HOUSTON Methodist RESEARCH INSTITUTE

Participant's Name:_____Subject ID Number:_____

Official Study 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).

Short Title: T-Regulatory Cells in ALS (Tregs in ALS)

Study Director: Stanley Appel, MD

Principal Investigator: Jason Thonhoff, MD, PhD

Sponsor: Houston Methodist Research Institute

Funding Agencies: ALS Association, ALS Finding a Cure, and MDA Foundation

Before consenting to participate in this research study, you should have enough time to read the information in this form, or have it read to you. A member of the research team will discuss it with you. Be sure to ask questions about anything that is not clear before giving consent.

CONCISE SUMMARY

We are asking you to consent to participation in a research study. Your participation is voluntary.

The purpose of this study is to determine if your own Regulatory T Cells (Tregs), after being grown in a special laboratory and delivered by infusion back to you, in combination with injections of Interleukin-2 (IL-2) are safe and tolerable, and can potentially improve Tregs numbers and function in your blood, and slow the progression of disease in patients with ALS. The study has two groups of patients called "cohorts." Group 1/Cohort 1 –up to six participants: They study is in two parts. During the first six months of the study, participants will be randomly assigned to either Treg infusions and IL-2 injections or placebos (inactive infusion and inactive injections). During the second 6 months of the study, all participants will receive Treg infusions and IL-2 injections. Group 2/Cohort 2 – up to 2 participants will be enrolled directly into the open label part of the study and receive Treg infusions and IL-injections.

There are risks to this study drug combination including the increased risk of infections, worsening of disease

You can choose not to participate at any time, even after starting the study, without any penalty or loss of benefits to which you are entitled.

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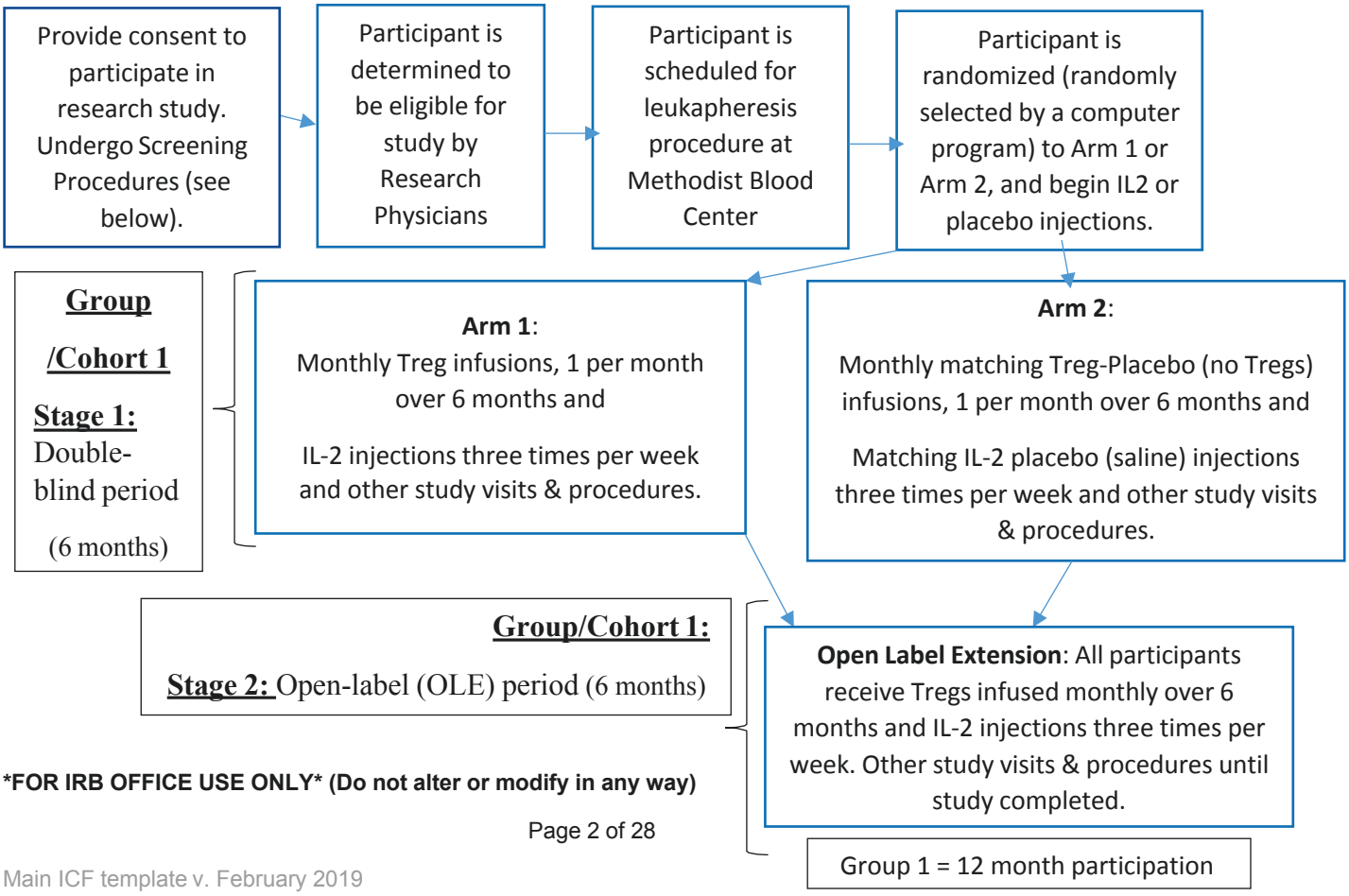
You will have procedures done that are considered research and may or may not be a part of the usual care for you condition. If you decide to participate, your private health information will be collected; however, the researchers in this study will take appropriate measures to ensure confidentiality of your information. In the case you are injured as a result of the study, medical treatment is available.

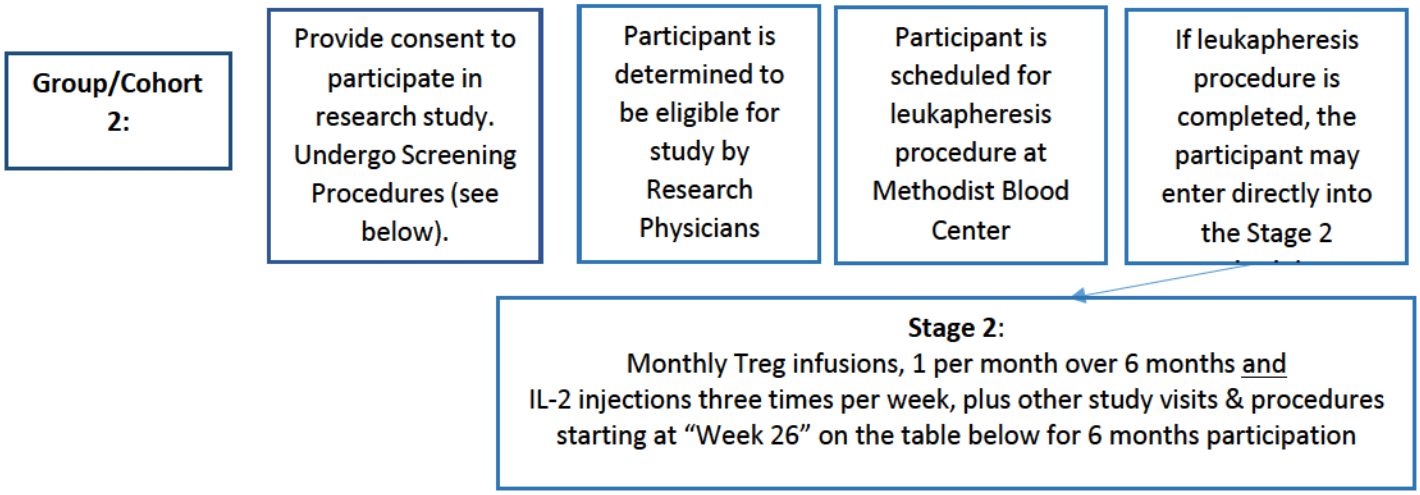
If you go to Houston Methodist or another healthcare facility or provider for any reason while participating in this study, you should inform them that you are involved in this research study, as it may impact the type(s) of care provided and protect your safety.

Why me: You are being asked to participate in a research study because you have ALS.

Study Summary: This study is a phase IIa double-blind, placebo-controlled study. "Double-blind" means that neither you, the study doctor, nor any of the study staff will know if you're receiving drug or placebo. "Placebo-controlled" means that you will be placed in one of two groups through a computer decision or similar to results from a flip of a coin, some people will receive a harmless, look-alike of the actual study drug. "Phase IIa study" means that a small number of people will participate and the main goal is to ensure safety with the doses of study medication you are receiving. Only 3 people have received this treatment before.

Study plan:





To obtain the Tregs, a routine procedure called leukapheresis performed in some hospital blood centers, such as the Houston Blood Center, will be done. The leukapheresis procedure is how the Tregs will be collected from your white blood cells, which is done by separating your white blood cells from the blood collected from one arm through an intravenous (IV) tube and the rest of your blood components without the white blood cells are returned to your body through another IV tube in your other arm. The white blood cells are removed for the blood to collect the T-regulatory cells (Tregs) which will be taken to the special cell manufacturing lab for manipulation to be infused back to you at a later date.

Two Groups/Cohorts

For Group/Cohort 1: Double-Blind Randomized Controlled Trial □ Open Label

Stage 1: During the first stage of the study (approximately six months) also known as the randomized controlled trial (RCT), you will receive either your own body's immune cells (Tregs) and injections of IL-2 or you will receive a matching "Treg placebo" which consists of a sterile mixture of salt and water (normal saline) that is similar to the salt concentration in blood, 5 % human serum albumin (blood protein), and 0.2% DMSO (cell product preservative) per infusion for the infusion to be the most similar to the active Treg infusion. The Treg placebo infusion will look exactly like the Treg infusion. Like the infusions, the IL-2 injection for the first stage of the study will have an active IL-2 injection and a matching IL-2 placebo (contains sterile saline and no drug), depending on the arm you were randomized to for the first 6 months of the study. Placebos are used in research studies as a control to determine if the results are due to the study drug or due to other reasons.

Stage 2: During the second stage of the study (approximately six months), also known as the ascending dose-open label extension (OLE), all eligible subjects enrolled will receive the active combination therapy (Tregs and injections of IL-2).

You have a 1:1 chance of being assigned to the treatment group and a 1:1 chance of being assigned to the placebo group. Participation will be approximately 12 months.

Group/Cohort 1 –Monthly Treg infusions and daily IL-2 injections for 6 months

OR

- – Monthly Placebo infusion and daily placebo injections for 6 months



OLE: For second six months participants in either of the above arms will be given Monthly Treg infusions and daily IL-2 injections for 6 months

Group/Cohort 2: Open Label (OLE) Monthly Treg infusions and daily IL-2 injections for 6 months

If you choose to take part in this study, you will be asked to sign this consent form prior to doing any study tests or procedures.

Approximately 8 study participants will be enrolled throughout the study: 3 at Houston Methodist and 3 Massachusetts General Hospital in Boston, MA, in the 12-month study; and 2 at Houston Methodist in the 6-month study.

Group 1/Cohort 1: The total time for study participation will be up to approximately 68 weeks for the 12-month study. You will receive the study drug for a total of 54 weeks in the 12-month study. During this time, we will ask you to participate in up to 25 study visits in the clinic and 12 phone calls. Overall, you may be in the research study for up to 17.5 months.

Group/Cohort 2: The total time for study participation for will be up to approximately 34 weeks for the 6-month study. You will receive the study drug for a total of 27 weeks in the 6-month. During this time, we will ask you to participate in up to 13 study visits in the clinic and 6 phone calls. Overall, you may be in the research study for up to 9 months.

What other choices do I have?

You do not have to participate in this study to receive care for ALS. Currently, there are 2 medications that are FDA approved; riluzole (Rilutek®) and edaravone (Radicava®). There may be other clinical research studies available to you. The study doctor can discuss with you other studies for which you might qualify. Your physician and/or the research doctor is able to discuss currently available treatments or procedures that may be of potential benefit outside of this study depending on your ALS symptoms, which may include no additional treatments.

What extra test and procedures will I have if I take part in the study?

You will undergo the following tests if you elect to participate in this research study. You may also be asked to undergo additional tests while participating in this study if the study doctor believes they are needed to diagnose or manage any medical issues.

Leukapheresis: This procedure is a healthcare procedure that will be performed at least once and possibly a second time during the study to collect your Tregs. A second leukapheresis may occur if the research physician determines you need more cells for the duration of the study. Your blood will be collected for the isolation of Tregs, the specific immune cells that will be taken to the lab to increase their numbers and delivered back into you. You will only receive your Tregs back in infusions when it is scheduled later in the study. To collect the Tregs, an intravenous (IV) line is placed in your arm to allow cycling of your blood through a machine. Your white blood cells (which contain Tregs) will be removed from your blood in the machine and your blood will be returned to your body through an IV line placed in your other arm. A substance called citrate, an anticoagulant or blood thinner, may be used in order to prevent the blood from clotting during the procedure. This process can take 4-7 hours.

Infusions of Regulatory T Cells (Tregs) or Matching Placebo: Infusions will take place every 4 weeks at Houston Methodist through an IV in your arm. Infusions will last approximately –1 hour and 10 minutes and you will be monitored for 4 hours after the first infusion and up to 4 hours for the next infusions. The Treg infusions will contain a small amount of dimethyl sulfoxide (DMSO), which helps preserve your Tregs while they are in a frozen state. Frozen Tregs will be thawed immediately prior to their infusion. The Matching Placebo infusion will contain a small amount of dimethyl sulfoxide (DMSO) and serum albumin to make it as close to the Tregs as possible, but without the Tregs. There will be a total of 12 infusions for Group/Cohort 1 and 6 infusions for Group/Cohort 2.

Injections of the drug Interleukin-2 (IL-2) or placebo: During the leukapheresis visit, the nurse or specially trained study personnel will teach you how to give yourself an under-the-skin injection of IL-2 or placebo. If no issues have occurred, your first injection will start at least 2 weeks and preferably as much as 4 weeks prior to the first Treg or placebo infusion. The study doctor will let you know when you can begin IL-2 or placebo. For Group/Cohort 1: You will give yourself the injection 3 days per week as directed by the study doctor, each week for 58 weeks. For Group/Cohort 2: You will give yourself injections 3 days per week as directed by the study doctor, each week for 29 weeks. This medication will be supplied to you to take home or will be delivered directly to your home.

Blood Draws: We will draw blood from a vein in your arm for standard laboratory tests and biomarker tests. Biomarkers are substances in your body that indicate your body's response to a specific treatment. Biomarkers can also indicate the status of your underlying disease. If there are any problems due to the study drug, you may have more blood drawn. Over the course of the

study, you will have a total of 1797 ml (or 3.75 pints) of blood drawn over the course of 15 or 7 months depending on which Group/Cohort you are in. As a reference, a typical blood donation is one pint of blood.

Screening laboratory tests: The screening tests will be performed locally and include HIV, Hepatitis B, Hepatitis C, Complete Blood Count with Differential, Full Chemistry Panel, International Normalized Ratio (INR), Prothrombin Time (PT), Partial Thromboplastin Time (PTT) and Pregnancy Test (urine) for Women of Childbearing Potential.

Safety laboratory tests: This will be performed prior to each leukapheresis and infusion, and at the final RCT, OLE and End-of-Study visits Safety laboratory tests include Complete Blood Count with Differential, Full Chemistry Panel, and Pregnancy Test (urine) for Women of Childbearing Potential. These samples will be processed locally. The Investigators may order additional testing, if needed, to further assess an adverse event (AE), or if there is any suspicion that a subject may be pregnant, throughout the course of the study.

Biomarker tests: Blood and urine samples will be obtained from each participant for biomarker analyses immediately prior to each leukapheresis, each infusion, during the final RCT and OLE visits; and during the Safety/End of Study visit. Biomarkers will be also collected on week 0 and week 30, 1(+3 days) and 7 days (±3 days) post-infusion. On week 20, week 38 and week 54, biomarkers will be collected 7 (±3 days) days post-infusion. Samples may be collected from subjects in a non-fasted state (you may eat before visits). Biomarkers for the Group/Cohort 2 will follow the OLE collection schedule.

Pregnancy Test: If you are a woman able to get pregnant, you will have a urine pregnancy test at the screening visit and other visits. If results show that you are pregnant (or if you are breastfeeding), you cannot participate in this research study. A second pregnancy test will be performed to confirm the positive urine test. About a half teaspoon of blood will be drawn for the confirmatory test. Pregnant and nursing women are not allowed to participate in this study.

Vital Signs: Vital signs include blood pressure, heart rate, respiratory rate, temperature, height - obtained at the first visit only, and weight. These assessments will be completed before and after each infusion.

Forced Vital Capacity (FVC) and Maximum Inspiratory Pressure (MIP): Reduction of pulmonary function is the primary cause of death in people with ALS. FVC and MIP testing is used to monitor your lung function. FVC measures the maximum amount of air you can blow out from the lungs after taking the deepest breath possible. FVC and MIP will be measured at the start of the study and at various times throughout the study.

The Appel ALS Score (AALS): The Appel ALS score consists of five sub-scores: bulbar (related to swallowing and speech function), respiratory, muscle strength, and arm and leg function. The rate of change in the Appel ALS score is a significant predictor of survival for people with ALS.

The ALSFRS-R (ALS Functional Rating Scale-Revised): The ALSFRS-R is a quickly administered questionnaire used to determine a person's assessment of their capability and independence in 12 different activities. The ALSFRS-R is used as a clinical assessment in ALS standard of care and in most ALS clinical trials.

Questionnaire tool called the Columbia Suicide Severity Rating Scale (C-SSRS): One of the study staff members will ask you questions about your mental health related to suicidal thinking and behavior. This questionnaire will take about 5-10 minutes to complete and will be done at every visit. The research team may refer you for counseling or follow-up if there is concern.

Fasciculations (muscle twitching) Diary: You will receive a diary at each visit (beginning at the Screening Visit) so that you can record how severe your fasciculations are each day. A study staff member will review this diary with you at each visit.

Neurological and Physical Examination: You will have a neurological exam to check your senses, reflexes, strength, balance, and coordination. A physical exam will include an exam of your heart, lungs, abdomen and skin. These will occur at the screening visit and at the end of the study.

Electrocardiogram (ECG): An ECG evaluates the electrical activity of your heart and will be performed at the screening visit, and at the middle and end of the study.

Data Storage & Sharing: Personal health information, such as name, date of birth, and medical record, will be shared with Research personnel at the University of Texas Health Science Center at Houston (UTHealth), who are manufacturing (producing) the Tregs with Methodist Hospital Researchers to ensure you receive only your Tregs back during an infusion and to comply with UTHealth Treg manufacturing requirements. This will include information such as name, date of birth (DOB) and the date of disease onset. At the end of this study, all shared data will be de-identified (meaning all identifying information, including dates, will be removed leaving the uniquely assigned code). The de-identified dataset, combined with information from other research studies and projects, will then become available for sharing with the research community.

Every effort will be made to protect your personal health information privacy and security.

Data from this study will be stored in a data repository at the Massachusetts General Hospital (MGH) Neurological Clinical Research Institute (NCRI).

Global Unique Identifier (GUID) Code: As part of your participation in the study, a unique subject number will be assigned to you that will allow researchers to see if you have been involved in more than one research study or have been entered into the database for patients with ALS through the North Eastern ALS Consortium that is part of the project. This subject number will allow your de-identified data to be combined with data from other research studies. Only this subject number and not your personal identifiable information will be accessible to other investigators. This unique code number may make it possible for a study doctor who used this unique code number in another study that you took part in to identify your coded information.

STUDY VISITS: - Please refer to the Schedule of Events at the end of this consent form.

SCREENING VISIT – VISIT 1: - all participants

During this visit, you will review, discuss, and sign the consent form, we will do some tests and procedures to see if you qualify to take part in this research study. The study doctor will review the results of these tests and procedures. If you do not qualify, the study doctor will tell you why.

Length of time = 3 Hours

At this visit, we will:

- Review this informed consent
- Check your eligibility to participate in this study
- You may not take part in this study if you have persistent asthma, have a prior history of a certain type of allergic reaction, a severe allergic response to any drug, or an allergic response that resulted in swelling of the face, tongue, airway, abdomen, arms, legs, or body.
- Review your ALS diagnosis history, medical history, medications, and demographic information
- Perform physical and neurological examinations and vital signs
- Obtain a 12-Lead Electrocardiogram (ECG)
- Collect screening and safety blood samples
- Complete questionnaires: C-SSRS assessment (Baseline), ALSFRS-R and Appel ALS Score
- Obtain Forced Vital Capacity (FVC) and Maximum Inspiratory Pressure (MIP) measurements
- Review instructions for and provide the fasciculations (muscle twitch) diary

LEUKAPHERESIS (1 & 2): - all participants

Length of time = 9-10 Hours

Between 3 to 6 weeks after you complete your screening visit, the leukapheresis procedure will be completed at the Methodist Blood Center. There is a possibility that a second leukapheresis procedure will need to be done based on how much Treg product is available for use in all infusions. We will let you know if this is needed as soon as we determine a second leukapheresis needs to be collected. The following procedures and tests will be performed during the leukapheresis procedure. The procedure will be performed in the Houston Methodist Blood Donor Center within the first:

- Re-review study eligibility, changes in your health condition, ask about medications and supplements
- Collect blood sample for biomarker and study drug testing and safety labs
- Perform Leukapheresis at HMH
- There is a possibility the second leukapheresis will not be needed, we will know when all participants have been enrolled
- Receive first IL-2 or placebo dose and dispense IL-2 or placebo administration, discuss and monitoring of injection sites
- Educate on study drug compliance and who to call for questions/concerns

Study Drugs

During Stage 1: you will receive Tregs or matching Treg placebo, once a month, and IL-2 or matching placebo injections under the skin, 3 times per week, for 24 weeks.

During Stage 2: all participants in Group/Cohort 1 and Group/Cohort 2 will receive increasing doses of their own Tregs once a month and active IL-2 under the skin injections 3 times per week for 24 weeks.

For all infusions, you will be asked about your health status prior to the infusion. If you have had a recent severe cold or flu before the infusion, you may need to be rescheduled to another day to allow your immune system to recover. You will be monitored for the potential for an allergic response to the infusion while you are in the infusion area and asked about the use of non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin, ibuprofen, or naproxen; or ACE inhibitors which are typically used for blood pressure management, heart conditions, or kidney protection related to diabetes. There are other medications in these classes. The research team will review your medications prior to each infusion to check on NSAID or ACE inhibitor use. We ask about these medications because they may impact how your body responds to the Treg or placebo infusion.

Prior to your infusion, you will be assessed for the need to pre-medication with medications such as diphenhydramine (Benadryl) and/or corticosteroids prior to the infusion to prevent an infusion

reaction. The research physician and team will review your past history of allergic response to medications or other products, your current health status, any recent severe cold or flu, and any other health issue that may impact your safety with the Treg or placebo infusion.

Please notify the research physician and/or team if you suspect any response to the Treg or matching placebo infusion during the infusion and any time after the infusion.

If you have an allergic response to the Treg or Matching Placebo infusion, you will be monitored and possibly asked to stay overnight at Houston Methodist for observation for your overall safety and to identify any delayed allergic response to the infusion.

Following all infusions, you will be contacted within 24 to 48 hours to ask you whether you have any changes, including an allergic response to the infusion or to your health status related to the infusion.

INFUSION VISIT - WEEK 0-Cohort 1-Double-Blind or "WEEK 26'-Cohort 2-Open label:

Length of time = 3-4 hours

The following will be performed:

- Review current health status, changes in your health condition, and ask about medications and supplements that you are currently taking and/or any therapies you are receiving
- Measure vital signs (blood pressure, heart and breathing rates, temperature) and weight
- Collect blood and urine for routine safety lab tests and biomarkers
- Complete C-SSRS assessment (since last visit), and ALSFRS-R and Appel ALS Score
- Obtain Forced Vital Capacity (FVC) and Maximum Inspiratory Pressure (MIP) measurements
- Intravenous Tregs or matching placebo infusion and monitoring
- Review study drug compliance
- Review plan for administering IL-2 or placebo injection and monitoring
- Review and dispense fasciculations (muscle twitch) diary

WEEK 54 (+ or – 7 days): - all participants

Length of time = 3-4 hours

The following will be performed:

- Perform a neurological examination
- Review any concerns, side effects, and/or changes in your health condition, ask about medications and supplements that you are currently taking

- Measure vital signs and weight
- Collect blood sample for Routine safety lab tests and biomarkers
- Complete C-SSRS assessment (since last visit), Perform ALSFRS-R and Appel ALS Score
- Obtain Forced Vital Capacity (FVC) and Maximum Inspiratory Pressure (MIP) measurements
- Intravenous Tregs infusion and monitoring
- Return IL-2 or matching placebo use
- Review and dispense fasciculations (muscle twitch) diary

FINAL VISIT/EARLY DISCONTINUATION VISIT - WEEK 58 – all participants

Length of time = 3-4 hours

The following will be performed:

- Perform physical and neurological examinations
- Perform a 12-Lead Electrocardiogram (ECG)
- Review concerns, any side effects, and changes in your health condition, ask about medications and supplements that you are currently taking
- Measure vital signs and weight
- Collect blood sample for routine safety lab tests and biomarkers
- Complete C-SSRS assessment (since last visit), and Perform ALSFRS-R and Appel ALS Score
- Obtain Forced Vital Capacity (FVC) and Maximum Inspiratory Pressure (MIP) measurements
- Review and dispense fasciculation (muscle twitch) diary
- Collect unused IL-2 vials

Additional study visits and procedures:
You will also be asked to return one day (+3 day) and 7 days (+/-3 days) after the first study infusion visit (Week 0) and the OLE, for Groups/Cohorts 1 and 2: (Week 34), and 7 days (+/- days) after your last infusion on at week 20 and the OLE (Week 50). The following will be performed:

- Review side effects and changes in your health condition
- Ask about medications and supplements that you are currently taking and/or any therapies you are receiving
- Collect blood sample for biomarkers
- Assess study drug compliance

Photographs and video:

For this study and to follow your progress, we are requesting photos and/or video while you are in the study. Collecting photos and/or video of you is not required to participate in the study. Photos and/or video of you will not be shared without your additional written permission. If your photo or video should be used in a scientific publication, you will not be identified by name and your face be covered to protect your identity.

___Yes, I consent to photos and/or video No, I do not consent to photos/video

Tissue donation

In the future, if you are interested in donating some of your tissue or organs for research after you die, this statement allows you to express your wishes to your family for an autopsy and your permission to donate your brain, spinal cord, and samples of skin, liver, spleen, diaphragm, skeletal muscle and nerve and connective tissue to Houston Methodist Neurological Institute. This statement for tissue donation is to alert the research team and your family of your wish to donate your organs. This statement is included because an individual in Texas cannot consent to an autopsy in advance; a legally valid consent can be obtained only after death and only from the next of kin. By signing here you are indicating your willingness to have tissue samples saved and stored for research testing at some future date.

□I agree to tissue donation upon my death (sign): _____ Date: _____

□I decline to donate tissue or organs (sign): _____

What risks will I face by taking part in the study and how will Researchers protect me from these risks?

This is a research study and there is no guarantee that this study regimen will be helpful for your condition. The study drug could make your condition worse. This process has only been conducted in 3 people previously and during study intervention their condition seemed to stabilize. At the end of study intervention, their disease progression resumed.

The combination therapy of Tregs and IL-2, or increasing your Tregs in a lab to infuse back into you, is not a U.S. Food and Drug Administration (FDA) approved drug. This means that Tregs and IL-2 together can only be used in research studies. However, IL-2 alone is a therapy that is FDA approved for the treatment of some cancers. It is important to note that in this study IL-2 will be given in much lower doses than typically used in other patient populations. In this study, IL-2 is being used to help the development, expansion, activity, and survival of Tregs.

As with any research study, there may be additional risks that are unknown or unexpected. If these become known, the study team will notify you in a timely manner of any changes that may alter your willingness to participate. If new information is provided to you after you have joined the study, it is possible that you may be asked to sign a new consent form that includes the new information.

If you experience side effects or worrisome changes in your health, please tell your study doctor right away. If you have a health emergency, seek emergency medical attention.

Risk of Taking Tregs Infusions: Treg infusions could cause an allergic or even anaphylactic reaction during or after the infusion process. It is very important that you let us know if you have ever had a severe allergic reaction, have persistent asthma, or ever experienced angioedema (hives, rapid swelling of the skin, face, throat, or other area of your body). If you have had any of these you should not participate in this study.

You will need to let the research physician and team know if you are using certain medications such as ACE inhibitors (certain blood pressure medications that we can check on with you) or frequent long-term use of non-steroidal anti-inflammatory medications such as ibuprofen, aspirin, or naproxen. These types of drugs may interfere with your body's response to the infusion and would require the research physician and you to determine whether you need pre-medications such as corticosteroids, diphenhydramine (Benadryl), or similar to prevent a potential adverse reaction to the Treg or Placebo infusion.

We also ask that you notify your research physician and team if you have experienced a severe upper respiratory infection (cold or flu) at least a day or more **prior to each infusion** so we can determine if you are able to have the Treg or matching placebo infusion on your scheduled day. We ask that you notify the research physician or team if you have had a recent severe cold or flu before your planned infusion. Having a recent cold or flu prior to an infusion may increase your chance of having an adverse infusion reaction, which we will avoid for your safety.

An anaphylactic reaction to the Tregs infusion would be an emergency and may require you to be immediately taken to the emergency department, have additional blood tests (blood culture, lactic acid level, white blood cell count), and remain in the hospital overnight or longer. A severe anaphylactic reaction can result in death. Infusions of Tregs could also potentially increase the rate of your disease progression, especially once the Treg infusions are stopped at the end of the study. In the first pilot study, all 3 subjects experienced clinical deterioration when the Treg infusions were stopped. In this study, we have had one participant have a serious allergic reaction after the second Treg or Placebo infusion. This participant's infusion response was managed and they were back to normal by the next day. Due to the allergic response, the participant was removed from the study. Tregs infusions, especially in combination with IL-2, could increase your risk of developing infections as both therapies will suppress your immune

response and ability to fight off an infection. The development of cancer is also a potential risk of long-term Treg administration.

Risk of Taking the Matching Treg Placebo Infusions: The Matching "Treg Placebo: (will contain normal saline, small amount of human serum albumin (blood protein) and small amount of DMSO, cell preservative, per infusion). The risks of having an IV infusion is discomfort, redness, and/or infection at the IV insertion site. The IV infusion will occur in an experienced infusion center that follows strict IV practices. Risks related to the normal saline in the infusion includes fluid retention, electrolyte imbalance, and increase in blood pressure. Adverse events related to human serum albumin is rare. However there are risks related to the human serum albumin which includes infusion site reactions, hypersensitivity reactions, fluid retention, pulmonary edema, a potential risk that the product could contain infectious agents, headache, a change in the sense of taste, myocardial infarction, disrupted heart rhythms low blood pressure, flushing, difficulty breathing, nausea, vomiting, rash, itching, fever, and chills.

You will be monitored closely throughout the infusion and the infusions to prevent these risks.

Possible side effects of Interleukin-2 (IL-2) Injections: Under the skin injections of IL-2 has been previously utilized in studies involving ALS patients. You will be taught how to self-administer the IL-2. The medication will be dispensed to you at each infusion or delivered directly to your home via commercial courier with temperature controls, and will be provided free of charge to study participants.

IL-2 known and common side effects include:

- Redness and mild discomfort at the site injection

Occasional:

- Fatigue, Fever, Nausea, Vomiting, Cough, Rash,
- Low blood counts (white blood cells, red blood cells, and platelets may temporarily decrease in number)
- Changes in mental status, such as confusion, drowsiness, and memory loss
- Fast heart rate
- Changes in liver function
- Generalized aches and pain
- Swelling of the face, ankles or legs

As with any drug, an allergic reaction can occur. Allergic reactions can be mild or more serious, and can even result in death. Common symptoms of an allergic reaction are rash, itching, skin problems, swelling of the face and throat, or trouble breathing. If you think you are having an

allergic reaction, call the study doctor right away. If you are having trouble breathing, call 911 immediately.

Risks to an Embryo or Fetus, or to a Breastfeeding Infant: The effects of Tregs on an embryo or fetus (a developing baby still in the womb), or on a breastfeeding infant, are unknown and may be harmful. Because of these unknown risks, women cannot take part in this study if they are pregnant, trying to become pregnant, or breastfeeding. A man of reproductive capacity, unwilling to use effective contraception for the duration of the trial and for 3 months after completing the study, cannot take part in this study.

If you are a post-menopausal woman and have not had a period for the past 12 months or more, you will not need to have a pregnancy test. Also, you will not need to have a pregnancy test if you have had a hysterectomy (surgical removal of your uterus and/or ovaries). All other female participants must have a negative pregnancy test before starting the study drug.

If you are sexually active and able to become pregnant, you must agree to use one of the birth control methods listed below. You must use birth control for the entire study and for 3 months after your last dose of study drug.

Acceptable birth control methods for use in this study are:

- Hormonal methods, such as birth control pills, patches, injections, vaginal ring, or implants
- Barrier methods (such as a condom or diaphragm) used with a spermicide (a foam, cream, or gel that kills sperm)
- Intrauterine device (IUD)
- Abstinence (no sex)
- One partner who is surgically sterile

If you miss a period, or think you might be pregnant during the study, you must tell the study doctor immediately. If you become pregnant, you must stop taking the study drug and stop taking part in the study. The study doctor may ask for your permission to collect information about the outcome of your pregnancy and the condition of your newborn.

For male subjects, if your female partner misses a period, or thinks she might be pregnant during the time you are in the study and 3 months after, you must tell the study doctor immediately. The study doctor may ask for your partner's permission to collect information about the outcome of her pregnancy and the condition of her newborn. You will not have to stop taking the study drug or stop taking part in the study if your partner becomes pregnant.

Risks of taking Tregs and IL-2 with Other Medications: There are no known medications that are absolutely contraindicated (not allowed) while taking Tregs and IL-2. However, taking any medication that suppresses the immune system (immunosuppressant) may result in a higher risk

of developing infections. Do not take any other investigational therapy during the course of this research study, as the therapy could be a risk to your health and would complicate the study results.

Risks of dimethyl sulfoxide (DMSO): DMSO has been used extensively for the preservation of autologous stem cell grafts (frozen stem cells grown from a person's blood). Infusions of products containing DMSO are associated with well-known side effects that increase with the amount of DMSO infused. Mild adverse events include nausea, vomiting, headache, flushing, chest tightness, hypotension (low blood pressure), bradycardia (low heart rate) and abdominal cramps. Serious side effects include hypertension (high blood pressure), arrhythmias (abnormal heart rhythms), cardiac arrest (heart attack), multi-organ failure, encephalopathy (brain dysfunction) and stroke. Autologous stem cell infusions usually consist of high volumes more than 50 mL (milliliters) containing 10 % DMSO. The amount of infused preservative with the Tregs will be 1 mL (about 1.7 ounces or 3.3 tablespoons) containing 10% DMSO (100 microliters of DMSO), which should reduce the risk of DMSO-associated adverse reactions.

Risks of Leukapheresis: Potential risks related to the leukapheresis procedure include nausea, vomiting, fainting or dizziness, hematoma at the access site, seizures, blood loss, pain, injection, hypotension, and tingling in the lips, hands, and face. Your clotting cells, the platelets, might temporarily decrease, however, the body normally replaces platelets in one to two days after the procedure. Most patients tolerate this procedure without any issues. It is unusual and not expected, however there is a possibility the Treg cells removed from during leukapheresis will not grow in the lab and will therefore be unable to be made into a Treg product for infusion. If this occurs, study discontinuation may occur.

Risks of citrate anticoagulation used during leukapheresis: Possible side effects include muscle cramping, numbness, sensation of feeling cold, tingling in the hands, lips, and face, feeling of anxiety, and red blood cell damage.

Risks of Blood Draw: You may have a bruise (a black and blue mark) or pain where the blood sample is drawn. There is also a small risk of infection, lightheadedness, and/or fainting.

Risks of Forced Vital Capacity (FVC) and Maximum Inspiratory Pressure (MIP): The risks and discomforts associated with FVC testing include feeling tired, light-headed or short of breath. These symptoms should disappear with rest.

Risks of Questionnaires: The ALSFRS-R, C-SSRS Questionnaires and Fasciculations assessment may cause you to feel sad or upset about how ALS has affected your abilities to perform daily activities and your quality of life. You may get tired or bored when we are asking you questions or you are completing questionnaires. You do not have to answer any question you do not want to answer. All of your answers will be kept confidential.

Confidentiality Risks: Every possible effort will be made to keep the research information strictly confidential. However, we cannot provide an absolute assurance that unintentional disclosures may not occur.

Other Risks: Reviewing health related information might be stressful or make you feel uncomfortable. You do not have to answer any questions you do not want to, and you may stop the interview at any time if it is too uncomfortable. There may be other risks to taking the drug used in this study that are not known yet. It is possible that the study drug may make your symptoms of ALS worse or make your ALS progress faster.

Incidental Findings: It is possible that the research procedures could uncover information related to your health that you did not know about before and that is unrelated to the study. For example, routine lab tests may detect other health conditions that may be of interest to you or your doctor. In case of any clinically important incidental findings, a study physician will be in contact to discuss these results with either you or a designated physician of your choice.

Unknown Risks: There may be additional risks that we are not aware of at this time. Please tell the researches in the contact section about any injuries, side effects, or other problems that you have during this study. You should also tell your regular doctors.

The researchers have taken steps to minimize the risks of this study through periodic phone calls, study visits, physical exams, and blood tests. If you experience any abnormal effects that feel life threatening please call 911 and then reach out to the research team.

Please tell the researchers in the contact section about any injuries, side effects, or other problems that you have during this study. You should also tell your regular doctors**.**

How could I and others benefit if I take part in this study?

We do not know if taking part in this study will benefit you. We hope that Tregs and IL-2 infusions taken at the right doses will slow the rate of progression of ALS, but we cannot guarantee that taking Tregs and IL-2 during the study will help you. No benefit is expected if you are assigned to receive placebo. Others with ALS or who will develop ALS in the future may benefit from what is learned in this research study.

What is the cost of participating in this study?

The funding agencies for the study will cover the cost of the research study procedures as noted above. Most of the exams, tests, and procedures you will have are part of the study. The study will cover the cost of all your study related procedures and study medications detailed in this consent form. However, some of your visits may occur at the same time as your usual follow-up visits for treatment of your ALS. These usual clinic visits will be billed as they are now to you or your insurance company. There are some extra tests, procedures and exams that you will need to

have if you take part in this study. These will be paid for by the study and you will not be responsible for any of these charges.

The Study team will review a list of procedures with you that show which are standard of care and which are research only. Unless a procedure is listed as 'research' you should expect that you and your insurance company will be responsible for payment of items and services. You will be responsible for your normal co-payments and co-insurance/deductibles. If you have a question about the cost of participation, ask for more information before deciding to participate in the study.

Will I be paid for participating in this study?

You will not be paid for participating in this research study. You will receive reimbursement for parking.

Who could profit or financially benefit from the study results?

This study is being funded by The ALS Association, ALS Finding a Cure and the MDA. There is no drug company that owns the study drug being tested or has any financial interest in the outcome of the study. Payments are made to Houston Methodist and the funds are used to cover the expenses of the study and related academic and research activities of the institution.

The investigator and Houston Methodist do not have any financial interest in the outcome of the study.

If commercial products or other valuable discoveries result from this research project, these products and discoveries could be patented, licensed, or otherwise developed for commercial sale by Houston Methodist or their respective designees. If this should occur, there are no plans to provide financial compensation to you. There are no plans for you to share in the patent rights, other ownership rights, or rights to control the commercial products and discoveries that may result from this research project.

If I take part in this study, can I also participate in other studies?

Being in more than one research study at the same time, or even at different times, may increase the risks to you. It may also affect the results of the studies. You should not take part in more than one therapeutic study at a time without approval from the researchers involved in each study. Non-interventional studies, such as biomarker studies, would be permitted during your participation. Please discuss your participation with your study doctor or study staff prior to enrolling in any other research studies.

If I want to stop participating in the study, what should I do?

If you wish to stop your participation in this research study for any reason you should let the principal investigator/study coordinator know as soon as possible so that you can stop safely. You may be asked why you are leaving the study and your reasons for leaving may be kept as part of the study record. If you decide to leave the study before it is finished, please tell one of the persons listed in "Contact Information".

Could the researchers take me out of the study even if I want to continue to participate?

The researchers could remove you from the study if:

- ✓ The researcher believes that it is not in your best interest to stay in the study.
- ✓ You become ineligible to participate.
- ✓ Your condition changes and you need treatment that is not allowed while you are taking part in the study.
- ✓ You do not follow instructions from the researchers.
- ✓ The study is suspended or canceled.

If you are taken out of active participation, ongoing follow-up may continue at your regularly scheduled clinic visits or with your permission, on the same schedule as if you were still in the study.

What happens if I get hurt, my condition worsens, or have other problems as a result of this research?

If you are injured as a direct result of this study, medical care is available. In general, no long-term medical care or financial compensation for research-related injuries will be provided by Houston Methodist. You do not waive (give up) any legal rights by signing this informed consent form.

What information about me could be seen by the researchers or by other people? Why? Who might see it? How will it be protected?

- Methodist Researchers, the UTHealth Treg manufacturing team, and the Massachusetts General Hospital Neurological Institute Coordinating Center involved in managing this study may see information about you and your study participation to be able to carry out this study and ensure your Tregs come back to you when it is time for infusion. The
- Regulatory bodies such as the institutional review board, data and safety monitoring personnel such as the Barrow Neurological Institute Monitoring group associated with the North Eastern ALS (NEALS) Consortium, auditors, study monitors, Federal agencies such as the FDA, and state and hospital agencies who may inspect your records to audit the research.
- Non-research staff within Methodist who need identifiable information to do their jobs for treatment, payment/billing or hospital operations.

The results of this study may be published in a journal or used to teach other. Your name or other identifying information will not be used. The research team, hospital personnel, UTHealth, MGH, NEALS have training on protecting your privacy and data security and will work diligently to protect your data by carefully guarding your data and , sharing your data with only those who need access to your data for research or hospital operations, and using a code to de-identify your data throughout the trial.

Release of Health Information – If you decide to participate in this study, information about your health may be used or disclosed (shared outside of the Hospital) for the purposes of conducting this study. This information may include information from your medical record that is relevant to this study, such as your medical history, medications, test results, diagnoses, treatments, operative reports (reports from operations that you have undergone), and discharge summaries. It may also include information relating to: Human Immunodeficiency Virus ("HIV") infection or Acquired Immunodeficiency Syndrome ("AIDS"); treatment for or history of drug or alcohol abuse; or mental or behavioral health or psychiatric care. Information collected by the study doctor and/or research staff specifically for this study, such as test results, blood samples, physical examinations, information about possible side effects, and surveys you might be asked to complete could also be used or disclosed.

Individuals that may use or release this information include: physicians, physicians' office staff, hospital staff, the study doctor, and authorized members of the study doctor's research staff. These individuals may release this information to the study doctor, authorized members of the study doctor's staff, the funding agencies of the study; the ALS Association, ALS Finding a Cure and the MDA, as well as its agents or contractors, other researchers, the Institutional Review Board (IRB), the United States Food and Drug Administration (FDA) and its representatives, and other government agencies.

In most cases, the information released to the above listed individuals or entities will not contain your name, social security number, or any other personal information. However, authorized representatives of your study doctor, IRB, FDA, or other government agencies may review records containing personal information to make sure that the study information is correct. Because of the need to provide information to these parties, absolute confidentiality cannot be guaranteed.

Use of Information – This information may be used to determine whether you meet all requirements for participation in the study, to monitor your healthcare during the study, to enable the sponsor to answer the scientific questions for which the study was designed, and to ensure that the study has been done properly. Examples of the use of this information are as follows: the sponsor may use the information in submissions to government agencies throughout the world, to request approval of the study drug or device; the sponsor may use the information for reporting adverse events to government agencies, such as the FDA; the sponsor may also transfer the information to business partners or companies it hires to provide study-related services; the sponsor may also provide overall study results, including your information, to other study doctors;

and the sponsor may reanalyze the data from this study in the future or combine it with data from other studies for analysis. In addition, both the sponsor and the study doctor may use the information to prepare reports or publications of the study results. However, when results of the research study are reported in medical journals or at scientific meetings, the people who were in the study are not named and identified. Therefore, your names would not be disclosed in any presentation or publication.

You need to understand that once your information has been released, it may no longer be protected by US federal regulations relating to data privacy and could be used or re-disclosed in ways other than those listed in this section of the consent form.

You have the right to see and copy your medical records but information relating to this study will be withheld until the end of this study.

Will my genetic information be protected?

Your genetic information will not be collected in this particular study, but some future studies may examine your DNA, the genetic information you inherited from your parents (genetic testing). Genes are substances in the body that are responsible for passing traits such as eye color from parents to children. Other genes affect the chances that a person will get a certain disease or the way their bodies respond to medicines. Because certain genes may have a different form from person to person, one person may respond differently to the same drug. This study could help researchers understand why people may react differently to the study drug and how the body uses the study drug.

Genetic Research Risks: The Sponsor has taken steps to safeguard your genetic testing information, so the risk of loss of confidentiality is small. However, if confidentiality is broken, results of genetic testing may become available to insurance carriers or employers. The knowledge of this information has the potential to lead to discrimination in employment or insurance. Someone with a known genetic condition indicating a susceptibility to develop a disease or condition might be denied a job or a promotion, or denied health or life insurance, because they are regarded as a health risks and therefore an economic risk. Carriers for a genetic disorder might be discriminated against and viewed as having the potential to have a child with a genetic condition.

A Federal law, called the Genetic Information Nondiscrimination Act (GINA) generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.

- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans had to follow this law by May 21, 2010. All employers with 15 or more employees had to follow this law by November 21, 2009. Be aware that this Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

What happens to information about me after the study is over or if I cancel my permission?

If you stop participating in this study, you also have the right to revoke (withdraw) your authorization to disclose and use your information. Revoking your authorization means taking back the permission you gave the study doctor to send information about you to the sponsor or other people and entities. If you revoke your authorization, your doctor will not use or release any more information about you after receiving your request, except to tell the sponsor that you have stopped early and have revoked your authorization. However, the sponsor and the study doctor can still keep and use any information that it has already received to the extent necessary to preserve the integrity of the research study. To revoke this authorization, contact the research team. The research team will accept either a written or verbal request.

For withdrawal of samples

If you agree to allow your tissue/blood/cells to be kept for future research with identifying information that could link your sample to you, you are free to change your mind at any time. We ask that you contact Dr. Appel or Dr. Thonhoff in writing and let him know you are withdrawing your permission for your identifiable tissue, blood or cells to be used for future research. His mailing address is 6560 Fannin St., Suite 802. Houston, TX 77030. At that time, we will ask you to indicate in writing if you want the unused identifiable tissue, blood or cells destroyed or if your samples (having all identifying information removed that would link the sample to you) could be used for other research.

When does my permission expire?

Because this information is being disclosed for research use, there is no expiration date for the authorization to disclose and use this information. The sponsor may keep and continue to use your study information for many years. Your study doctor may need to add to or correct information about you even after your study participation is over; including providing updates of your health status if that is important to the purpose of the study. The review of your medical records may

also take place after the study is over. This authorization will remain in effect unless you revoke it.

Authorization– By signing this consent form, you authorize the use and disclosure of personal information to, and review of your medical records by, the people and entities described above. You do not have to authorize this disclosure of information. However, if you do not, you will not be able to participate in this study.

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights.

For questions about your rights as a research participant, or if you have complaints, concerns, or questions about the research, please contact Susan M. Miller, M.D., M.P.H., Chair, Houston Methodist Research Institute Institutional Review Board for the Protection of Human Subjects, at 713-441-2750 or Ethan Natelson, MD, Chair, Houston Methodist Research Institute Institutional Review Board for the Protection of Human Subjects, at 713-441-5154. You may also contact the Director, HMRI Office of Research Protections at HMRI Office of Research Protections, 6670 Bertner Avenue, Houston, Texas 77030, Ph.: 346-356-1400The research team will take proper precautions to ensure that any information regarding your identity obtained in connection with this research will remain confidential; however, confidentiality cannot be guaranteed.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Where can I get more information?

If you have any questions regarding your participation in this study, please ask us. If you have any additional questions later, please contact the researchers listed below to:

Principal Investigator: Jason R. Thonhoff, M.D., Ph.D
Mailing Address: 6560 Fannin Street, Suite 802, Houston, Texas 77030
Telephone: 713-441-7310

Office of Neuromuscular Research – Clinical Research Team
713-441-5855, 713-441-9120, 713-441-5192, 713-441-3420

Signatures

Study Participant

I have read this consent form or had it read to me. I have discussed it with the study team and my questions have been answered. I will be given a signed copy of this form. I agree to take part in this study including any options where I checked 'yes'.

Signature:_____**Date**:_____**Time**:_____

Name (Print Legal Name): _____

Person Obtaining Consent:

I have given this research subject information about this study that I believe is accurate and complete. The subject has indicated that he or she understands the nature of the study and the risks and benefits of participating.

Name:_____Title: _____

Signature:_____Date:_____Time: _____

Translation Service: I verbally translated the informed consent process and the conversation between the person obtaining consent and the study participant.

Name:_____Organization: _____

Signature:_____Date:_____Time: _____

Witness ***(required if 'short form' used for translation, or when participant physically unable to read, write, talk or see):*** I was present as an impartial witness (not a member of the research team or family) for the informed consent process. I observed the above subject (or his/her legally authorized representative, if applicable) indicate consent.

If applicable participant has capacity to consent but is unable to sign, how did he or she indicate consent: _____

Witness Name: _____

Signature:_____ Date: _____

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	V1 Screen-ing	V2 Leuka-pheresis	V3 RCT 1			V4[12] RCT 2	V5[12] RCT 3		V6[12] RCT 4	V7[12] RCT 5	V8[12] RCT 6			V9[12] Follow- Up Visit
		Week -6 to -3	Infusion Week 0 Day 0	Wk 0 Day 1 +3 Days	Wk 1 Day 0 ±3 Days	Infusion Week 4 ±7 Days	Infusion Wk 8 ±7 Days	Wk 9 ± 3 Days	Infusion Week 12 ±7 Days	Infusion Week 16 ±7 Days	Infu-sion Week 20 ±7 Days	Wk 20 Day 1 + 3 day	Wk 21 Day 0 ±3 Days	Week 24 ±7 Days
Consent	X													
Medical	X	X												
Safety Labs	X													
Vital Signs, Height Weight	X		X			X	X		X	X	X			X
Physical Exam	X													X
EKG	X													X
Questionnaires	X		X			X	X		X	X	X			X
Breathing Tests	X		X			X	X		X	X	X			X

1.2 SCHEDULE OF ACTIVITIES (SOA) – Study Table for Period 1 – Randomized Controlled Trial (RCT)

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			
Teaching of IL-2 or placebo administration[9] (administer prior to infusion; at least 2 weeks to 4 weeks –		X~												
First dose of IL-2 or placebo[10] (3x/wk dosing throughV9.IL-2 dosing for all subjects restarting at least 14 days prior to Week 26(V11) through Week 50 (V17) follow-up visit)		X~												
Return of IL-2 or placebo supplies			X			X	X		X	X	X			X
Discuss fasciculations diary	X		X			X	X		X	X	X			
IL2 or placebo accountability compliance			X			X	X		X	X	X			X
Blindedness Questionnaire														X

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

	V10[12] Leukapheresis #2– if needed	**V11 OLE 1**			**V12 OLE 2**	**V13 OLE 3**		**V14 OLE 4**	**V15 OLE 5**	**V16 OLE 6**		
	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**	**Week 46** Day 1 + 3 Days	**Wee 47** ±3 Day
Written Informed												
Vital Signs, Height and Weight		X			X	X		X	X	X		
Physical Exam												
Neurological												
12-Lead ECG												
C-SSRS[4]		X			X	X		X	X	X		
ALSFRS-R and Appel ALS Scale		X			X	X		X	X	X		
Forced Vital Capacity (FVC) and maximum inspiratory pressure (MIP) Measurements		X			X	X		X	X	X		
Assess Survival	X	X	X	X	X	X	X	X	X	X	X	
Concomitant	X	X	X	X	X	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	

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Safety Labs[5]	X	X			X	X		X	X	X	
Leukapheresis	X										
Blood & Urine biomarkers[6]	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	
Review fasciculations diary		X			X	X		X	X	X	
Drug accountability compliance		X			X	X		X	X	X	
Exit Questionnaire[12]											

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