patients whose values are in the normal range as determined by the laboratory norms based on age and sex will be allowed to participate. Exceptions may be made for borderline normal laboratory values manifesting no clinical symptoms at the discretion of the Principal Investigator.
- Use of systemic chemotherapeutic or anti-mitotic medications within three months of study start date due to the possibility of interference with bone marrow procedure
- Any patients with a history of or with active malignancy
- Use of steroids within three months of the study start date, as this would suggest an active disease state
- History of cirrhosis due to increased risk of CNS infection
- Significantly uncontrolled hypertension because of increased risk for stroke or CNS hemorrhage.
- Patients with active thyroid disease resulting in hyperthyroidism or hypothyroidism (Only well controlled patients with labs in the normal range will be included) because of hormone influence on cell growth
- History of central nervous system infection or immunodeficiency syndromes due to increased risk of CNS infection
- Preexisting blood disease (such as bone marrow hypoplasia, leukopenia, thrombocytopenia, or significant anemia) due to invasive nature of bone-marrow aspiration
- Previous or current history of a coagulation disorder
- Any metal in the body, which is contraindicated for MRI studies
- Allergy to any of the antibiotics used in this study, e.g.tobramycin, vancomycin, or gentamicin
- Allergy to, or have had adverse reactions to gadolinium or other contrast agents to be used in the study
- Patients with alcohol or other substance abuse problems that may affect stem cell growth; habitual drug (including marijuana and nicotine) abusers, will be excluded from the study
- Other major disease that, in the opinion of the Principal Investigator, would preclude participation in the study
- Patients with HBV, HCV, syphilis, HIV-1, or HIV-2.

- Any evidence of significant cognitive dysfunction based on a screening history and physical examination because it would preclude giving a truly informed consent
- Patients who are enrolled in another clinical trial for MS treatment or who have received any study drug/biologics within the last 6 months. Additionally, while in the trial, patients may not enroll in any other clinical trial for MS or any other condition.
- Patients who are anticipated to have difficultly accessing the intrathecal space related to scoliosis, obesity, or any other relevant factors determined by the PI.

5. Study Drug(s):

Drug: Clinical-grade autologous bone marrow mesenchymal stem cell-derived neural progenitors (MSC-NPs) are manufactured according to Chemistry, Manufacturing and Controls (CMC) section of IND 13889 (approved by FDA in August 2013). Briefly, mesenchymal stem cells (MSCs) are isolated from the bone marrow mononucleated cell fraction based on plastic adherence, and expanded in media containing 5% human platelet lysate (PLT). MSCs were cryopreserved after 2 and 3 passages, generating a stock of cells sufficient for at least 10 subsequent expansions. For each treatment, a vial of MSCs is thawed, expanded for 2 to 3 more passages, then cultured for 2-3 weeks in neural progenitor maintenance medium containing growth factors EGF and bFGF to generate neural progenitors (MSC-NPs). Just prior to the injection, MSC-NPs are collected, washed, counted, and resuspended in saline for immediate intrathecal administration. All manufacturing procedures were conducted in a cGMP clean-room facility by specifically trained personnel.

Product Release testing: Quality testing is conducted at multiple steps during MSC-NP manufacturing. Bone marrow-derived MSCs are tested for MSC characteristics including growth, morphology, cell surface expression ($CD105^+/CD73^+/CD90^+/CD45^-/CD34^-/CD14^-/CD20^-/HLA\text{-}DR^-$), osteogenic and adipogenic differentiation potential, and sterility. During each expansion of cells for dosing, cells were tested for chromosomal stability, neurosphere morphology of MSC-NPs, cell number, cell viability, and sterility (fungal and bacterial/mycoplasma). In addition, identity testing of MSC-NPs is performed to confirm gene upregulation of CXCR4, Nestin, TLR2, and HGF and downregulation of SMA (smooth muscle isoform of alpha actin) and Thy1 (CD90) in MSC-NPs compared to the MSCs from which they were derived.

Dose: Between 5 to 10 million MSC-NPs will be administered intrathecally in each dose. 10 million cells is the maximum dose. Treatment will consist of 6 doses spaced 2 months apart.

5.1 Study drug compliance/adherence

All lumbar punctures will be performed by the treating clinician (the study PI, Dr. Sadiq) on an outpatient basis at Tisch MSRCNY. These appointments will be confirmed months in advance to ensure compliance. Whether the subject is receiving intrathecal injections of autologous MSC-NPs or getting placebo (only undergoing a lumbar puncture), each procedure will take approximately 20 minutes. Prior to all lumbar punctures, a trained infusion nurse will infuse patients with 1 gram of vancomycin and 80 mg of tobramycin to prevent the occurrence of meningitis. This infusion takes approximately four hours. Vancomycin is the drug of choice for S. epidermidis, a potential contaminant from the skin during the lumbar puncture procedure. It is also the drug used to treat penicillin-resistant S. pneumoniae. Vancomycin is also indicated in empiric use in meningitis until an organism has been identified. Tobramycin is synergistic with vancomycin and is also the treatment of choice for pseudomonas infections. Vancomycin and tobramycin are indicated for empiric treatment of neurosurgery-related meningitis at most institutions. Vital signs will be performed on both groups to monitor safety. These visits will last approximately 5 hours. Study subjects will be instructed to stay in bed and limit any physical activity for the 24 hours following each procedure. Patients will be asked to take Tylenol every 6 hours for two days after each treatment. Based on the data from our Phase I trial it is anticipated that patients may show signs of headache when given stem cells. Hence providing patients with Tylenol will help maximize patient blinding.

5.1.1 Withdrawal of subjects due to non-compliance/adherence

Subjects have the right to voluntarily withdraw from the study at any time for any reason. If a subject becomes non-compliant, defined as failure to complete study treatment visits, they will be discontinued from the trial and a new subject from the same EDSS block would receive the non-compliant subject's treatment allocation. Additionally, a subject could become non-compliant if a urine screen tests positive suggesting abuse of drugs, including nicotine. If this were to happen, they will be discontinued from the trial and a new subject would receive the non-compliant subject's treatment allocation.

5.2 Study drug supplies

5.2.1 Formulation and packaging

Treatment group: Freshly harvested autologous MSC-NPs will be resuspended in approximately 1.0 ml preservative-free saline and transferred to a sterile 1.5ml tube, which is capped and placed inside a sterile, pre-labeled transport

tube (50ml conical tube). The cell suspension has the appearance of a white cloudy suspension. The study subject will not see the drug prior to administration. The transport tube will be labeled as follows:

ID:_____ Date:_____ Sample: MSCNP cells in saline Cell number:_____ Viability:_____ Expiration: for use on the above date only FOR AUTOLOGOUS USE ONLY CAUTION: New Drug - Limited by Federal (or United States) law to investigational use

Placebo group: Approximately 1.0ml of saline will be placed in 1.5ml tube, which will be placed inside a pre-labeled transport tube. The study subject will not see the tube. The contents of the tube will not be administered. Placebo will be labeled with the following label:

ID:_____ Date:_____ Sample: saline Cell number: n/a Viability: n/a Expiration: for use on the above date only NOT FOR ADMINISTRATION CAUTION: New Drug - Limited by Federal (or United States) law to investigational use

5.2.2 Drug administration

Treatment group: Intrathecal injections of autologous MSC-NPs will be performed by the treating clinician (the study PI, Dr. Sadiq), according to the following standard procedure. However, if necessary, a board-certified neurologist (clinical fellow) at the IMSMP will perform the LP procedure. Any personnel involved in product/placebo administration will not be performing subject assesments, and will not be involved in the study otherwise. The patient is either seated and bent over or positioned lying down with their knees to their chest. A nurse will assist to maintain this position. The lumbar region is cleaned with betadine and a sterile drape is applied. As a local anesthetic, 1 cc of lidocaine (1%) is administered subcutaneously at the L3-L4 interspace. A sterilized lumbar puncture adult tray (CareFusion/ Henry Schien, Cat. number 4301C) will be used. A standard LP kit has a 20 gauge needle; however, in this study a 24 gauge, non-traumatic BD Whitacre pencil point Spinal needle (Ref number 405133) will be used to lower the incidence of headaches associated with spinal taps. Dr. Sadiq will aspirate 10 mL of CSF at the L3-L4 level using a sterile 24 gauge needle to confirm accurate access to the intrathecal space. CSF will be transferred to a 15ml specimen tube

and transported to the laboratory for processing. If access is limited at the L3-L4 level, the L2-L3 level will be used as an alternative site. The cells will be removed from the 1.5 ml transport vial using a 22 gauge needle 10cc syringe, re-suspended in 3 mL of preservative-free sterile saline and gently mixed by passage through a 22 gauge needle several times to break up any remaining cell clusters to ensure a uniform suspension. The cell/saline suspension will be injected intrathecally and chased by up to 7 mL of preservative-free sterile saline to ensure complete deposition of stem cells into the intrathecal space and to prevent material from concentrating at the site of injection. This procedure will take approximately 20 minutes. The entirety of the procedure will take place posterior to the study subject, thus ensuring they remain blinded to the treatment.

Patients who have an intrathecal Medtronic pump installed for spasticity and pain management will be given the stem cells through the side port of the pump. The patient will be lying flat for the procedure with their pump exposed. The abdominal region where the pump is located is cleaned with betadine and a sterile drape is applied. A sterilized Medtronic Catheter Access Port Kit (Medtronic Inc product number 8540) will be used. The side port will be accessed with a 25-gauge non-coring needle. Dr. Sadiq will aspirate 1.5 mL of fluid from the side port to clear the catheter of any therapeutic drug in pathway that will be disposed of. CSF will be transferred to a 15ml specimen tube and transported to the laboratory for processing. The cells will be removed from the 1.5 ml transport vial using a 22-gauge needle 10cc syringe, re-suspended in 3 mL of preservative-free sterile saline and gently mixed by passage through a 22 gauge needle several times to break up any remaining cell clusters to ensure a uniform suspension. After the cells are injected through the side port, extra saline will be flushed through to make sure that the stem cells have been cleared out of the side port catheter. Following the procedure, a prime bolus will be administered via Medtronic programmer to ensure continuous supply of the patient's medicine. For the entirety of the procedure, the subject will be blindfolded, thus ensuring they remain blinded to the treatment.

Placebo group: For the six placebo treatment visits, subjects will undergo standard lumbar punctures as described above but with no cell injections. Ten mL of CSF will be collected for biomarker analysis at each lumbar puncture. The treating physician (Dr. Sadiq) will perform a mock procedure to imitate cell resuspension and delivery. Three ml of preservative-free sterile saline will be injected, and this will be given with up to 7 mL flush of preservative-free sterile saline. The entire procedure will take approximately 20 minutes. All subjects in the placebo group will get treated with antibiotics as well in the same regimen indicated above for the treatment group. The entirety of the procedure will take place posterior to study subject, thus ensuring they remain blinded to the treatment.

Patients who have an intrathecal Medtronic pump installed for spasticity and pain management will be given the placebo through the side port of the pump as described above.

5.4 Study drug storage and accountability

The study drug (autologous MSC-NPs) is a biologic and each batch of drug is prepared according to each study subject's treatment schedule. Each study subject is given a unique alphanumeric ID that is used to label all cells derived from that patient. Each ID is recorded in a study subject ID assignment log that documents the patient's name and information. The log will be kept in a secure data base accessible only by un-blinded investigators.

Cryopreserved cells (MSCs) can be stored indefinitely with no expiration date. Any unused, cryopreserved cells will be donated for research purposes after the trial is completed, as detailed in the informed consent form. Expanded cells (MSC-NPs) are harvested just prior to the injection and injected within an hour after preparation. There is no storage period and no shipping. A sample of the final product MSCNP cells is preserved for quality testing purposes. These cells are lysed for RNA extraction. All commercial reagents will be used within manufacturers' expiration dates.

5.5 Concomitant Medications

Patients will be allowed to continue with their regular course of therapy excluding any systemic chemotherapeutic and anti-mitotic agents. Patients who are on such medications will not be enrolled in this study. Patients are excluded from the study if there is a change of disease modifying agent <12 months prior to beginning treatment, or if there is a change in MS symptom management treatment, including Ampyra (dalfampridine) <6 months prior to beginning treatment. Additionally, no changes to disease modifying agent or MS symptom management treatment will be made during the course of the study. However, if there is clinical improvement, a patient may discontinue their MS symptom management drug. Patients will be asked to take Tylenol every 6 hours for two days after each treatment. Based on the data from our Phase I trial it is anticipated that patients may show signs of headache when given stem cells. Hence providing Tylenol will help maximize patient blinding.

5.6 Specimen Collection

CSF and blood specimens will be collected from all patients for exploratory biomarker discovery and validation. Approximately 10 mL of CSF will be collected at each of the 12 lumbar puncture procedures (treatment and placebo visits), just prior to intrathecal injections of the same volume of cells or saline. One additional CSF sample will be withdrawn at the month 27 follow up visit. Blood samples will also be collected at the same time as CSF collection. One approximately 6 mL blood sample will be collected in a tube with EDTA for

plasma isolation, and another approximately 6 mL blood sample will be collected in a tube without EDTA for serum isolation. All specimens will be used for research purposes to identify and/or verify biomarkers that correlate with disease progression or clinical response to IT-MSC-NP therapy. Candidate protein biomarkers, including neurofilament light, will be analyzed by single analyte ELISA or multi analyte Luminex.

5.7 MRI Protocol

Brain, cervical and thoracic spine MRI scans will be scheduled at baseline, and at months 13, 27, and 36 using 3Tesla Siemens MAGNETOM VERIO scanners. Assessments will include the following:

- Proton density and T2-weighted two-dimensional (2D) multislice turbo/fast spin-echo
- T1-weigted three-dimensional (3D) spoiled gradient recalled echo both pre-contrast and after a 10 minute delay following injection of gadolinium post-contrast sequencing
- 2D T2 weighted Fluid -Attenuated Inversion Recovery (FLAIR)
- MTR MR tractography dependent on the number of tracts followed
- DTI – A T2 SPC 3D Flair sagittal sequence, EP diffusion, and a gradient filed mapping

Using the CorTechs NeuroQuant software package a multi-structure atrophy report will be generated for all brain MRI scans as well as a Lesion Quant Flair assessment of total number and volume of lesions; the total number and volume of enlarging lesions and the total number and volume of new lesions.
All lesions to be counted would have to be at least 3 voxels in size. For lesions to be considered enhancing an increase in intensity by at least 20% on the post-contrast images compared to the baseline T1 sequence lesions.
The total number of new or enlarging T2 hyperintense lesions as detected by MRI will be assessed by a negative binomial model. Percentage change in total brain volume from baseline will be assessed using an MMRM analysis.

6. Research Study Procedures:

Please see Schedule of activities (Study Table) Section 6.4 for the schedule of assessments performed during the study.

6.1 Screening procedures (Month 4; within 4 months prior to first treatment):

- Candidates believed to meet the inclusion/exclusion criteria will first attend a pre-enrollment visit where the details of the informed consent form (ICF) will be explained in depth.

- Subjects will be told that their participation is voluntary and that their regular MS treatment will continue whether or not they enter the study. Consent for participation in the study will be obtained by any of the examining neurologists, the clinical trials manager, or by one of the study nurses, and documented in the subject's study file during the initial screening visit. An explanation and justification for the study will be given to the participant at this time. Consenting will occur after having had ample time to read the ICF, speak to family and friends, and review the consent with and ask questions of the consenting neurologist or study nurse. Patients will be given a copy for their records.
- Subjects will then undergo the following examinations to confirm eligibility for study participation
    - Complete physical examination with vital signs
    - Complete neurological examination
    - EDSS
    - Infectious Disease Questionnaire
    - Pregnancy test (for female patients of child-bearing age)
    - Infectious disease testing including HIV Test
    - Urine tests for drug/nicotine abuse (In addition to the screening period, a drug/nicotine urine test could be done at any time during the study for random testing)

- The EDSS evaluations will be done by the examining neurologists at Tisch MSRCNY. Every attempt will be made to have the same examining neurologist for each patient for the study duration. Examining neurologists will perform objective physical and neurological examinations during the study to evaluate efficacy, and will be blinded to the treatment
- All patients will be required to go to a clinical laboratory facility to undergo a panel of infectious disease screening. The results will be sent to the screening neurologist, and any patient positive for any infectious diseases will be excluded from the study.
- Screening Blood Draw
- The initial screening will last 1 hour.

6.2 Study drug procedures

Bone Marrow Visit (Month -3):

- This visit will occur approximately one month after enrollment and approximately 3 months prior to the first treatment.
- Vital signs will be documented
- A bone marrow aspiration will be performed by Dr. Gabriel Sara, an experienced, board certified hematologist affiliated with the Tisch MSRCNY. Dr. Sara will obtain a bone marrow aspirate from the sternum or the iliac crest under sterile conditions following standard procedure. An adult bone marrow kit with a heparinized syringe will be used.

- Bone marrow aspirate will be obtained from the manubrium of the sternum, at the level of the second intercostal space. Alternatively, aspirate may be obtained from the iliac crest. Bone marrow will be aspirated using an adult bone marrow kit with a heparinized syringe.
- Each research subject's sterile bone marrow sample will be placed in a pre-labeled bag and immediately transferred from the clinic to the stem cell culture laboratory. Patients will be told not to shower or get their bandage wet for 24 hours following the procedure. In order for patients to move to the next phase of the study, their cells must be viable and pass bone marrow release testing. If for some reason cells do not thrive, the patient's involvement in the study will be reevaluated.
- Total duration of the Bone Marrow Visit is approximately 2 hours and will be conducted at the Tisch MSRCNY due to necessary proximity to the cell manufacturing laboratory.
- Cells from subjects who have had bone marrow harvested under the long-term ongoing research protocol, "Harvesting Bone Marrow for Neural Stem Cells" (Former St. Luke's-Roosevelt IRB# 08-049) (Current WIRB# 20162012), can be used for implantation as long they are deemed acceptable for use (and eliminate any risk from an unnecessary marrow harvesting procedure). This includes bone marrow harvested under this long-term ongoing research protocol harvested prior to enrollment in this study.

Baseline Visit (Month-1; within one month prior to first treatment):

- The purpose of the baseline visit is to establish baseline values of outcome assessments prior to treatment.
- The following baseline visit exams will be conducted by study subject's examining neurologist and study coordinator (duration approximate 1 hour)
- Vital signs-Only if not performed at screening
- Physical exam-Only if not performed at screening
- Neurological exam-Only if not performed at screening
- Headache pain scale
- EDSS-Only if not performed at screening
- MSFC
- 6 Minute Walk Test
- 12 Item MS Walking Scale
- Bladder Survey
- Urinalysis and Culture to screen for UTI
- The following exams will be scheduled to occur within one month prior to the first treatment
- MRI (brain, C and T spine) ± Gadolinium enhancement
- Urodynamic testing of bladder compliance (ability of bladder to fill without accompanying rise in bladder pressure)

Treatment Phase visits, year 1 (Month 0, 2, 4, 6, 8, and 10):

- Treatment/placebo visits will be conducted at the Tisch MSRCNY every 2 months (± 1 week)
- Details regarding treatment/placebo procedures detailed in section 5.2.2 above
- Treatment visits will be conducted by the treating neurologist, Dr. Sadiq
- Duration of treatment visits will last approximately 5 hours
- Study subjects will be instructed to stay in bed and limit any physical activity for the 24 hours following each procedure.
- CSF for biomarker analysis discussed in 5.2.2
- Blood draw for serum and plasma biomarker analysis
- Vital Signs
- EDSS and MSFC (Month 6 Only) Follow-up by phone will occur 48 hours, 1 week and 1 month after each treatment visit. The purpose of the follow-up phone call is safety assessment and to document any adverse events. Calls will be made either by the clinical trial manager, the clinical research assistants, or the adverse event physician. Assessment will include the following:
- Headache pain scale

Outcome assessments I (Month 13)

- The purpose of outcome assessment I visit is to evaluate effects of the treatment compared to the baseline visit, as well as to serve as a baseline visit for the crossover treatment phase.
- The following exams will be conducted 3 months following the 6th treatment phase visit (± 1 week)
    - Vital signs
    - Physical exam
    - Neurological exam
    - Headache pain scale
    - EDSS
    - MSFC
    - 6 Minute Walk Test
    - 12 Item MS Walking Scale
    - Bladder Survey
    - Urinalysis and Culture to screen for UTI

- The following exams will be scheduled to occur 3 months following the 6th treatment visit (± 1 week)
    - MRI (brain, C and T spine) ± Gadolinium enhancement
    - Urodynamic testing of bladder compliance (ability of bladder to fill without accompanying rise in bladder pressure)

Treatment Phase visits, year 2 (Month 14, 16, 18, 20, 22, and 24):

- Crossover treatment/placebo visits will be conducted at the Tisch MSRCNY every 2 months (± 1 week)
- Details regarding treatment/placebo procedures detailed in section 5.2.2 above
- Treatment visits will be conducted by the treating neurologist, Dr. Sadiq
- Duration of treatment visits will last approximately 5 hours
- Study subjects will be instructed to stay in bed and limit any physical activity for the 24 hours following each procedure.
- CSF for biomarker analysis discussed in 5.2.2
- Blood draw for serum and plasma biomarker analysis
- Vital Signs
- EDSS and MSFC (Month 20 Only). Follow-up by phone will occur 48 hours, 1 week, and 1 month after each treatment visit. The purpose of the follow-up phone call is safety assessment and to document any adverse events. Calls will be made either by the clinical trial manager, the clinical research assistants, or the adverse event physician. Assessment will include the following:
- Headache pain scale

Outcome assessments II (Month 27):

- The purpose of outcome assessment II visit is to evaluate effects of the treatment group in year 2 compared to the baseline visit at the beginning of year 2. In addition, these assessments will be used to evaluate any sustained effects of the treatment in year 1.
- The following exams will be conducted 3 months following the 12th treatment visit (± 1 week) by the study subject's examining neurologist and study coordinator
- Vital signs
- Physical exam
- Neurological exam
- Headache pain scale
- EDSS
- MSFC
- 6 Minute Walk Test
- 12 Item MS Walking Scale
- Bladder Survey
- Urinalysis and Culture to screen for UTI
- Lumbar Puncture with CSF Aspiration for biomarker analysis
- Blood draw for serum and plasma biomarker analysis
- The following exams will be scheduled to occur 3 months following the 12th treatment visit (± 1 week)
- MRI (brain, C and T spine) ± Gadolinium enhancement
- Urodynamic testing of bladder compliance (ability of bladder to fill without accompanying rise in bladder pressure)

6.3 Follow-up procedures

- Treatment phase follow-up: Follow-up by phone will occur 48 hours,1 week and 1 month after each treatment visit. A total of 24 follow-up calls will be made over the duration of the study. The purpose of the follow-up phone call is safety assessment and to document any adverse events. Calls will be made either by the clinical trial manager, the clinical research assistants, or the adverse event physician. Assessment will include the following:
    - Headache pain scale

- Long-term follow-up (Month 36, ± 3 weeks): The purpose of the follow-up is to assess long-term safety and efficacy at the end of the study. The following exams will be conducted by the study subject's examining neurologist and study coordinator: (duration approximate 1 hour)
- Vital signs
- Physical exam
- Neurological exam
- Headache pain scale
- EDSS
- MSFC
- 6 Minute Walk Test
- 12 Item MS Walking Scale
- MRI (brain, C and T spine) ± Gadolinium enhancement will be scheduled to occur at end of study

6.4 Schedule of activities (Study Table)

	Pre-treatment Phase			Treatment Phase, year 1[2]		Safety FU	Outcome assess-ments I[2]	Treatment Phase, year 2[2]		Safety FU	Outcome assess-ments II[2]	Final FU[3]
Tests and Assessments	Screening Visit Month -4 (Within 4 months prior to treatment phase)	Bone marrow visit Month -3	Baseline visit Month -1	Treatment group Months 0, 2, 4, 6, 8, 10	Placebo group Months 0, 2, 4, 6, 8, 10	FU by phone 48 hrs and 1 week after each treatment or placebo	All groups Month 13	Treatment group (crossover from placebo) Months 14, 16, 18, 20, 22, 24	Placebo group (crossover from treatment) Months 14, 16, 18, 20, 22, 24	Follow-up by phone 48 hrs and 1 week after each treatment or placebo	All groups Month 27	End of study follow-up Month 36
ICF	X											
Serum and Plasma Biomarker Analysis Blood draw				X	X			X	X		X	
Screening Blood Draw[1]	X											
Bone marrow aspiration		X										
LP Procedure				X	X			X	X		X	
CSF aspiration for biomarker analysis				X	X			X	X		X	
Vital signs	X	X	X[4]	X	X		X	X	X		X	X
Physical exam	X		X[4]				X				X	X
Neurol. exam	X		X[4]				X				X	X
Headache pain scale			X			X (12 total)	X			X (12 total)	X	X
EDSS	X		X[4]	X[6]	X[6]		X	X[7]	X[7]		X	X
MSFC (T25FW, 9HPT, PASAT)			X	X[6]	X[6]		X	X[7]	X[7]		X	X
6 Minute Walk Test (6MW)			X				X				X	X
UD			X				X				X	
Brain, Cervical, and Thoracic MRI ±Gd			X				X				X	X
Twelve Item MS Walking Scale			X				X				X	X
Infectious disease test	X											
Pregnancy test (females)	X											
Drug & Nicotine Urine Testing	X[5]											
Urinalysis & Culture			X				X				X	
Bladder Survey			X				X				X	
Infectious Disease Questionnaire	X											

[1]Screening Blood Draw will include CBC with differential, ESR, Comprehensive Metabolic Panel, PT/PTT, and Thyroid Function Tests
[2]+/- 1 Week Window allowed for all Treatment Phase Visits and Outcome Assessments
[3]+/- 3 Week Window for all Final Follow-Up
Abbreviations: ICF, informed consent form; FU, follow-up; LP, lumbar puncture; UD, urodynamic testing
[4]Only required if not done during screening visit
[5]In addition to the screening period, a drug & nicotine urine testing could be done for random testing at any time during the study
[6]Only in month 6
[7]Only in month 20

7. Safety and Effectiveness Assessments:

7.2 Safety assessments

Research subjects will be routinely assessed for safety at all study visits and during follow-up telephone calls. Subjects will also be consistently asked to complete the headache pain scale. Safety assessments include monitoring for adverse events, vital signs, physical and neurological examination, and MRIs.

Information regarding a history of a previous positive COVID-19 test or inoculation with a COVID-19 vaccine will be collected.

7.2 Effectiveness assessments

Research subjects will be evaluated for effectiveness by EDSS, urodynamic testing, MSFC, 12 item MS walking scale, time to disability progression, progression on MRI (total T2 lesion volume and total brain volume), and 6-minute walk test.

8. Adverse Event Reporting:

8.1 Adverse event definitions

Adverse event means any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related.

Adverse reaction means any adverse event caused by a drug.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction."

- *Reasonable possibility.* For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.

Life-threatening, suspected adverse reaction. A suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator) or Sponsor, its occurrence places the patient or

research subject at immediate risk of death. It does not include a suspected adverse reaction that had it occurred in a more severe form, might have caused death.

Serious, suspected adverse reaction. A suspected adverse reaction is considered "serious" if, in the view of the Investigator (i.e., the study site principal investigator) or Sponsor, it results in any of the following outcomes: death, a life-threatening adverse reaction, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.

- Important drug-related medical events that may not result in death, be life-threatening, or require hospitalization may be considered "serious" when, based upon appropriate medical judgment, they may jeopardize the research subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

  Unexpected, suspected adverse reaction. A suspected adverse reaction is considered "unexpected" if it is not listed in the general investigational plan, clinical protocol, or elsewhere in the current IND application; or is not listed at the specificity or severity that has been previously observed and/or specified.

8.2 Recording/Reporting requirements

8.2.1 Eliciting adverse event information

Research subjects will be routinely questioned about adverse events at all study visits and during follow-up telephone calls. Subjects will also be routinely asked to complete the headache pain scale.

8.2.2 Recording requirements

All observed or volunteered adverse events (serious or non-serious) and abnormal test findings, regardless of study group or suspected causal relationship to the study drug(s) will be recorded in the subjects' case histories. For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a *serious adverse event*) and; 2) an assessment of the casual relationship between the adverse event and the study drug(s).

Adverse events or abnormal test findings felt to be associated with the study drug(s) will be followed until the event (or its sequelae) or the abnormal test

finding resolves or stabilizes at a level acceptable to the safety assessing neurologist (Dr. Williams or Dr. Stark). Whichever neurologist is seeing the subject for assessments will not be the neurologist assessing safety for that subject. Each subject will have a separate examining neurologist and safety assessing neurologist. Monitoring and adjudication of all adverse events (serious or non-serious) will be performed in a sequential reporting manner. All patients will be provided a 24-hour hotline for reporting of any adverse effects. Initial screening will be performed by the clinical nursing team and all adverse events will be reported to the corresponding safety assessing neurologist for further evaluation. The treating and examining neurologists will not be involved in the notification of any adverse effects. Adjudication of all adverse events (serious or non-serious will be determined by one of the safety assessments neurologist (sub-investigators Dr. Williams or Dr. Stark). Additionally, all serious adverse events will be reported to the DSMB immediately (24 to 48 hours) and they will adjudicate the adverse event and recommend unblinding, if necessary. Any non-serious adverse events will be reported to the DSMB on a half-yearly basis.

8.2.2.1 Abnormal test findings

An abnormal test finding will be classified as an *adverse event* if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy
    - Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.
- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study
- The test finding is considered an adverse event by the Sponsor-Investigator of the IND application

8.2.2.2 Causality and severity assessment

One of the safety assessments neurologist (sub-Investigators Dr. Williams or Dr. Stark) of the trial will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s); and 3) if the adverse event meets the criteria for a *serious adverse event*.

If the final determination of causality is “unknown and of questionable relationship to the study drug(s)”, the adverse event will be classified as *associated with the use of the study drug(s)* for reporting purposes. If the final determination of causality is “unknown but not related to the study drug(s)”, this determination and the rationale for the determination will be documented in the respective subject’s case history.

8.3 Reporting of adverse reactions

8.3.1 Reporting of adverse reactions to the FDA

8.3.1.1 Written IND Safety Reports

The Sponsor-Investigator will submit a written IND Safety Report (i.e., completed FDA Form 3500 A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be a *serious and unexpected, suspected adverse reaction*. Each IND Safety Report will be prominently labeled, “IND Safety Report”, and a copy will be provided to all participating investigators (if applicable) and sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the Sponsor-Investigator’s receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

For each written IND Safety Report, the Sponsor-Investigator will identify all previously submitted IND Safety Reports that addressed a similar suspected adverse reaction experience and will provide an analysis of the significance of newly reported, suspected adverse reaction in light of the previous, similar report(s) or any other relevant information.

Relevant follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the information is available and will be identified as such (i.e., “Follow-up IND Safety Report”).

If the results of the Sponsor-Investigator’s follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Sponsor-Investigator will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

8.3.1.2 Telephoned IND Safety Reports – Fatal or life-threatening suspected adverse reactions

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Sponsor-Investigator will notify the responsible review division of the FDA by telephone or facsimile transmission of any *unexpected, fatal or life-threatening suspected adverse reaction.*

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

8.3.2 Reporting adverse events to the responsible IRB

In accordance with applicable policies of WIRB (Western Institutional Review Board), the Sponsor-Investigator will report, to the IRB, any observed or volunteered adverse event that is determined to be 1) *associated with the investigational drug or study treatment(s)*; 2) *serious*; and 3) *unexpected*. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the sponsor-investigator's receipt of the respective information. Adverse events which are 1) *associated with the investigational drug or study treatment(s)*; 2) *fatal or life-threatening*; and 3) *unexpected* will be reported to the IRB within 24 hours of the Sponsor-Investigator's receipt of the respective information.

Follow-up information to a reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the Sponsor-Investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the Sponsor-Investigator will report the adverse event to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

8.4 Withdrawal of subjects due to adverse events

If any of the following events occur during treatment, patients will be discontinued from the study:

- Acute worsening of MS symptoms
- Any neurological deficit that is unexplained by another condition and is non-reversible within 24 hours of its development
- Any sign of nervous system infection
- Any changes in cognitive function
- Any unexpected adverse medical event that is unexplained by another medical condition
- Any time the Data Safety Monitoring Board feels a patient should be withdrawn from the study
- Patient meets an exclusion criterion
- Pregnancy

Should a patient meet any of the above criteria for early termination, he or she will be discontinued from the trial and will be replaced. Subjects that are discontinued from the study will continue to undergo the follow-up visits relative to their last treatment. They will also undergo long-term follow-up safety visits as described. All data gathered at these appointments will be included in the CRF and data set. Should a study subject terminate the study due to pregnancy, no MRI will be performed during the course of the pregnancy.

9. Statistical Methods/Data Analysis:

Detailed descriptions relating to data handing and statistical analysis is presented in the Statistical Analysis Plan (SAP).

9.1 Study endpoints

9.1.1 Primary endpoint(s)

Primary efficacy outcome will be a composite score based on EDSS (our EDSS assessments will be based only on pyramidal and brainstem FSS, and performed by a blinded examiner), T25FW, and 9HPT (EDSS-Plus) [6]. Improvement will be defined by at least one of the following three measures: ≥0.5 improvement in EDSS (if EDSS at entry is ≥ 6.0) or ≥ 1.0 improvement in EDSS (if EDSS at entry is ≤5.5), ≥20% improvement in T25FW, or ≥20% improvement in 9HPT in either dominant or non-dominant upper limb. Scores will be compared within patients between the baseline and various post-treatment visits. Scores will also be compared between different treatment groups at various post-treatment visits. Assessments will be made at Baseline (within 4 months of first treatment/placebo), Month 6 (just prior to 4th

treatment/placebo), Month 13 (3 months post 6th treatment/placebo), Month 20 (just prior to 10th treatment/placebo), and Month 27 (3 months post 12th treatment/placebo). Long-term follow-up assessment will be conducted at Month 36. The primary outcome will be the comparison of the composite scores at Month 13 (3 months after the 6th treatment/placebo) compared to baseline.

9.1.2 Secondary endpoints

- EDSS (Improvement defined by ≥0.5 improvement in EDSS if EDSS at entry is ≥ 6.0, or ≥ 1.0 improvement in EDSS if EDSS at entry is ≤5.5)
- T25FW (≥20% improvement)
- 9HPT (≥20% improvement)
- MSFC
- 12-item MS Walking Scale
- Urodynamic Studies
- Progression on MRI (measured by percent change from baseline in total volume of T2 Lesions and percent change from baseline in total brain volume at month 13, month 27, and month 36)
- Time to Progression (measured at month 13, month 27, and month 36)
- Six Minute Walk Test

9.2 Sample size determination

Power analysis was conducted by Dr. Linda Gerber, Professor of Healthcare Policy & Research and Professor of Epidemiology in Medicine at Weill Cornell Medicine, who will provide statistical consultations throughout the study. Power analysis was based on 20 participants in each group of the Phase II study. Group sample size of 20 in the treatment group and 20 in the placebo group achieve more than 80% power to detect a difference between the group proportions of 35%, using a two-sided Z-test with pooled variance and using a significance level of 0.05. This calculation assumes that 40% of patients in the treatment group and 5% of patients in the placebo group achieve at least one of the EDSS-Plus components. The prevalence of patients with improved outcome is informed by the results of our Phase I trial, in which 40% of patients experienced a decrease of 0.5 or more on EDSS. A total of 50 patients (25 patients in each group) will initially be enrolled to account for an expected attrition rate of 20%.

9.3 Effectiveness analysis

All statistical analysis will be performed by Dr. Linda Gerber and her team. Descriptive statistics will be calculated for patients at baseline for the entire cohort

as well as by treatment status. Treatment and placebo group patients will be compared on continuous and discrete baseline characteristics to assess randomization. The EDSS-Plus scale [6] will be used as our primary endpoint with improvement on one or more of the 3 components (EDSS, T25FW and 9HPT). Improvement is defined as ≥1point decrease from baseline EDSS if EDSS at entry is ≤5.5, and a ≥0.5 point decrease from baseline EDSS if EDSS at entry is ≥6.0, ≥ 20% positive change in T25FW, and/or ≥ 20% positive change in 9HPT confirmed. The primary endpoint of improvement on one or more of the 3 EDSS-Plus categories between treatment and placebo groups will be assessed bi-variately using the chi-square or Fisher's exact test, as appropriate, at year 1, 2, and 3 of the study. The three components of the EDSS-Plus composite score will also be assessed individually at all time points. A longitudinal linear mixed-effect model will be used to assess longitudinal change (percentage change in mean time) from baseline to 3 years for T25FW and 9HPT, with random intercepts for patients and taking into account treatment status (treatment vs placebo), progression status (SPMS vs PPMS) and time-progression status interaction. Secondary endpoints including 12-item walking scale and urodynamic studies (binary, ≥20% improvement in bladder capacity vs < 20%) will also be analyzed. Linear regression models for continuous outcomes and logistic regression models for discrete outcomes will be used to assess the independent effect of treatment status on secondary outcomes, controlling for co-variates. All analyses will be performed in SAS Version 9.4 (SAS Institute Inc., Cary, NC).

Any deviations from the previously described statistical plan will be described and justified in a protocol amendment and/or in the final report submitted to the IND application.

9.4 Safety analysis

Safety analysis will be performed on the analysis population. Safety will be assessed through summaries of serious adverse events, minor adverse events including headache and fever, and neurological disease worsening (neurological assessments and MRI). All adverse events occurring on or after the first lumbar puncture will be coded and summarized by NCI CTCAE v4.0 criteria.

9.5 Data and Safety Monitoring Committee

A Data Safety Monitoring Board (DSMB) will review safety data and make recommendations to the investigators about any existing or potential problems. The Data Safety Monitoring Board will be comprised of four members. The first three include: Norman Latov, MD, PhD, Elliot Frohman, MD, and Timothy Vartanian, MD, PhD (Chairman) with expertise in medicine, neurology, basic research, stem-cell research, and ethical and legal matters. The fourth member, Martin Lesser, PhD is a biostatistician independent of our trial, who has valuable expertise to add given his long history of serving on DSMB boards. The board will include members with

no conflict of interest and are blinded to patient identity. DSMB procedures will be conducted according to the DSMB charter. The DSMB will meet by teleconference at the beginning of the study and yearly thereafter. All serious adverse events will be reported to the DSMB within 24-48 hours. An ad hoc meeting of the DSMB may be called at any time by the DSMB Chair or the investigator if participant safety issues arise. If a significant safety concern arises during the study, or upon withdrawal of a study participant from the study, the DSMB Chair may convene a meeting to review safety and any other aspect of the study. At meetings, they will review safety data from all subjects enrolled. The DSMB has the discretion to remove an individual from the study or terminate the study if need be. The Data Safety Monitoring Board will address clinical protocol violations if they arise. Violations in clinical protocol will be monitored by the DSMB. The Board will speak with the clinical researchers about protocol violations and will have the authority to investigate if they feel there is a violation occurring. Violations in the laboratory protocol will also be monitored by the Data Safety Monitoring Board. The Board will speak to the head of the laboratory research and will have the authority to investigate if it is suspected that a violation has occurred.

10. Quality Control and Quality Assurance:

Monitoring of the clinical study for protocol and GCP compliance will be conducted periodically by the DSMB

The Sponsor-Investigator will permit direct access of the DSMB and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

Study will be monitored to insure:

- Accuracy and Completeness
- Compliance with the FDA, HIPAA, and other appropriate regulations
- Compliance with ICH GCP and related standards
- Compliance with the protocol requirements
- Compliance with IRB requirements
- Compliance with tissue processing and related standards and practices
- Accuracy of Data Management practices

11. Data Handling and Record-Keeping:

11.1 Data recording/Electronic Case Report Forms

An electronic Case Report Form (eCRF) will be completed for each subject enrolled into the clinical study. The Sponsor-Investigator will review, approve and sign/date each completed CRF; the Sponsor-Investigator's signature serving as attestation of the Sponsor-Investigator's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic.

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in *Source Documents*. *Source Documents* are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. When applicable, information recorded on the eCRF shall match the *Source Data* recorded on the *Source Documents*.

Each study subject will have an electronic file electronic Case Report Form (eCRF). The eCRF will contain results from all screens used to assess patients. These include results from:

- ICF
- Physical examinations
- Vital signs
- Neurological examinations
- Each EDSS
- Each MSFC
- Each 6 Minute Walk Test
- Each 12 Item MS Walking Scale
- Each Headache Pain Scale
- Each Infectious Disease Questionnaire
- Bladder Survey
- Lab and Urine Results
- MRI Reports
- Pregnancy test for female patients of child-bearing age
- Urodynamics testing reports
- Bone Marrow Aspiration Record
- Lumbar Puncture Record

11.2 Record maintenance and retention

The Sponsor-Investigator will maintain records in accordance with Good Clinical Practice guidelines; to include:

- FDA correspondence related to the IND and clinical protocol, including copies of submitted Safety Reports and Annual Reports
- IRB correspondence (including approval notifications) related to the clinical protocol; including copies of adverse event reports and annual or interim reports
- Current and past versions of the IRB-approved clinical protocol and corresponding IRB-approved consent form(s) and, if applicable, subject recruitment advertisements
- Copies of initial and continuing IRB approval notifications
- Signed FDA Form 1572 Statements of Investigator
- Financial disclosure information (i.e., for the Sponsor-Investigator; also for all study site sub-investigators who will be involved in the administration of the study drugs and/or the evaluation of research subjects [i.e., who will contribute significantly to the study data])
- Curriculum vitae (i.e., for the Sponsor-Investigator and investigators responsible for the conduct of the clinical research study)
- Certificates of required training; e.g., human subject protections, Good Clinical Practice, etc. (i.e., for the Sponsor-Investigator and investigators responsible for the conduct of the clinical research study; also for all study site sub-investigators who will be involved in the administration of the study drugs and/or the evaluation of research subjects [i.e., who will contribute significantly to the study data])
- Listing of printed names/signatures. (i.e., for the Sponsor-Investigator and investigators responsible for the conduct of the clinical research study; also for all study site sub-investigators who will be involved in the administration of the study drugs and/or the evaluation of research subjects [i.e., who will contribute significantly to the study data])
- Normal value(s)/range(s) for medical/laboratory/technical procedures or tests included in the clinical protocol
- Laboratory certification information for study site
- Instructions for on-site preparation and handling of the investigational drug(s), study treatment(s), and other study-related materials (i.e., if not addressed in the clinical protocol)
- Responsibility delegation log
- Signed informed consent forms
- Completed electronic Case Report Forms; signed and dated by Sponsor-Investigator
- Source Documents or certified copies of Source Documents
- DSMB reports for study site
- Copies of Sponsor-Investigator correspondence (including notifications of safety information) to sub-investigators
- Subject screening and enrollment logs
- Subject identification code list
- Investigational drug accountability records
- Final clinical study report
- Decoding procedures
- Master randomization list

- Retained biological specimen log
- Interim data analysis report(s)
- Copies of Sponsor-Investigator correspondence (including notifications of safety information) to Investigators responsible for the conduct of the clinical research study

The Sponsor-Investigator will retain the specified records and reports for up to 2 years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until 2 years after investigations under the IND have been discontinued and the FDA so notified.

12. Ethics:

12.1 Institutional Review Board (IRB) approval

The Sponsor-Investigator will obtain, from Western IRB (WIRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the Sponsor-Investigator will promptly notify WIRB of the deviation.

The Western IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (CGP).

In the event that WIRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of the Sponsor-Investigator's decision to modify the previously accepted clinical protocol:

- The Sponsor-Investigator will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to the Phase protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study.

12.2 Ethical and scientific conduct of the clinical research study

The clinical research study will be conducted in accordance with the current IRB-approved clinical protocol; ICH GCP Guidelines adopted by the FDA; and relevant policies, requirements, and regulations of the Western IRB, and applicable state and federal agencies.

12.3 Subject informed consent

The Sponsor-Investigator will make certain that an appropriate informed consent process is in place to ensure that potential research subjects are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The Sponsor-Investigator, or a sub-investigator(s) designated by the Sponsor-Investigator, will obtain the written, signed informed consent of each subject prior to performing any study-specific procedures on the subject. The date and time that the subject signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The Sponsor-Investigator will retain the original copy of the signed informed consent form, and a copy will be provided to the subject, or to the subject's authorized representative.

The Sponsor-Investigator will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the Sponsor-Investigator will obtain the informed consent of enrolled subjects for continued participation in the clinical study

13. <u>Study Discontinuation Criteria</u>:

13.1 Discontinuation of individual research subjects (refer also to sections 5.1.1 and 8.4)

If any of the following events occur during treatment, patients will be discontinued from the study:

- Acute worsening of MS symptoms
- Any neurological deficit that is unexplained by another condition and is non-reversible within 24 hours of its development
- Any sign of debilitating infection or central nervous system infection
- Any changes in cognitive function
- Any unexpected adverse medical event that is unexplained by another medical condition
- Any time the Data Safety Monitoring Board feels a patient should be withdrawn from the study

- Patient's desire to withdraw
- Patient's inability to comply properly with study requirements (inability to make scheduled appointments, etc.)
- Patient meets an exclusion criterion
- Patient becomes incarcerated
- Pregnancy

Should a patient meet any of the above criteria for early termination, he or she will be discontinued from the trial and will not be replaced. Subjects that are discontinued from the study will continue to undergo the follow-up visits relative to their last treatment. They will also undergo follow-up long term safety visits as described. All data gathered at these appointments will be included in the CRF and data set. Should a study subject terminate the study due to pregnancy, no MRI will be performed during the course of the pregnancy.

13.2 Sponsor-Investigator discontinuation of the clinical research study

The investigator has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- Unsatisfactory enrollment
- Incidence of Adverse Events indicating potential risk to subjects

The DSMB has the right to terminate a site at any time. Reasons for closing a site may include, but are not limited to the following:

- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with ICH guideline for GCP
- Slow Recruitment

14. References:

1. Harris, V.K., R. Faroqui, T. Vyshkina, and S.A. Sadiq, Characterization of autologous mesenchymal stem cell-derived neural progenitors as a feasible source of stem cells for central nervous system applications in multiple sclerosis. Stem Cells Transl Med, 2012. 1(7): p. 536-47.
2. Harris, V.K., Q.J. Yan, T. Vyshkina, S. Sahabi, X. Liu, and S.A. Sadiq, Clinical and pathological effects of intrathecal injection of mesenchymal stem cell-derived neural progenitors in an experimental model of multiple sclerosis. J Neurol Sci, 2012. 313: p. 167-177.
3. Harris, V.K., T. Vyshkina, and S.A. Sadiq, Clinical safety of intrathecal administration of mesenchymal stromal cell-derived neural progenitors in multiple sclerosis. Cytotherapy, 2016. 18(12): p. 1476-1482.
4. Harris, V.K., J. Stark, T. Vyshkina, L. Blackshear, G. Joo, V. Stefanova, G. Sara, and S.A. Sadiq, Phase I Trial of Intrathecal Mesenchymal Stem Cell-derived Neural Progenitors in Progressive Multiple Sclerosis. EBioMedicine, 2018. 29: p. 23-30.
5. Kurtzke, J.F., Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology, 1983. 33(11): p. 1444-52.
6. Cadavid, D., J.A. Cohen, M.S. Freedman, M.D. Goldman, H.P. Hartung, E. Havrdova, D. Jeffery, R. Kapoor, A. Miller, F. Sellebjerg, D. Kinch, S. Lee, S. Shang, and D. Mikol, The EDSS-Plus, an improved endpoint for disability progression in secondary progressive multiple sclerosis. Mult Scler, 2017. 23(1): p. 94-105.