Statistical Analysis Plan

Autologous, Bone Marrow-Derived Mesenchymal Stem Cell-Derived Neural Progenitor Cells, Expanded Ex Vivo; Administered Intrathecally

Phase II Clinical Trial

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IND: 13889

Clinicaltrials.gov NCT 03355365

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Project Organizational Chart, Personnel

Name	Role	Responsibility
Saud A. Sadiq, MD	Principal Investigator, Neurologist, Chief Research Scientist (TMSRCNY)	Experimental design and implementation, performs all study treatments (unblinded)
Violaine Harris, PhD	Senior Research Scientist (TMSRCNY)	Subject randomization, coordination of cell manufacturing (unblinded)
Armistead Williams, MD	Neurologist (TMSRCNY)	Examining neurologist, baseline screening, adverse event assessments (for subjects examined by Dr Stark)
James Stark, MD	Neurologist (TMSRCNY)	Examining neurologist, baseline screening, adverse event assessments (for subjects examined by Dr Williams)
Gabriel Sara, MD	Hematologist (Mount Sinai)	Bone marrow aspiration procedures
Neil Grafstein, MD	Urologist (Mount Sinai)	Urodynamics testing
Linda Gerber, PhD	Biostatistician (Weill Cornell)	Biostatistical analysis
Yuqing Qiu, MS	Biostatistician (Weill Cornell)	Biostatistical analysis
Aleksandra Wawrzyniak, BA	Clinical Trials Manager (TMSRCNY)	Informed consent, coordination of patient screening and all study visits
Daisy Ramos, RN	Research nurse (TMSRCNY)	Informed consent, nursing support for all clinical procedures
Samantha McKillip, RN	Research nurse (TMSRCNY)	Informed consent, nursing support for all clinical procedures
Michaela Malin, BA	Study Coordinator (TMSRCNY)	Informed consent, coordination of patient screening and all study visits
Morgan Roche, BA	Study Coordinator (TMSRCNY)	Informed consent, coordination of patient screening and all study visits

SAP Revision History

Version #	Version date	Reason for Revision
Version 1	Started Nov 2019 – Issued Dec 2020	Objective of SAP: To describe planned analysis of study objectives prior to data analysis (anticipated initial data analysis to be performed December 2021).
Version 2	July 2021	Finalize SAP for submission to FDA: update personnel list, update study design to indicate parallel group design, more detailed description of statistical analysis added.

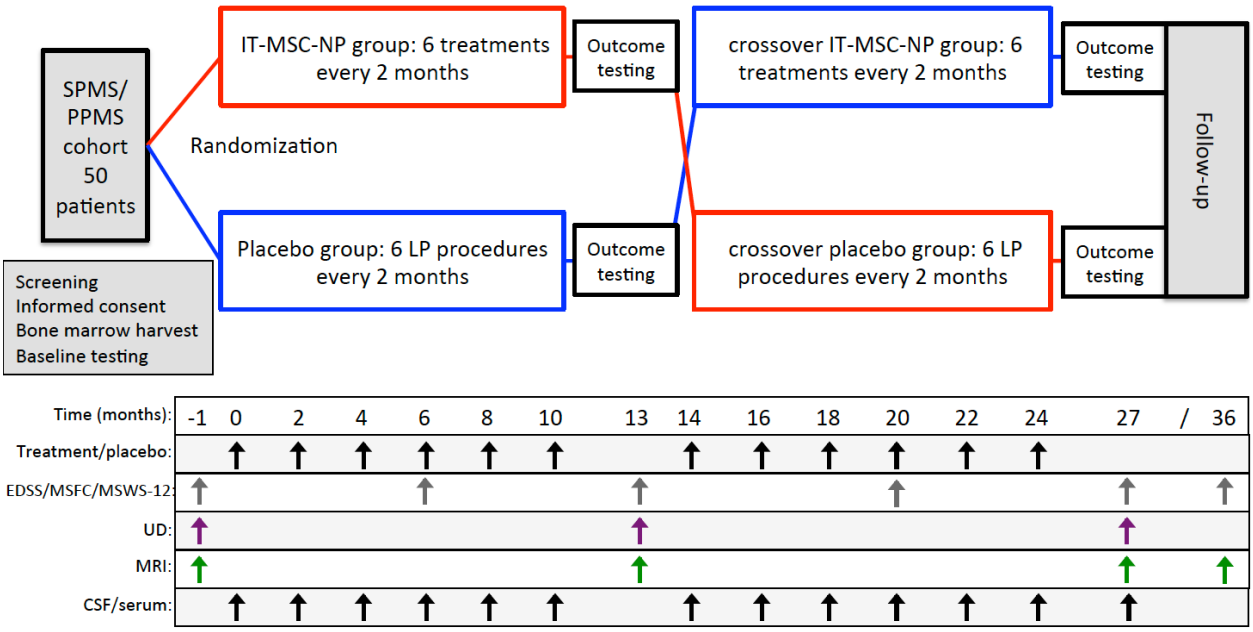
1 BACKGROUND AND STUDY DESCRIPTION

1.1 Objective

The primary aim of the study is to determine the efficacy of multiple intrathecal (IT) administrations of autologous bone marrow mesenchymal stem cell-derived neural progenitors (MSC-NP) compared to placebo through assessment of disability outcomes in patients with progressive multiple sclerosis (MS). The secondary aim is to determine continued safety and tolerability of multiple IT injections of MSC-NPs in patients with progressive MS.

1.2 Study Design

The study is a Phase II, double-blind, placebo-controlled, randomized, parallel group design with a compassionate crossover element. The IT-MSC-NP treatments and all clinical assessments take place at a single center (Tisch MSRCNY). Both the patient and the examining neurologist are blinded to the treatment. The treating clinician (Dr. Sadiq, principal investigator) and the cell manufacturing coordinator (Dr, Harris, co-PI) are not blinded. The total study duration is 3 years upon enrollment. Each subject will initially attend 1 screening visit, 1 visit for bone marrow extraction, and 1 baseline visit. In the first year, each subject will receive 6 intrathecal injections of MSC-NPs or placebo spaced 2 months apart, followed by an outcome assessment visit 3 months after the 6^{th} treatment. In the second year, subjects will cross-over into the opposite group, and will receive 6 intrathecal injections of placebo or MSC-NPs spaced 2 months apart, followed by an outcome assessment visit 3 months after the 12^{th} treatment. There is an additional follow-up visit at the end of year 3.



1.3 Primary and Secondary Outcomes

1.3.1 Primary Outcome

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). 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. 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).

1.3.2 Secondary Outcomes

- 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

1.4 Eligibility Criteria

1.4.1 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) ≥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

1.4.2 Exclusion Criteria

- 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.

1.5 Sample Size and Randomization

A total of 50 study subjects will be enrolled based on power analysis 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%.

Subjects will be stratified according to baseline EDSS score (3.0-4.0, 4.5-5.5, 6.0, and 6.5) and disease subtype (SPMS or PPMS), and block randomized within each stratum. The total number of subjects to be enrolled in each subgroup is shown below. Subjects are stratified and block randomized into placebo or treatment group in order of date of enrollment based on randomization scheme designated by Dr. Linda Gerber. Study subjects are randomized in an equal fashion to study treatment and placebo at initial randomization.

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**

2 VARIABLES FOR ANALYSIS

2.1 Analysis of Baseline Data

- Gender (M/F)
- Age at study (years)
- MS subtype (SPMS/PPMS)
- Disease duration (years)
- EDSS score

3 HANDLING OF MISSING DATA AND STUDY BIAS

Appropriate methods to deal with missing values will be chosen after exploring the data. Patients with missing outcomes and patients without missing outcomes will be compared to determine the type of missingness (missing at random/missing completely at random/missing not at random). Knowledge from researchers who implement the study will also be considered to determine type of missingness. Based on type and amount of missingness, deletion and/or imputation might be applied.

4 STATISTICAL ANALYSIS OF THE PRIMARY AND SECONDARY STUDY OBJECTIVES

4.1 Analysis of the Primary Study Objective

Primary Outcome:

The primary analysis will include all subjects meeting inclusion and exclusion criteria and who have completed a year of treatment/placebo and the 3 month post-treatment assessment. EDSS Plus composite score from 3 month post-treatment assessment will be compared to baseline.

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 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. Adjusted odds ratio (95%CI) between treatment and placebo groups at each time point will be estimated through mixed effect logistic regression with fixed effect of time, treatment, relevant covariates, and random effect of patients. 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), disease subtype (SPMS vs PPMS) and time-progression status interaction.

Under parallel design, we will compare the outcomes of interest between the two study treatments, with outcomes at 1 year as the primary time point, secondary analyses will evaluate outcomes at years 2 and 3.

For comparisons via association tests, number (%) of events in the two groups and p-values from Chi-squared or Fisher's exact test will be reported. For regressions, odds ratios (95% CI) between the two groups and p-values from the mixed-effect model will be reported.

Appropriate method to deal with missing values will be chosen after exploring the data collected. Based on reason(s) and amount of missingness, deletion and/or imputation might be applied.

Secondary Outcomes:

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.

- MSFC → T25FW (time in seconds) + 9HPT (time in seconds) + PASAT (time in seconds)
    - MSFC score = {Zarm, average + Zleg, average + Zcognitive} / 3.0
    - reduction in score indicates improvement
- EDSS
    - ranges from 3 to 6.5 for our study (scale ranges from 0 to 10). No units.
    - reduction in score indicates improvement
- T25FW (timed 25-foot walk)
    - time in seconds
    - reduction in time indicates improvement
- 9HPT (9-hole peg test)
    - time in seconds
    - reduction in time indicates improvement
- 12-item MS Walking Scale
    - Total score out of 60, and %
    - reduction in scores or % means improvement
- Urodynamic studies
    - PVR
    - Cystometry with simultaneous measurements of bladder, urethral and subtracted rectal pressures for first sensation
    - Maximum bladder capacity
    - Urethral pressure profile with functional length measurement maximum closing pressure
    - Bladder pressure was assessed with filling and sphincter relaxation and synergic voiding assessed

    - Urinary peak flow rate was also measured.
- 6-Minute Walk Test
    - Distance in meters
    - Increase in distance indicates improvement

Exploratory Outcomes:

Individual MRI measures obtained from NeuroQuant software will be analyzed as an exploratory outcome. Brain, cervical and thoracic spine MRI scans will be obtained at baseline, and at months 13, 27, and 36. 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 to month 13 will be assessed using an MMRM analysis.

CSF biomarker analysis will also be included as an exploratory outcome. CSF, plasma, and serum samples will be obtained at the time of each treatment, and 3 months following the 12th treatment, for a total of 13 samples of each type.

4.2 Analysis of the Secondary Study Objective

Safety Analysis

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 and submitted for yearly reporting to the DSMB and FDA. For safety analysis, the following will be tabulated:

- Incidence of Adverse Events
- Relationship pf Adverse Events to Investigational Product

- Severity of Adverse Event
- Serious Adverse Events
- Adverse Events Leading to Treatment Discontinuation
- Death
- Adverse Events of Special Interest

Summary descriptive statistics will be reported for each adverse event. For continuous outcome, mean(sd)/median(IQR) for whole sample and each group will be reported along with p-values from two-sample two-sided t-test or Wilcoxon rank-sum test as appropriate. For binary outcomes, number (%) of events will be reported along with p-values from Chi-squared test or Fisher's exact test as appropriate. Linear or logistic regression will be applied to estimate the treatment effect on each safety outcome. Analyses will be performed at the end of each of the three years.

All analyses will be performed in SAS Version 9.4 (SAS Institute Inc., Cary, NC) or R version 4.0.3 (2020-10-10).