STATISTICAL ANALYSIS PLAN (SAP)

Title A Randomized, Placebo-Controlled Phase IIa Trial to Evaluate the Biological Activity, Safety, and Tolerability of Autologous Regulatory T Lymphocytes (Tregs) Expanded Ex-Vivo and Returned Intravenously in Combination with Low-Dose IL-2 in People with Amyotrophic Lateral Sclerosis (ALS)

Project Medical Director Stanley H. Appel, MD

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SAP APPROVAL SIGNATURES

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SAP REVISION HISTORY

Version	Date	Description of Changes
1.0	30 Jul 2021	Initial version

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1. Governing Documents

This statistical analysis plan (SAP) specifies the outcome measures, analysis samples, and planned analyses for the T-Reg IIa trial. The SAP supplements the clinical protocol. Please refer to the clinical protocol for details on the rationale for the intervention, eligibility criteria, conduct of the trial, clinical assessments and the timing of their use in the trial, definitions and reporting of adverse events, data management conventions, and regulatory oversight and compliance procedures. In case of discrepancies between the SAP and the clinical protocol concerning matters of data analysis, the SAP is authoritative. On all other matters, the clinical protocol is authoritative.

2. Study Design

2.1 Overview

This is a phase IIa, two-period, multicenter trial to evaluate the biological activity, safety, and tolerability of autologous regulatory T lymphocytes (Tregs) expanded ex-vivo and returned intravenously in combination with low-dose IL-2 in people with amyotrophic lateral sclerosis (ALS). Participants in Cohort 1 enrolled in a six-month, two-arm, double-blind, placebo-controlled randomized controlled trial (RCT, Period 1) followed by a six-month open-label extension (OLE, Period 2) with exposure to three dosage levels. Participants in Cohort 2 enrolled directly into the OLE. The trial is registered at Clinicaltrials.gov as study NCT04055623.

2.2 Study Objectives

Primary Objective:

- To evaluate the biological activity of IV infusion of an expanded population of autologous regulatory T lymphocytes and low-dose IL-2 in people with ALS by evaluating Treg suppressive function.

Secondary Objectives:

- To evaluate the biological activity of IV infusion of an expanded population of autologous regulatory T-lymphocytes and low-dose IL-2 in people with ALS by evaluating Treg number.
- To evaluate the safety and tolerability of IV infusion of an expanded population of autologous regulatory T lymphocytes and low-dose IL-2 in people with ALS.

Exploratory Objectives:

- To characterize the effects of IV infusion of an expanded population of autologous regulatory T lymphocytes and low-dose IL-2 on clinical outcome measures of ALS, including Appel ALS Rating Scale (AALS) and ALS Functional Rating Scale Revised (ALSFRS-R) scores, forced vital capacity (FVC), maximum inspiratory pressure (MIP), and fasciculations. To assess efficacy, the Combined Assessment of Function and Survival (CAFS) will be evaluated.

2.3 Study Population

Individuals eligible for trial participation are men or women at least 18 years old who meet the El Escorial criteria of possible, laboratory-supported probable, probable, or definite for a diagnosis of sporadic or familial ALS, experienced a decline in ALSFRS-R total score of at least two points in the 90 days prior to screening or at least four points over the 180 days prior to screening, possess a forced vital capacity (FVC) at least 65% of that expected based on age, sex, and height, are treatment naïve or on a stable dose of riluzole, edaravone, or both, lack unstable clinical or psychiatric conditions, and were not recently exposed to other investigational treatments. Detailed inclusion and exclusion criteria are specified in the protocol.

Participants will be recruited from two centers that are part of the Northeast ALS (NEALS) Consortium: Houston Methodist Hospital (HMH), Houston, TX and Massachusetts General Hospital (MGH), Boston, MA.

2.4 Participant Flow

Cohort 1 participation included screening, leukapheresis, initiation of subcutaneous IL-2 at $2x10^5$ IU/m^2/injection or placebo three times weekly, randomization, initiation of monthly Treg intravenous infusions at $1x10^6$ cells/kg/infusion or placebo, follow-up visits every 4 weeks to 24 weeks with additional biospecimen sampling 1 and 7 days post-infusion at selected visits, followed by entry into OLE, initiation of subcutaneous IL-2 at $2x10^5$ IU/m^2/injection three times weekly, a dose escalation of monthly Treg infusions at $1x10^6$ cells/kg/infusion for two infusion, at $2x10^6$ cells/kg/infusion for two infusion, and at $3x10^6$ cells/kg/infusion for two infusion, and follow-up visits every 4 weeks to 54 weeks with additional biospecimen sampling 1 and 7 days post-infusion at selected visits.

Cohort 2 participation included screening, leukapheresis, initiation of subcutaneous IL-2 at $2x10^5$ IU/m^2/injection three times weekly, a dose escalation of monthly Treg infusions at $1x10^6$ cells/kg/infusion for two infusions, at $2x10^6$ cells/kg/infusion for two infusions, and at $3x10^6$ cells/kg/infusion for two infusions, and follow-up visits every 4 weeks to 28 weeks (corresponding to Week 54 for Cohort 1 participants) with additional biospecimen sampling 1 and 7 days post-infusion at selected visits.

Detailed descriptions of study procedures and timing are specified in the protocol.

2.5 Treatment Allocation

Prior to the baseline visit, eligible Cohort 1 participants were randomly allocated in a 1:1 ratio to active or placebo treatment. Randomizations followed a permuted-block schedule, stratified by site. The randomization schedule was prepared by computer program by an unblinded study statistician.

2.6 Treatment Administration

Details of treatment administration are described in the protocol.

2.7 Allocation Concealment

Allocation to treatment group of Cohort 1 participants is concealed to participants and investigators. The randomization schedule is known only to the unblinded study statistician who generated the schedule and personnel at the central cGMP laboratory responsible for processing

cells harvested by leukapheresis and generating the population of expanded autologous regulatory T lymphocytes and tracking completed activities. An independent Data and Safety Monitoring Board (DSMB) received unblinded data as described in the DSMB Charter. Concealment of the true treatment assignment of specific participants was achieved by using matching placebo syringes containing saline in place of syringes containing IL-2 and using matching infusion bags containing saline, 5% human serum albumin, and 0.2% DMSO in place of infusion bags containing Tregs.

[continued on next page]

2.8 Schedule of Assessments

2.8.1 Study Table for Period 1 – Randomized Controlled Trial (RCT) – Cohort 1 Only

	V1 Screening	V2 Leuka-pheresis	V3[13] RCT 1 w/in 8 weeks from screening & 6 weeks of Leukapheresis			V4[13] RCT 2	V5[13] RCT 3		V6[13] RCT 4	V7[13] RCT 5	V8[13] RCT 6			V9[13] Follow-Up Visit
	Week -8 to -3	Week -6 to -3	Infusion Week 0 Day 0	Week 0 Day 1 +3 Days ^^	Week 1 Day 0 ±3 Days	Infusion Week 4 ±7 Days	Infusion Week 8 ±7 Days	Week 9 ± 3 Days	Infusion Week 12 ±7 Days	Infusion Week 16 ±7 Days	Infusion Week 20 ±7 Days	Week 20 Day 1 +3 days	Week 21 Day 0 ±3 Days	Week 24 ±7 Days
Written Informed consent	X													
Eligibility Review	X	X												
Medical history	X	X												
Demographics	X													
Screening Labs[1]	X													
ALS Diagnosis History	X													
EI Escorial Criteria	X													
Vital Signs, Height and Weight[2]	X		X			X	X		X	X	X			X
Physical Exam	X													X
Neurological Exam[3]	X													X
12-Lead ECG (Electrocardiogram)	X													X
C-SSRS[4]	X		X			X	X		X	X	X			X
ALSFRS-R and Appel ALS Scale	X		X			X	X		X	X	X			X
FVC and MIP Measurements	X		X			X	X		X	X	X			X
Assess Survival		X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X

	V1 Screening	V2 Leuka-pheresis	V3[13] RCT 1 w/in 8 weeks from screening & 6 weeks of Leukapheresis			V4[13] RCT 2	V5[13] RCT 3		V6[13] RCT 4	V7[13] RCT 5	V8[13] RCT 6			V9[13] Follow-Up Visit
	Week -8 to -3	Week -6 to -3	Infusion Week 0 Day 0	Week 0 Day 1 +3 Days ^^	Week 1 Day 0 ±3 Days	Infusion Week 4 ±7 Days	Infusion Week 8 ±7 Days	Week 9 ± 3 Days	Infusion Week 12 ±7 Days	Infusion Week 16 ±7 Days	Infusion Week 20 ±7 Days	Week 20 Day 1 +3 days	Week 21 Day 0 ±3 Days	Week 24 ±7 Days
Safety Labs[5]	X		X			X	X		X	X	X			X
Leukapheresis		X												
Blood & Urine biomarkers[6]	X		X+	X	X	X	X	X	X	X	X	X	X	X
Randomization[7]		X ~												
Intravenous Treg or placebo infusion[8]			X			X	X		X	X	X			
Teach IL-2/placebo administration[9]		X~												
First dose of IL-2 or placebo[10]		X~												
Return of IL-2 or placebo supplies			X			X	X		X	X	X			X
Shipping of IL-2 (Pharmacy*)		X~	X			X	X		X	X	X			
Distribute fasciculations diary	X		X			X	X		X	X	X			
Review fasciculations diary			X			X	X		X	X	X			X
IP accountability compliance and Diary Review & IL2-re-education			X			X	X		X	X	X			X
Blindedness Questionnaire														X

2.8.2 Study Table for Period 2 – Open Label Extension (OLE) ^ – Cohorts 1 and 2

	Optional V10 Leuka-pheresis #2 (if needed) ****	V11 OLE 1			V12 OLE 2	V13 OLE 3		V14 OLE 4	V15 OLE 5	V16 OLE 6			V17 OLE 7	V18 Final Safety Visit
	□Not needed Week 25 ±7 Days	Week 26 +14 Days *****	Week 26 Day 1 +3 Days	Week 27 Day 0 ±3 Days	Week 30 ±7 Days	Week 34 ±7 Days	Week 35 Day 0 +3 Days	Week 38 ±7 Days	Week 42 ±7 Days	Week 46 ±7 Days	Week 46 Day 1 +3 Days	Week 47 ±3 Days	Week 50 ±7 Days	End of Participation/End of Study Week 54 ±7 Days
Written Informed consent														
Vital Signs, Height and Weight[2]		X			X	X		X	X	X			X	X
Physical Exam														X
Neurological Exam[3]													X	X
12-Lead ECG														X
C-SSRS[4]		X			X	X		X	X	X			X	X
ALSFRS-R and Appel ALS Scale		X			X	X		X	X	X			X	X
FVC and MIP Measurements		X			X	X		X	X	X			X	X
Assess Survival	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Labs[5]	X	X			X	X		X	X	X			X	X

	Optional V10 Leuka-pheresis #2 (if needed) ****	V11 OLE 1			V12 OLE 2	V13 OLE 3		V14 OLE 4	V15 OLE 5	V16 OLE 6			V17 OLE 7	V18 Final Safety Visit
	□Not needed Week 25 ±7 Days	Week 26 +14 Days *****	Week 26 Day 1 +3 Days	Week 27 Day 0 ±3 Days	Week 30 ±7 Days	Week 34 ±7 Days	Week 35 Day 0 +3 Days	Week 38 ±7 Days	Week 42 ±7 Days	Week 46 ±7 Days	Week 46 Day 1 +3 Days	Week 47 ±3 Days	Week 50 ±7 Days	End of Participation/End of Study Week 54 ±7 Days
Leukapheresis	X													
Blood & Urine biomarkers[6]	X	X+	X	X	X	X	X	X	X	X	X	X	X	X
Intravenous Treg infusion[8]		X			X	X		X	X	X				
Teaching of IL-2 administration[9&11]	X													
First dose of IL-2[10]	X													
Shipping of IL-2	X	X			X	X		X	X	X				
Return of IL-2 supplies		X			X	X		X	X	X			X	X
Review fasciculations diary		X			X	X		X	X	X			X	X
Drug accountability compliance		X			X	X		X	X	X				
Exit Questionnaire[12]														X

1. Screening laboratory tests will be performed locally and include HIV, Hepatitis B, Hepatitis C, Complete Blood Count with Differential, Full Chemistry Panel (Sodium, Potassium, Chloride, Blood Urea Nitrogen, Creatinine, Glucose, Calcium, Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline Phosphatase, Total Bilirubin, Total Protein, Albumin, and Carbon dioxide/bicarbonate), Urine, International Normalized Ratio/Prothrombin Time/Partial Thromboplastin Time, and Pregnancy Test (urine) for Women of Childbearing Potential.

2. Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature. Height is only recorded once at the Screening Visit. During Treg infusions, vital signs will be obtained pre-infusion and monitored and recorded once every hour from the infusion start time for up to 4 hours. There is a +/- 10-minute window for each hour vital signs are taken during the infusion.
3. The standard Neurological Exam will be used for all subjects.
4. C-SSRS Screening Version to be completed at Screening Visit only. C-SSRS since Last Visit version to be completed at all other visits.
5. Safety laboratory labs will be performed locally and include Complete Blood Count with Differential, Full Chemistry Panel (Sodium, Potassium, Chloride, Blood Urea Nitrogen, Creatinine, Glucose, Calcium, Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline Phosphatase, Total Bilirubin, Total Protein, Albumin, and Carbon dioxide/bicarbonate), Urinalysis, and Pregnancy Test (urine) for Women of Childbearing Potential.
6. Biomarker blood and urine samples (non-fasting) will be obtained from each participant for biomarker analyses and labeled with a subject identifier code and date at/with:
    - Immediately prior to each infusion at Screening, the final RCT-week 24 visit, OLE-week 50, and the End of Study Visit -week 54;
    - Weeks 0, 20, 26, and 46 - 1 day +3 days & 7 days ± 3 days following the infusion day/date;
    - Week 8 & Week 26 - 7 days ±3 days following the infusion day/date.
    - Specimen collection: (six tubes) 10 mL sodium heparin green tops, (one tube) 10 mL serum red top, (one tube) 4 mL sodium heparin green top, and a urine sample (5 mL to 10 mL).
        - Five (5) 10 mL sodium heparin green tops will be used at the site of collection to perform the Treg Suppression Assay per SOP. Serum will be stored at -80°C at the site of collection per SOP.
        - One (1) 10 mL sodium heparin green top and the 4 mL sodium heparin green top will be shipped overnight at room temperature to HMH for flow cytometry and messenger RNA collection per SOP. Urine specimens will be frozen at -80°C at the site of collection per SOP. All biospecimens will be labeled with a subject identifier code and date.
    - Primary Biomarkers: Treg suppressive function;
    - Secondary biomarkers: Treg numbers by flow cytometry; and
    - Exploratory biomarkers: Banking of urine, serum, plasma, and messenger RNA.
    - These samples will be collected and stored at -80°C. Banked samples will be analyzed after the study has completed.
    - After all analyses have been completed, the remaining samples will be transported to the NEALS biorepository.

    + Participants will also return to their enrollment sites 1 day (+3 days) and 7 days (± 3 days) after the first infusion of the RCT and OLE (Week 0, Week 20, Week 26 and week 46) to collect the same amount of blood and urine for post-infusion analyses of blood biomarkers.
7. Randomization will occur after the Leukapheresis procedure is completed within 7 days.
8. Safety monitoring & TReg or matching placebo infusion: For the RCT, 4 hours post-infusion monitoring is required for the first infusion but is otherwise optional at the discretion of the Investigator. For the OLE, 4 hours post-infusion monitoring is required following the first infusion. Post-infusion monitoring for at least 1 hour is then required after each escalating Treg dose in the OLE but is otherwise optional at the discretion of the Investigator. Following each infusion, the PI or study staff will provide a follow up phone call within 24 to 48 hours to query subjects for symptoms of a delayed anaphylaxis response.

9. Il-2 or Matching Placebo: The teaching of IL-2 subcutaneous administration will be provided to all participants following the manufacturer's directions on the days of leukapheresis. The dispensation of IL-2 or placebo and diary will take place at the enrollment site. For additional syringe dispensation, IL-2 and placebo for subjects will be shipped directly to the subjects from the HMH or MGH, respectively. Sites will provide the subjects with a sharps container and instructions on discarding used syringes and returning unused syringes with their IL-2/Placebo dosing diary to the next visit.
10. During RCT and OLE, IL-2 or placebo will begin after Leukapheresis and will start as early as 4 weeks or at least 2 weeks prior to the first Treg infusion. IL-2/placebo will be administered subcutaneously 3 times per week and then discontinued 4 weeks after the last Treg infusion of the RCT (Week 24) and at the final visit for the OLE phase (Week 50).
11. For MGH patients, teaching of IL-2 will occur at screening.
12. If performed as part of an early termination visit before the V9 Week 24 Follow-Up Visit, the Exit Questionnaire will include the Blindedness Questionnaire. If performed as part of an early termination visit after the V9 Week 24 Follow-Up Visit or as part of the V18 Week 54 Final Safety Visit, the Exit Questionnaire will not include the Blindedness Questionnaire.
13. RCT applies to Cohort 1 only

~ These activities will only apply to HMH patients.

* IL-2 or matching placebo administration is ongoing throughout the study and not associated with a study-specific visit.

** Any study visit that cannot be done in person will be done by phone, collecting as much safety data as possible.

*** Early termination visits for randomized subjects who have received at least one dose of investigational product; where possible perform safety labs, physical and neurological exam, vital signs, ECG, AE, and SAE assessment, collect IL-2 or placebo syringes and materials and collection of fasciculation diary.

**** Second leukapheresis will be performed ONLY if Treg collection from initial leukapheresis is insufficient.

***** OLE 1 for Cohorts 1 & 2 must occur at least 14 days after participant has initiated IL-2 dosing for the OLE phase.

^ For subjects in Cohort 1, visit windows for the OLE are to be calculated from Week 24 of the RCT. For subjects in Cohort 2, visit windows for the OLE are to be calculated from the Week 26 infusion (i.e., the Week 26 V11 OLE1 will be Day 0). Visit windows for visits following infusions are relative to the date of the infusion.

3. General Considerations for Data Analysis

3.1 Statistical Software

Statistical analyses will be performed using SAS (SAS Institute, NC, USA) or R (R Foundation for Statistical Computing, Vienna, Austria).

3.2 Summary Statistics

Data will be summarized with respect to disposition, demographics, pre-treatment characteristics, efficacy endpoints, safety endpoints, and tolerability. Summary statistics for continuous variables will include the number of observations, the mean, median, standard deviation, inter-quartile range, and range. Summaries of categorical data will include counts, denominators, and percentages.

3.3 Precision

Results will generally be reported to 3 significant figures. Percentages will generally be reported to 0.1 percentage points. P-values will be reported to two digits when greater than or equal to 0.095, to three digits when greater than or equal to 0.00095 and less than 0.095, and as <0.001 for all smaller values.

3.4 Transformations

Data that are strictly positive, continuous, and strongly right skewed will typically be log-transformed prior to any inferential testing. Skewness greater than 3 will be used as a guide in determining which variables to transform. Original, untransformed values will be used for all summaries.

3.5 Multiplicity Adjustments

A single primary endpoint, 24-week change in Treg suppression function during Period 1, is specified and will be tested at two-tailed $p < 0.05$. A single secondary endpoint of biological activity, 24-week change in Treg cell number during Period 1, will be tested at two-tailed $p < 0.05$ using a closed testing procedure to maintain an overall two-tailed family-wise error rate less than 5%. Tests of clinical response are exploratory. Nominal comparison-wise p-values will be reported for all analyses.

3.6 Missing Data

If baseline values of a given measure are missing, the last observed pre-treatment value will be used. Missing baseline covariates will be imputed using the mean of the respective covariate. Off-schedule results, e.g., those collected as part of an Early Termination Visit, will be used in place of the closest missing scheduled visit (while preserving visit sequence) for analyses that depend on visit-specific data. Other results will not be carried forward.

The planned mixed model analyses, where applied, yield estimates that are unbiased conditional on the observed values under a missing at random assumption.

4. Study Endpoints

4.1 Endpoints of Biological Activity

- Change in Treg suppressive function in the blood from baseline to week 24 during Period 1 (overall primary endpoint) and from start to finish of Period 2 and each dosage level during Period 2, and
- Change in Treg numbers in the blood from baseline to week 24 during Period 1 (overall key secondary endpoint) and from start to finish of Period 2 and each dosage level during Period 2.

4.2 Exploratory Clinical Endpoints

- Change in Appel ALS Rating Scale (AALS) score from baseline to week 24 during Period 1 and from start to finish of Period 2,
- Change in ALS Functional Rating Scale Revised (ALSFRS-R) from baseline to week 24 during Period 1 and from start to finish of Period 2 and each dosage level during Period 2,
- Change in forced vital capacity (FVC) and maximum inspiratory pressure (MIP) from baseline to week 24 during Period 1 and from start to finish of Period 2 and each dosage level during Period 2,
- Tracheostomy-free survival between baseline and week 24 during Period 1 and from start to finish of Period 2 and each dosage level during Period 2,
- Proportion of days free of fasciculations between baseline and week 24 during Period 1 and from start to finish of Period 2 and each dosage level during Period 2, and
- Combined Assessment of Function and Survival from baseline to 1-year after each participant's first Treg infusion.

4.3 Safety and Tolerability Endpoints

- Safety: Defined as the occurrence of adverse events (AEs), serious adverse events (SAEs), treatment-emergent adverse events (TEAEs), and clinically significant abnormalities in clinical and laboratory values.
- Tolerability: Defined as the percentage of participants who complete the 6-month RCT (RCT Tolerability) and who complete the 6-month OLE and each ascending dose on study treatment (OLE Tolerability).

5. Measurement Definitions

5.1 AALS

The Appel ALS Rating Scale (AALS, Appel et al. 1987) is a 19-item clinician-completed instrument for assessing participant function in five groups: bulbar, respiratory, muscle strength, lower extremity function, and upper extremity function. Each group is scored based on one to six individual assessments (bulbar = 2, respiratory = 1, muscle strength = 4, lower extremity function = 6, upper extremity function = 6). Group scores are calculated as the sum of all items

within the group. A group score of six (6) points indicates no dysfunction, and group scores of 30 to 36 points (maximum group score: bulbar = 30, respiratory = 30, muscle strength = 36, lower extremity function = 35, upper extremity function = 33) indicate maximal dysfunction. The AALS total score is the sum of all items (range 30 for healthy subjects to 164 for those with maximum impairment). Any missing item causes the corresponding group score and the total score to be missing.

5.2 ALSFRS-R

The ALS Functional Rating Scale Revised (ALSFRS-R, Cedarbaum et al. 1999) is a 12-item instrument completed by clinician interview for assessing participant function in four domains: bulbar, fine motor, gross motor, and respiratory. Each item is scored from 0 to 4 with higher scores indicating greater function. The ALSFRS-R total score is the sum of all items (range 0 to 48). Domain scores are the sum of their respective items (range 0 to 12): bulbar function = Q1-Q3, fine motor function = Q4-Q6, gross motor function = Q7-Q9, and respiratory function = QR1-QR3 (Q10-Q12). Any missing item causes the domain score and the total score to be missing.

The average rate of disease progression prior to the Baseline Visit as measured by ALSFRS-R (delta-FRS) will be calculated as 48 minus the ALSFRS-R total score measured at the Baseline Visit, that difference divided by the number of months from onset of symptom weakness due to ALS to the date of the Baseline Visit (with months calculated as days x 12 / 365.25).

5.3 FVC

Forced vital capacity (FVC) is the maximum volume of air that can be forcefully exhaled after maximal inhalation. Trained technicians coach participants through 3 to 5 maneuvers using a spirometer. A minimum of 2 completed FVC maneuvers are required to calculate an estimate of maximum FVC for a given participant visit. The maximum volume expired is converted to percent of predicted normal using normal values calculated based on age, sex, age, and height (Knudson et al. 1983). Higher values indicate greater respiratory function.

5.4 MIP

Maximum inspiratory pressure (MIP) is the maximal pressure achieved by inspiration in a seated position. Trained technicians coach participants through 3 to 5 maneuvers using a spirometer. A minimum of 2 completed MIP maneuvers are required to calculate an estimate of maximum MIP for a given participant visit. MIP is reported in units of cm H_2O. Higher values indicate better pulmonary function.

5.5 Survival

Survival will be measured by time to the earlier of death or tracheostomy. Follow-up for analysis of survival during Period 1 among participants who do not die or receive a tracheostomy prior to the Week 24 Visit will be censored at the Week 24 Visit, if completed, the date of consent withdrawal, if withdrawn, or the last date at which vital status is known prior to the end of the Week 24 Visit window for participants lost to follow-up. Follow-up for analysis of survival during Period 2 among participants who do not die or receive a tracheostomy prior to the Week 54 Visit will be censored at the Week 54 Visit, if completed, the date of consent withdrawal, if

withdrawn, or the last date at which vital status is known prior to the end of the Week 54 Visit window for participants lost to follow-up.

6. Statistical Methodology

6.1 Analysis Sets

Two analysis sets will be used for summaries and analyses of biological activity, safety, and tolerability:

- RCT Set: Cohort 1 participants who were eligible, randomized during Period 1, and received at least one double-blind Treg infusion, and
- OLE Set: Cohort 1 and 2 participants who were eligible and received at least one open-label Treg infusion.

6.2 Baseline Characterization

Each analysis set will be summarized overall and by treatment group in the RCT set and by cohort and treatment group (for Cohort 1) in the OLE set. Baseline characteristics of both the RCT and OLE sets are those assessed at the Screening Visit. The following characteristics will be summarized: enrollment site; age, sex, race, ethnicity, weight, El Escorial diagnosis, years since ALS symptom onset, delay between symptom onset and ALS diagnosis, site of ALS symptom onset, use of noninvasive ventilation, use of riluzole, use of edaravone, Treg suppression function, Treg cell number, AALS total score, ALSFRS-R total score, delta-FRS, FVC, and MIP.

6.3 Biological Activity and Clinical Endpoints

6.3.1 Primary Analysis Model

Treatment groups will be compared for progression of continuous endpoints (Treg suppressive function, Treg cell number, AALS total score, ALSFRS-R total score, FVC, and MIP) during Period 1 using a shared-baseline, linear mixed model in the RCT set. The model will include fixed terms for discrete visit and treatment group × post-screening visit interaction and random participant-level intercepts and slopes with unstructured covariance. Omitting a treatment-group main effect enforces a shared-baseline assumption. Use of a shared baseline reflects the true state of the population sampled prior to randomization and has the advantage of adjusting for any chance differences at baseline and increasing efficiency in a manner similar to analysis of covariance (Liang and Zeger, 2000). To enforce the shared-baseline assumption, the interaction between treatment group and visit will be restricted to post-baseline visits by including a numeric indicator variable (0 pre-treatment, 1 post-treatment) in the interaction.

The following SAS code specifies the model:

```
proc mixed data=xxx method=reml;
  class regimen id trtrnd visit;
  model Value = visit post*trtrnd*visit / solution;
  random intercept month / subject=id type=un;
```

where `id` is a participant study identifier, `trtrnd` is the randomly assigned treatment group, `visit` is the visit identifier, `Value` is value of the efficacy endpoint being tested for a given

participant at a given visit, `post` is the indicator of visits occurring after baseline, `dFRS` is delta-FRS centered at the sample median, and `month` is time in months from the Screening Visit (assuming 12 months in an average of 365.25 days per year). The primary estimate will be the treatment-dependent difference in change from the Screening Visit to the Week 24 Visit. The estimate and its 95% Wald confidence bounds will be obtained by a linear contrast of adjusted means. The following SAS code specifies the linear contrast for Treg suppressive function assessed at the Screening Visit and 12 follow-up visits assuming that the sort order for treatment group has the active group last and visits are sorted chronologically:

```
estimate "3|Act vs Plb|dWk 24"
  post*trtrnd*visit 0 0 0 0 0 0 0 0 0 0 0 0 -1  0 0 0 0 0 0 0 0 0 0 0 0 1 / cl;
```

A significant positive difference in 24-week change would support inference that the infusion of autologous T regulatory cells improved Treg suppressive function over 24 weeks.

6.3.2 OLE Analysis Model

Progression of continuous endpoints (Treg suppressive function, Treg cell number, AALS total score, ALSFRS-R total score, FVC, and MIP) during Period 2 will be estimated as a single group using a linear mixed model in the OLE set. The model will include fixed terms for discrete visit and random participant-level intercepts and slopes with unstructured covariance. Data from the Screening Visit will be used as the start of follow-up for both Cohort 1 and 2 participants, imputed at the time of the Week 24 Visit for Cohort 1 participants. Estimates of change from the Screening Visit and comparisons between dosage levels will be obtained by linear contrasts of adjusted means of applicable visits.

6.3.3 Survival

The proportion of participants who die or receive a tracheostomy will be summarized overall and by treatment group in the RCT set and by cohort and treatment group (for Cohort 1) in the OLE set. Kaplan-Meier product-limit estimates with complimentary log-log confidence bounds will be estimated in the RCT set at 24 weeks (end of Period 1), 34 weeks (end of OLE $1x10^6$ cells/kg/infusion dosage interval), 42 weeks (end of OLE $2x10^6$ cells/kg/infusion dosage interval), 50 weeks (end of OLE $3x10^6$ cells/kg/infusion dosage interval), and 54 weeks (end of Period 2). Time to death or tracheostomy will be compared between treatment group in the RCT set by log-rank test.

6.3.4 Fasciculations

The proportion of days per week with no fasciculations, fasciculations only after exertion, fasciculations at rest or after exertion, and fasciculations that substantially interfere with daily activities will be summarized as means and standard deviations by treatment group and week in the RCT and OLE sets.

6.3.5 CAFS

To account for potentially informative loss to follow-up due to death, treatment groups will be compared by the Combined Assessment of Function and Survival (CAFS, Berry et al. 2013) in the RCT set to the end of Period 1. CAFS is a joint-rank test (Finkelstein and Schoenfeld 1999) of a composite endpoint combining function as measured by change in ALSFRS-R total scores and time to death or tracheostomy. The test consists of calculating a rank-sum score for each individual relative to pair-wise comparisons with all other participants. Participants are ranked

according to time to death or tracheostomy when that is observed for both members of a pair or when one is censored after the observed event time for the other. Pairs that cannot be ranked by time to death or tracheostomy are ranked by absolute change from baseline in ALSFRS-R total score at the maximum follow-up time at which both participants have an observation. Inference of treatment-dependent difference in the hierarchical composite of death and disease progression is drawn by calculating a U-statistic from the rank-sum scores and testing as a Z-score.

6.3.6 Correlations between Biological Activity and Clinical Endpoints

Changes in measures of biological activity (Treg suppressive function and Treg cell number) and measures of clinical progression (AALS total score, ALSFRS-R total score, FVC, and MIP) will be compared by Spearman correlation. The correlation of change from the Screening Visit to the Week 24 Visit will be estimated among participants in the RCT set as a single group. The correlation of change from the Screening Visit to the Week 54 Visit will be estimated among all participants in the OLE set as a single group.

6.4 Safety and Tolerability Summaries

6.4.1 Treatment-emergent Adverse Events

The incidence of TEAEs will be summarized by the number of events of a given classification experienced by participants in each treatment group and by the number and proportion of participants experiencing such an event in each treatment group in the RCT and OLE sets. TEAEs will be summarized by MedDRA system organ class and preferred term for all TEAEs and all serious TEAEs.

Aggregate summaries of TEAE characteristics in the RCT and OLE samples will include: (a) seriousness, (b) severity, (c) relationship to IL-2/placebo injection, (d) action taken with respect to IL-2/placebo, (e) relationship to Treg infusion, (e) action taken with respect to Treg infusion, (f) use of a concomitant medication, and (g) outcome. For each level of a given TEAE characteristic, summaries will include the number of events of a given classification and the number and proportion of participants for which that level of a characteristic was the worst they experienced. Any unknown characteristic of a TEAE will not be classified as worst. The level for participants who experienced no TEAE will be coded as the zero-level of each characteristic.

6.4.2 Safety Labs

The absolute level and the absolute change from baseline for each safety laboratory assay will be summarized as means, standard deviations, medians, and ranges at each visit by treatment group in the RCT and OLE sets. The proportion of participants with abnormal and clinically significant safety lab levels will be summarized by treatment group by visit and at any post-baseline visit in all safety samples.

6.4.3 Vital Signs and Weight

The absolute level and the absolute change from baseline for vital signs and weight will be summarized as means, standard deviations, medians, and ranges at each visit by treatment group in the RCT and OLE sets.

6.5 Other Summaries

6.5.1 Participant Disposition

The number of Cohort 1 participants consented to the study, failed screening, determined eligible, completing leukapheresis, randomized, initiated on IL-2/placebo study drug, receiving an initial Treg/placebo infusion, completed scheduled Period 1 follow up, prematurely terminated study participation due to TEAE, withdrawal of consent, or administrative termination, and entered into the OLE will be summarized overall and by treatment group for the RCT set. The number of OLE participants initiated on IL-2, completing leukapheresis for Period 2 participation, receiving an initial Treg infusion, completed scheduled Period 2 follow up, and prematurely terminated study participation due to TEAE, withdrawal of consent, or administrative termination will be summarized overall and by cohort for the OLE set. Reasons for screen failure and for withdrawal from study will be presented.

6.5.2 Tolerance

Tolerability will be defined for Period 1 as the percentage of participants who complete the 6-month RCT on study treatment. RCT Tolerability will be compared between treatments in the RCT set. Tolerability will be defined for Period 2 as the percentage of participants who complete the 6-month OLE on study treatment. OLE Tolerability will be compared between cohorts in the OLE set.

6.5.3 Concomitant Medication Use

Concomitant medications taken during the study period will be coded using the World Health Organization (WHO) Drug Dictionary Enhanced and Default Anatomical, Therapeutic, and Chemical (ATC) class. The percentage of participants who initiate a concomitant medication after first Treg/placebo infusion will be summarized by WHO drug classified medication and each ATC class of medications overall and by treatment group in the RCT set and overall and by cohort in the OLE set.

7. References

Appel V, Stewart SS, Smith G, Appel SH. A rating scale for amyotrophic lateral sclerosis: description and preliminary experience. Ann Neurol. 1987 Sep;22(3):328-33.

Berry JD, Miller R, Moore DH, Cudkowicz ME, van den Berg LH, Kerr DA, Dong Y, Ingersoll EW, Archibald D. The Combined Assessment of Function and Survival (CAFS): a new endpoint for ALS clinical trials. Amyotroph Lateral Scler Frontotemporal Degener. 2013 Apr;14(3):162-8.

Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, Nakanishi A. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci. 1999 Oct 31;169(1-2):13-21.

Finkelstein DM, Schoenfeld DA. Combining mortality and longitudinal measures in clinical trials. Stat Med. 1999 Jun 15;18(11):1341-54.

Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal expiratory flow-volume curve with growth and aging. Am Rev Respir Dis. 1983 Jun;127(6):725-734.

Liang KY, Zeger S. Longitudinal data analysis of continuous and discrete responses for pre–post designs. Sankhyā Ser B. 2000 Apr;62(1):134–48.

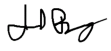
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