USES OF REGULATORY T CELLS FOR TREATING AMYOTROPHIC LATERAL SCLEROSIS

RELATED APPLICATIONS

[0001] This application claims priority to, and the benefit of, U.S. Provisional Application Nos. 63/567,034, filed on March 19, 2024. The contents of the aforementioned patent application are incorporated herein by reference in their entirety.

TECHNICAL FIELD

[0002] The disclosure is related to medical uses of T regulatory cells for treatment of amyotrophic lateral sclerosis.

BACKGROUND

[0003] Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that affects motor neurons, with the typical time from symptom onset to death of 2-3 years (Hardiman et al., (2011) *Nat Rev Neurol* 7(11): 639-649; Westeneng et al., (2018) *Lancet Neurol* 17(5): 423-433). Among the neurodegenerative disorders, ALS is the most rapidly fatal (Kiernan et al., (2011) *Lancet* 377(9769): 942-955). To date, there are no available treatments that slow the decline of skeletal muscle function, and in particular, slow the decline of respiratory function. There is a need for additional ALS treatments.

SUMMARY

[0004] Provided herein is a method for treating amyotrophic lateral sclerosis (ALS) in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq$ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and $\leq$ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0005] Provided herein is a method for treating amyotrophic lateral sclerosis (ALS) in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq$ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the

$CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0006] Provided herein is a method for treating amyotrophic lateral sclerosis (ALS) in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0007] Provided herein is a method for ameliorating a symptom of ALS in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0008] Provided herein is a method for ameliorating a symptom of ALS in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0009] Provided herein is a method for ameliorating a symptom of ALS in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0010] In some embodiments of the methods provided herein, the symptom of ALS is weakness, fatigue, paralysis, decline of skeletal muscle function, or decline of respiratory function.

[0011] In some embodiments of the methods provided herein, the subject has the following: (a) ALS onset ≤ 5 years; (b) upright Slow Vital Capacity (SVC) as adjusted for sex, age and height ≥ 50% predicted; and (c) ALSFRSR (Revised ALS Functional Rating Scale) score of 36-45 at baseline.

[0012] In some embodiments, the method further comprises administering to the subject one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol.

[0013] In some embodiments, the method further comprises measuring: (a) the presence and/or levels of a set of cytokines; and/or (b) the presence and/or levels of neurofilament light chain and/or neurofilament heavy chain, in the peripheral blood and/or cerebrospinal fluid (CSF) of the subject. In some embodiments, the measuring is performed at one or more of: baseline, 5 weeks after the first dose of Treg cells, 13 weeks after the first dose of Treg cells, 25 weeks after the first dose of Treg cells, 36 weeks after the first dose of Treg cells, 48 weeks after the first dose of Treg cells, and 4 weeks after last dose of Treg cells.

[0014] In some embodiments, the set of cytokines comprises: 6CKine, BCA-1, CTACK, EGF, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, FGF-2, Flt3L, Fractalkine, G-CSF, GM-CSF, GROα, I-309, IFNα2, IFNγ, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17E/IL-25, IL-17F, IL-18, IL-20, IL-21, IL-22, IL-23, IL-27, IL-28, IL-33, IP-10, LIF, MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, MDC, MIG, MIP-1α, MIP-1β, MIP-1δ, PDGF-AA, PDGF-AB/BB, RANTES, sCD40L, SCF, SDF-1α+β, TARC, TGFα, TNFα, TNFβ, TPO, TRAIL, TSLP, and VEGF-A.

[0015] In some embodiments, the set of cytokines comprises: TGFb1, TGFb2, and TGFb3.

[0016] In some embodiments, the set of cytokines comprises: BDNF, Cathepsin D, MPO, NCAM, PAI-1 (total), PDGF-AA, PDGF-AB/BB, RANTES, sICAM-1, and sVCAM-1.

[0017] In some embodiments, the set of cytokines comprises: sCD30, sEGFR, sgp130, sIL-1RI, sIL-1RII, sIL-2RA, sIL-4R, sIL-6R, sRAGE, sTNFRI, sTNFRII, sVEGFR1, sVEGFR2, and sVEGFR3.

[0018] In some embodiments, the set of cytokines comprises: HCKP1-16-10 - BTLA, CD27, CD28, CD40, CD80, CD86, CTLA4, GITR, GITRL, HVEM, ICOS, LAG3, PD-1, PD-L1, TIM-3, and TLR-2.

[0019] In some embodiments, the set of cytokines comprises: Granzyme A, Granzyme B, Perforin, sFas, and sFas Ligand.

[0020] In some embodiments, the set of cytokines comprises: MMP-1 (Collagenase 1), MMP-2 (Gelatinase A), MMP-3 (Stromelysin 1), MMP-7 (Matrilysin), MMP-8 (Collagenase 2),

MMP-9 (Gelatinase B), MMP-10 (Matrix Metalloproteinase 10), MMP-12 (Macrophage Metalloelastase), MMP-13 (Collagenase 3), TIMP-1, TIMP-2, TIMP-3, and TIMP-4.

[0021] In some embodiments, the set of cytokines comprises: ACTH, DKK-1, FGF-23, IL-1β, IL-6, Insulin, Leptin, PTH, OC, OPG, OPN, SOST, and TNFα.

[0022] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0023] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0024] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0025] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0026] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0027] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0028] In some embodiments of the cryopreserved, allogeneic, cord blood derived Treg cells for use provided herein, the symptom of ALS is weakness, fatigue, paralysis, decline of skeletal muscle function, or decline of respiratory function.

[0029] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0030] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0031] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0032] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0033] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0034] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0035] In some embodiments of the combinations for use provided herein, the symptom of ALS is weakness, fatigue, paralysis, decline of skeletal muscle function, or decline of respiratory function.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] FIG. 1 depicts a study design schematic for a Phase 1/1b, open-label study of CK0803 in amyotrophic lateral sclerosis.

[0037] FIG. 2 depicts a decision tree for the safety cohort of the Phase 1/1b, open-label study of CK0803 in amyotrophic lateral sclerosis. "TLT" = treatment-limiting toxicity. "DMC" = data monitoring committee.

[0038] FIG. 3 is a schematic of the CK0803 Treg dual selection process.

[0039] FIG. 4 depicts results from an analysis of the dual selected CK0803 CB Treg cell population phenotype.

[0040] FIG. 5 depicts results from a Tcon cell suppression assay.

[0041] FIG. 6 depicts results from an assay measuring IL-10 secretion by CK0803.

[0042] FIG. 7A – FIG. 7B depict a transwell migration assay analyzing CK0803 cell migration in response to ICAM-1. **FIG. 7A** depicts a diagram of the transwell insert. **FIG. 7B** is a bar graph depicting results from the assay.

[0043] FIG. 8 depicts results from a Treg cell subset analysis comparing total Tregs in CK0803 and control CB Tregs.

[0044] FIG. 9A – FIG. 9D depict results from a Treg cell subset analysis comparing Th17.1 Tregs (**FIG. 9A**), Th1 Tregs (**FIG. 9B**), Th2 Tregs (**FIG. 9C**), and Th17 Tregs (**FIG. 9D**) in CK0803 and control CB Tregs.

[0045] FIG. 10 depicts results from an analysis of expression of CXCR3 in CK0803 Treg cells.

[0046] FIG. 11A is bar graph of the average GVHD score calculated in control mice (PBMC only) or infused with exemplary CB Treg (PBMC+Treg).

[0047] FIG. 11B is a collection of representative histology sections of various tissues from GVHD model mice treated with exemplary CB Treg (PBMC+Treg) or vehicle (PBMC only).

[0048] FIG. 11C are bar graphs of inflammation biomarkers measured from blood sera from control mice (PBMC only) or infused with exemplary CB Treg (PBMC+Treg).

[0049] FIG. 12A shows representative skin histology sections from a xenogeneic systemic lupus erythematosus (SLE) mouse with (treatment) and without (control) CB Treg infusions.

[0050] FIG. 12B shows levels of CD8+ T cells in peripheral blood, spleen, lung, and liver of an SLE mouse model with (treatment) and without (control) CB Treg infusions, as explained in Example 3 herein.

[0051] FIG. 12C shows the distribution of different T cell populations in an SLE mouse model with (treatment) and without (control) CB Treg infusions, as explained in Example 3 herein.

[0052] FIG. 13 is a survival probability curve of COVID-19 patients infused with placebo, 100 million exemplary CB Tregs, or 300 million exemplary CB Tregs.

[0053] FIGs. 14A and **14B** show CK0803 treatment schedules used with subjects with severe ALS. Further details on the treatment schedule are described in Example 7 below.

[0054] FIGs. 15A-15D are plots of the ALSFRSR scores over time of 4 subjects with severe ALS treated with CK0803. Further details on the treatment are described in Example 7 below.

[0055] FIGs. 16A and **16B** are plots of the ALSFRSR score (**FIG. 16A**) and neurofilament concentration (FIG. 16B) over time in a subject with fast-progressing ALS treated with CK0803. Further details on the treatment are described in Example 7 below.

[0056] FIGs. 17A and **17B** are line plots over time of ALSFSRS scores (**FIG. 17A**) and forced vital capacity (**FIG. 17B**) of a subject with lung-dominant ALS treated with CK0803. Further details on the treatment are described in Example 7 below.

[0057] FIG. 17C is a line plot over time of the percentage of peripheral blood Treg cells in a subject with lung-dominant ALS treated with CK0803. Further details on the treatment are described in Example 7 below.

[0058] FIGs. 17D and **17E** are correlation plots of neurofilament concentration (**FIG. 17D**) and ALSFSRS score (**FIG. 17E**) to percentage of peripheral blood Treg cells in a subject with lung-dominant ALS treated with CK0803. Further details on the treatment are described in Example 7 below.

[0059] FIG. 18 shows the biodistribution of CB Tregs administered to a xenogeneic GVHD mouse model, as explained in Example 3 herein.

[0060] FIGs. 19A-19D are graphs showing trajectories of ALSFRS-R scores in ALS participants receiving TREG infusion therapy. **FIG 19A** shows ALSFRS-R scores over time for individual ALS participants. The x-axis shows months relative to the first infusion, and the y-axis shows the ALSFRS-R score. Shaded regions indicate treatment intervals: pre-treatment (before any infusion), on-treatment (receiving infusions), and post-treatment (after all infusions). Vertical dashed lines show timing of TREG infusions. Spearman correlation coefficients between ALSFRS-R scores and time are shown for each participant and treatment phase. **FIG. 19B** shows the distribution of ALSFRS-R slopes during each treatment interval, with colors corresponding to specific participants. Negative slopes indicate functional decline, while positive slopes suggest stabilization/improvement. **FIG. 19C** shows the correlation between NfL and ALSFRS-R trajectories in ALS participants receiving TREG infusion therapy. Normalized trajectories of ALSFRS-R scores (points) and NfL concentrations (triangles) over time for individual participants. Left four panels show plasma NfL, right four panels show CSF NfL. Shaded regions and vertical lines as in FIG. 1A. Spearman correlation coefficients between ALSFRS-R and NfL are shown for each participant and fluid type (rho = Spearman correlation, SD = standard deviation, n = number of paired measurements). **FIG. 19D** shows the correlation between NfL and ALSFRS-R trajectories in ALS participants

receiving TREG infusion therapy. Spearman correlation coefficients between ALSFRS-R scores and NfL concentrations for individual participants. Point shading indicate correlation strength from blue to red. Circle sizes represent correlation precision (1 / confidence interval width), with larger circles indicating higher precision.

[0061] FIG. 20A and FIG. 20B shows the correlation of Plasma and CSF Biomarker Trajectories with ALSFRS-R Scores in ALS Participants Receiving TREG Infusion Therapy. **FIG. 20A** is a heatmap showing Spearman correlation coefficients between trajectories of plasma biomarkers and ALSFRS-R scores for individual ALS participants receiving TREG infusion therapy. The color scale ranges from blue (negative correlation) to red (positive correlation). Circle size represents correlation precision (1 / confidence interval width), with larger circles indicating higher precision. Rectangles represent correlations where the confidence interval crosses zero, and demonstrate no statistical significance. The neurofilament light chain (NfL) biomarker is highlighted in green. **FIG. 20B** is a heatmap showing Spearman correlation coefficients between trajectories of CSF biomarkers and ALSFRS-R scores, formatted as in **FIG. 20A**. Participants and biomarkers are clustered hierarchically based on correlation patterns. Note that correlations are exploratory and not adjusted for multiple comparisons. A box shape for a particular biomarker-participant combination indicates a confidence that overlaps zero and the possibility of no correlational relationship can't be ruled out.

[0062] FIGs. 21A-21D show exploratory analysis of Key biomarker correlations with ALSFRS-R scores in ALS participants receiving TREG infusion therapy. **FIG. 21A** shows the comparison of median Spearman correlations between biomarkers and ALSFRS-R scores in CSF and plasma. Each point represents a biomarker, with x-axis showing median correlation in CSF and y-axis showing median correlation in plasma. Point size and shading intensity represent the precision of the correlation (1/overall median confidence interval width). The dashed red line represents the line of equality. **FIG. 21B** shows trajectories of MIP-1δ concentrations (triangles) and ALSFRS-R scores (circles) over time for individual participants. Top panels show CSF measurements, bottom panels show plasma measurements. The x-axis represents months since the first infusion, and the y-axis represents normalized concentrations or scores. Shaded regions indicate treatment intervals: pre-treatment, on-treatment, and post-treatment. Vertical dashed lines denote TREG infusions. Spearman correlation coefficients (rho) and sample sizes (n) are shown for each participant and fluid type. **FIG. 21C** shows trajectories of CTACK concentrations and ALSFRS-R scores, formatted as in FIG. 21B. **FIG.**

21D shows trajectories of GROα concentrations and ALSFRS-R scores, formatted as in FIG. 21B.

[0063] FIG. 22 shows Longitudinal plots of safety measures for each participant over the course of TREG treatment. The x-axis represents months since first infusion, and the y-axis shows the measured values. Top panels: Blood WBC count (10^3 cells/μl). Middle panels: CSF protein concentration (mg/dL). Bottom panels: CSF total nucleated cell count (cells/μl). Each participant is represented in a separate column. Shaded regions indicate treatment intervals: pre-treatment (red), on-treatment (green), and post-treatment (blue). Vertical dashed lines denote TREG infusions. Horizontal dashed lines represent the normal range for each measure (WBC: 3.12-8.44 x 10^3 cells/μl; CSF protein: 15-45 mg/dL; CSF cells: 0-5 cells/μl). Points outside the normal range are highlighted.

[0064] FIG. 23 shows various measurements over the course of the study described in Example 8 herein. The top left panel of FIG. 23 shows longitudinal increase in peripheral blood Tregs over time (n=1). The top right panel of FIG. 23 shows ALSFRS-R score over time during TREG treatment. The middle left panel of FIG. 23 shows ALS quality of life score (ALSQoL) over time during TREG treatment. The middle right panel of FIG. 23 shows Forced Vital capacity (FVC) over time during TREG treatment. The bottom left panel of FIG. 23 shows Edinburgh Cognitive and Behavioral ALS Screen (ECAS) over time during TREG treatment.

[0065] FIG. 24A shows the percentage of Tregs cells in the CSF as measured by flow cytometry, at Baseline and an on-treatment timepoint (Follow Up, 6M for TREG_4 and 1M for TREG_5) (n=2). **FIG. 24B** shows the percentage of CD8+ cells in the CSF as measured by flow cytometry, at Baseline and an on-treatment timepoint (Follow Up) (n=2).

DETAILED DESCRIPTION

[0066] Provided herein are therapies for human subjects with Amyotrophic Lateral Sclerosis (ALS). The therapies comprise administering CD11a-enriched T regulatory (Treg) cells to a subject. The CD11a-enriched Treg cells are thought to address neuroinflammation, which is a key driver in ALS. Continuous expression of CXCR3 (chemokine (C-X-C motif) receptor 3) on T cells and expression of its ligand, CXCL10 (interferon (IFN)-g-inducible protein of 10 kDa (IP-10)/CXC chemokine ligand 10) have been shown to be elevated in neuroinflammatory conditions. The CD11a-enriched Treg cells target the CXCR3/CXCL10 axis.

[0067] CD11a (also known as ITGAL) is the alpha-chain of $\alpha_L\beta 2$ integrin (also known as leukocyte function associated Ag 1, lymphocyte function-associated molecule 1, or LFA-1)

(see, *e.g.*, Bose et al., (2014) *J Immunol* 193(6): 2863–2872). LFA-1 integrin activation is crucial for inflammatory cell adhesion and is regulated by complementary mechanisms involving affinity alterations due to rapid conformational changes, as well as affinity-independent mechanisms such as integrin lateral mobility, resulting in valency and avidity increases (Choi et al. (2008) *Blood* 111(7): 3607-3614). Cytokines released over the course of inflammation may induce expression of ICAM-1 (Intercellular Adhesion Molecule 1) on neurons, allowing them to be targeted by leukocytes expressing the appropriate receptors (Birdsall (1991) *Am J Pathol* 139(6): 1341-1350). LFA-1 (via CD11a) is a cell surface adhesion receptor for ICAM-1 (Edwards et al., (1995) *J Biol Chem* 270(21): 12635-12640). Furthermore, Th17 cells infiltrate into the brain parenchyma of Parkinson's disease mice through a lesioned blood–brain barrier and exert a neurotoxic property by promoting glial activation and importantly by direct damage to neurons depending on LFA-1/ICAM-1 interaction (Liu et al., (2017) *Mol Neurobiol* 54(10): 7762-7776). Similarly, Treg cells depend on LFA-1 (CD11a) to enter CNS (Glatigny et al. (2015) *Sci Rep* 5: 7834).

[0068] Therefore, we hypothesize that CD11a expressed on allogeneic CB Treg cells presents itself as an ideal neurotropism candidate that will allow for the infused CB Treg cells to home to the areas of neuroinflammation in subjects suffering from ALS. Once in the tissue, these CD11a-expressing CB Treg cells could potentially block the cytotoxic T cells, decrease microglia injury, and restore immune balance. Furthermore, a steady state concentration of these healthy CD11a-expressing Treg cells in the body will be ensured by multiple doses infused over time, allowing these cells to impact any residual disease or escaped cells ensuring disease remission.

[0069] The methods, uses, and pharmaceutical combinations provided herein comprise use of a Treg cell population referred to as "CK0803". CK0803 is a population of cryopreserved, allogeneic, cord blood-derived Treg cells that are CD11a-enriched. CK0803 is cryopreserved at a dose of 100 million Treg cells per dose. CK0803 is produced from an umbilical cord blood unit that is not HLA-matched to the intended recipient. Exemplary characteristics of CK0803 are shown in **Table 1**.

Table 1. Exemplary CK0803 characteristics

Manufacturing Process	
Starting Material	CBU
CMV status CBU	CMV positive or negative
HLA Matching	No HLA Matching
Enrichment	Dual selection, CD8+ depletion followed by CD25+ selection on Day 0

Culture media	OpTmizer with 1% Glutamine and 10% human AB serum
Culture cytokine	IL-2
Culture beads	CD3/28
Culture duration	14 +/- 2 days
Bead removal	Remove beads (CTS DynaMag) Wash and Concentrate (LOVO) Add cryoprotectant solution & Fill Frozen Final Product in Bags Controlled Rate Freeze
Rapid Release	N/A
Appearance	Liquid suspension, clear to slightly opaque, colorless to slightly yellow, with no visible extrinsic particulates
Sterility	No Growth
Endotoxin	< 5EU/mL
Mycoplasma	Negative
Residual beads	< 100 per 3xe6 nucleated cells
%Viability of Total Nucleated Cells	>70%
$CD3^+CD4^+CD25^+$ T-reg cells	≥60%
$CD3^+CD4^+CD25^+CD11a^+$ T-reg cells	≥70%
$CD3^+CD4^+CD25^+CXCR4^+$ T-reg cells	NA
$CD3^+CD4^-CD8^+$ Cytotoxic/ suppressor cells	≤ 10%
Product formulation	Plasma-Lyte A with 0.5% Albumin (Human), which results in a final DMSO concentration of 5% (v/v).
Cryopreservation	Yes cryopreservation.
Final DMSO concentration	5% (v/v)
Storage package	cryobag
Storage temperature	stored at ≤ -150°C in vapor phase LN2, until immediately before thaw and administration
MOA	Suppression of inflammation
Indirect mechanisms	Secretion of IL-10 Depletion of IL-2 APC priming
Direct mechanism	Secretion of Perforin/ Granzyme
Target receptor	CXCR3/CXCL10 axis
Tissue target	CNS

Clinical	
Disease of interest	Amyotrophic lateral sclerosis
Route of administration	intravenously
Reformulation/ dilution prior to infusion	Not needed
Dose	Fixed dose: 100 million cells
Cell state at infusion	Thawed
Infusion instructions	Infused by gravity, intravenously and within 60 minutes of thawing. Total time (60 minutes) includes bag rinse.
Treatment plan	Multiple infusions: Weekly infusions x 4 followed by monthly infusions x 5

[0070] CK0803 Treg cells have the phenotype of: $\geq$ 60% $CD3^+CD4^+CD25^+$ (T regulatory phenotype), wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and $\leq$ 10% $CD4^-CD8^+$ (T-cytotoxic/suppressor phenotype). Treg cell phenotype is determined by flow cytometry analysis.

[0071] In some embodiments, CK0803 Treg cells further have the phenotype of about 85% $CD3^+CD4^+CD25^+FoxP3^+$, about 93% $CD3^+CD4^+CD25^+CD11a^+$, about 85% $CD3^+CD4^+CD25^+Helios^+$, and/or about 3% $CD3^+CD4^-CD8^+$. In some embodiments, CK0803 Treg cells are at least 91% or at least 92% $CD3^+/CD4^+CD25^{hi}$.

[0072] In some embodiments, each lot of CK0803 is manufactured from a single allogeneic umbilical cord blood unit (CBU) collected and banked from a healthy, normal donor who has met the U.S. Food and Drug Administration (FDA) requirements for screening and testing for transmissible disease, in compliance with 21 CFR Part 1271, Subpart C. The manufacturing process involves $CD8^+$ T cell depletion followed by CD25 selection, thereby isolating a pure population of Treg cells that express neurotropic homing markers including CD11a, followed by culture-expansion. A schematic of the manufacturing process is shown in **FIG. 3**. The resulting expanded Treg cells are formulated in a cryoprotectant solution, placed into cryobags, cryopreserved, then stored and shipped in the vapor phase of liquid nitrogen until they are ready to be thawed and infused into a patient.

[0073] The CK0803 final product is provided as a frozen suspension of cells, at a cell dose of 100 million Tregs expressing neurotropic markers, in an infusible cryopreservation solution containing dimethyl sulfoxide (DMSO) and Dextran 40, contained in a sealed Ethyl Vinyl Acetate (EVA) plastic freeze bag. After the product is thawed at the clinical site, the bag's port can be accessed using the plastic spike of a conventional intravenous (IV) administration.

[0074] CK0803 must be maintained continuously in the frozen state at ≤ -150°C during storage and transport, until immediately before thaw and infusion. In some embodiments, CK0803 is thawed rapidly by immersion in a 37°C water bath. The thawed product should not be diluted, washed, or otherwise manipulated prior to infusion. Once thawed, the product must be held and transported at controlled room temperature and infused as soon as possible. The cells are infused into a subject within 60 minutes of being thawed.

[0075] In some embodiments, CL0803 is administered by gravity flow through standard infusion tubing set without any filters, at a rate of approximately 5mL per minute. In some embodiments, an infusion pump is not used.

[0076] In some embodiments, a subject is pre-medicated 30 to 60 minutes prior to infusion of CK0803 with a histamine blocker such as diphenhydramine (*e.g.*, 25 mg). In some embodiments, a subject is pre-medicated prior to infusion of CK0803 with acetaminophen (*e.g.*, 650 mg).

[0077] In some embodiments, a single dose of CK0803 Treg cells is about 1 x 10^8 Treg cells.

[0078] In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses. In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days ± 2 days for 4 doses, and then administered once every 28 days ± 2 days for 5 doses. In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days ± 1 day for 4 doses, and then administered once every 28 days ± 1 day for 5 doses. In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days for 4 doses, and then administered once every 28 days for 5 doses.

[0079] In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses. In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days ± 2 days for 4 doses, and then administered once every 28 days ± 2 days for at least about 5 doses. In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days ± 1 day for 4 doses, and then administered once every 28 days ± 1 day for at least about 5 doses. In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days for 4 doses, and then administered once every 28 days for at least about 5 doses.

[0080] In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses. In some embodiments, a dose of CK0803 Treg cells is administered

to a subject once every 7 days ± 2 days for 4 doses, and then administered once every 28 days ± 2 days for about 5 doses to about 22 doses. In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days ± 1 day for 4 doses, and then administered once every 28 days ± 1 day for about 5 doses to about 22 doses. In some embodiments, a dose of CK0803 Treg cells is administered to a subject once every 7 days for 4 doses, and then administered once every 28 days for about 5 doses to about 22 doses.

[0081] Provided herein is a method for treating ALS in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1×10^8 Treg cells.

[0082] Provided herein is a method for treating ALS in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1×10^8 Treg cells.

[0083] Provided herein is a method for treating ALS in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1×10^8 Treg cells.

[0084] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1×10^8 Treg cells.

[0085] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0086] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0087] Provided herein is a method for ameliorating a symptom of ALS in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0088] Provided herein is a method for ameliorating a symptom of ALS in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0089] Provided herein is a method for ameliorating a symptom of ALS in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0090] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0091] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0092] Provided herein are cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0093] In some embodiments, a symptom of ALS is weakness, fatigue, paralysis, decline of skeletal muscle function, or decline of respiratory function.

[0094] In some embodiments, the subject has the diagnosis of ALS, meeting the possible, laboratory-supported probable, probable, or definite criteria for diagnosing ALS according to the Revised El Escorial Criteria for ALS (Brooks et al., (2000) *Amyotroph Lateral Scler Other Motor Neuron Disord* 1(5): 293-299).

[0095] In some embodiments, the subject has one, two, or three of the following characteristics: (a) ALS onset ≤ 5 years; (b) upright Slow Vital Capacity (SVC) as adjusted for sex, age and height ≥ 50% predicted; and (c) ALSFRSR (Revised ALS Functional Rating Scale) score of 36-45 at baseline.

[0096] In some embodiments, a method provided herein further comprises administering a concomitant medication to the subject. In some embodiments, a concomitant medication is a medication that treats ALS. In some embodiments, a concomitant medication is riluzole (*e.g.*, RILUTEK®). In some embodiments, a concomitant medication is edaravone (*e.g.*, RADICAVA®). In some embodiments, a concomitant medication is sodium phenylbutyrate and taurursodiol (*e.g.*, RELYVRIO® or ALBRIOZA™). In some embodiments, a subject is

on a stable dose of riluzole, edaravone, and sodium phenylbutyrate and taurursodiol for ≥ 30 days prior to the first dose of Treg cells.

[0097] In some embodiments, a concomitant medication is a combination of one or more of: riluzole, edaravone, and sodium phenylbutyrate and taurursodiol. In some embodiments, a method provided herein comprises further administering to a subject riluzole and edaravone. In some embodiments, a method provided herein comprises further administering to a subject riluzole and sodium phenylbutyrate and taurursodiol. In some embodiments, a method provided herein comprises further administering to a subject edaravone and sodium phenylbutyrate and taurursodiol. In some embodiments, a method provided herein comprises further administering to a subject riluzole, edaravone, and sodium phenylbutyrate and taurursodiol.

[0098] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1×10^8 Treg cells.

[0099] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1×10^8 Treg cells.

[0100] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in treating a subject having ALS, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then

administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0101] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0102] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0103] Provided herein is a pharmaceutical combination comprising: (a) cryopreserved, allogeneic, cord blood derived Treg cells, wherein the Treg cells have the phenotype of: ≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and ≤ 10% $CD4^-CD8^+$; and (b) one or more of: (i) riluzole; (ii) edaravone; and (iii) sodium phenylbutyrate and taurursodiol; for use in ameliorating a symptom of ALS in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses; wherein each dose is about 1 x 10^8 Treg cells.

[0104] In some embodiments, a method provided herein further comprises measuring the presence and/or levels of neurofilament light chain and/or neurofilament heavy chain, in the peripheral blood and/or cerebrospinal fluid (CSF) of a subject.

[0105] In some embodiments, a method provided herein further comprises measuring the presence and/or levels of a set of cytokines in the peripheral blood and/or CSF of a subject. In some embodiments, the cytokines are inflammatory cytokines.

[0106] In some embodiments, measuring (a) the presence and/or levels of a set of cytokines; and/or (b) the presence and/or levels of neurofilament light chain and/or neurofilament heavy chain, is performed at one or more of: baseline, 5 weeks after the first dose of Treg cells, 13 weeks after the first dose of Treg cells, 25 weeks after the first dose of Treg cells, 36 weeks after the first dose of Treg cells, 48 weeks after the first dose of Treg cells, and 4 weeks after last dose of Treg cells.

[0107] In some embodiments, the set of cytokines comprises: 6CKine, BCA-1, CTACK, EGF, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, FGF-2, Flt3L, Fractalkine, G-CSF, GM-CSF, GROα, I-309, IFNα2, IFNγ, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17E/IL-25, IL-17F, IL-18, IL-20, IL-21, IL-22, IL-23, IL-27, IL-28, IL-33, IP-10, LIF, MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, MDC, MIG, MIP-1α, MIP-1β, MIP-1δ, PDGF-AA, PDGF-AB/BB, RANTES, sCD40L, SCF, SDF-1α+β, TARC, TGFα, TNFα, TNFβ, TPO, TRAIL, TSLP, and VEGF-A. This set is referred to as "Set A".

[0108] In some embodiments, the set of cytokines comprises: TGFb1, TGFb2, and TGFb3. This set is referred to as "Set B".

[0109] In some embodiments, the set of cytokines comprises: BDNF, Cathepsin D, MPO, NCAM, PAI-1 (total), PDGF-AA, PDGF-AB/BB, RANTES, sICAM-1, and sVCAM-1. This set is referred to as "Set C".

[0110] In some embodiments, the set of cytokines comprises: sCD30, sEGFR, sgp130, sIL-1RI, sIL-1RII, sIL-2RA, sIL-4R, sIL-6R, sRAGE, sTNFRI, sTNFRII, sVEGFR1, sVEGFR2, and sVEGFR3. This set is referred to as "Set D".

[0111] In some embodiments, the set of cytokines comprises: HCKP1-16-10 - BTLA, CD27, CD28, CD40, CD80, CD86, CTLA4, GITR, GITRL, HVEM, ICOS, LAG3, PD-1, PD-L1, TIM-3, and TLR-2. This set is referred to as "Set E".

[0112] In some embodiments, the set of cytokines comprises: Granzyme A, Granzyme B, Perforin, sFas, and sFas Ligand. This set is referred to as "Set F".

[0113] In some embodiments, the set of cytokines comprises: MMP-1 (Collagenase 1), MMP-2 (Gelatinase A), MMP-3 (Stromelysin 1), MMP-7 (Matrilysin), MMP-8 (Collagenase 2), MMP-9 (Gelatinase B), MMP-10 (Matrix Metalloproteinase 10), MMP-12 (Macrophage Metalloelastase), MMP-13 (Collagenase 3), TIMP-1, TIMP-2, TIMP-3, and TIMP-4. This set is referred to as "Set G".

[0114] In some embodiments, the set of cytokines comprises: ACTH, DKK-1, FGF-23, IL-1β, IL-6, Insulin, Leptin, PTH, OC, OPG, OPN, SOST, and TNFα. This set is referred to as "Set H".

[0115] In some embodiments, a method provided herein further comprises measuring the presence and/or levels of a set of cytokines in the peripheral blood and/or CSF of a subject, wherein the set of cytokines comprises one or more of Sets A-H.

[0116] References to methods of treatment in this description are to be interpreted as references to compounds, pharmaceutical compositions and medicaments of the present invention for use in those methods.

[0117] In some embodiments, the subjects to be treated using the methods of the present disclosure are at least 18 years of age.

[0118] In some embodiments, the subjects to be treated using the methods of the present disclosure have experienced ALS onset within about 1 year prior to treatment, or within about 2 years prior to treatment, or within about 3 years prior to treatment, or within about 4 years prior to treatment, or within about 5 years prior to treatment. In some embodiments, the subject treated using the methods of the present disclosure have experienced ALS onset within about 3 years prior to treatment. In some embodiments, the subject treated using the methods of the present disclosure have experienced ALS onset within about 5 years prior to treatment.

[0119] In some embodiments, the subjects to be treated using the methods of the present disclosure have a slow vital capacity (SVC) or greater than or equal to 50%. In some embodiments, the SVC value is adjusted for sex, age, and or height of the subject. In some aspects, the SVC is measured using an upright sitting position.

[0120] In some embodiments, the subjects to be treated using the methods of the present disclosure have a Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) score of about 36 to about 45 prior to the initiation of treatment.

[0121] In some embodiments, the subjects to be treated using the methods of the present disclosure have been previously treated with riluzole, edaravone, albrioza, or any combination thereof. In some aspects, the subjects have been treated with riluzole, edaravone, albrioza, or any combination thereof at a stable dose for at least about 30 days prior to the initiation of treatment using CK803.

DEFINITIONS

[0122] Unless otherwise noted, the terms used herein have definitions as ordinarily used in the art. Some terms are defined below, and additional definitions can be found within the rest of the detailed description.

[0123] The term "a" or "an" refers to one or more of that entity, *i.e.*, can refer to plural referents. As such, the terms "a," "an," "one or more," and "at least one" are used interchangeably herein. In addition, reference to "an element" by the indefinite article "a" or "an" does not exclude the possibility that more than one of the elements is present, unless the context clearly requires that there is one and only one of the elements.

[0124] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device or the method being employed to determine the value, or the variation that exists among the samples being measured. Unless otherwise stated or otherwise evident from the context, the term "about" means within 10% above or below the reported numerical value (except where such number would exceed 100% of a possible value or go below 0%). When used in conjunction with a range or series of values, the term "about" applies to the endpoints of the range or each of the values enumerated in the series, unless otherwise indicated. As used in this application, the terms "about" and "approximately" are used as equivalents.

[0125] As used herein, the terms "treat," "treating" or "treatment of" (and grammatical variations thereof) mean that the severity of the subject's condition is reduced, at least partially improved or stabilized and/or that some alleviation, mitigation, decrease or stabilization in at least one clinical symptom is achieved and/or there is a delay in the progression of the disease or disorder.

[0126] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include" and "comprise" are used synonymously.

[0127] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited herein, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. In the event that one or more of the incorporated documents or portions of documents define a

term that contradicts that term's definition in the application, the definition that appears in this application controls. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment, or any form of suggestion, that they constitute valid prior art or form part of the common general knowledge in any country in the world.

[0128] The disclosure will be further clarified by the following examples, which are intended to be purely exemplary of the disclosure and in no way limiting.

EXAMPLES

Example 1. Phase 1 safety Run-in Study and Phase 1b Randomized, Double Blind, Placebo Control Trial of CK0803 in Amyotrophic Lateral Sclerosis

[0129] This is a two-part study consisting of safety run-in of 6 subjects followed by a randomized controlled trial (RCT) of additional 30+30 subjects for a total of 66 subjects treated as follows:

• one dose administered once every 7 days (+/-3) x 4; and thereafter one dose administered once every 28 days (+/-3) x 5

[0130] The first 6 subjects will be part of a safety run-in period with specific guidelines for continuation of treatments to the RCT cohort. The investigational agent is CK0803. Each subject will receive a single infusion of CK0803 at a fixed dose of 100 million Treg cells administered as per the schedule (**FIG. 1**). CK0803 is an allogeneic cord blood (CB) derived T regulatory (Treg) cell product that is enriched in the cell surface markers that allow for its preferential homing to the areas of neuroinflammation in subjects suffering from amyotrophic lateral sclerosis (ALS).

1. OBJECTIVES AND ENDPOINTS

1.1 Objective

[0131] 1.1.1. Primary Objective. The primary objective of the phase 1 Safety Study is to determine safety of CK0803 in subjects with ALS and the primary objective of the phase 1b randomized controlled trial is to determine extended safety and efficacy of CK0803 in subjects with ALS. The safety, tolerability and treatment related toxicities will be determined as described in Section 2.1.

[0132] 1.1.2. Secondary Objective. The secondary objective of this study is to determine the efficacy of CK0803 in subjects suffering from ALS. The response assessment will be defined as in Section 6.3.

[0133] 1.1.3. Exploratory Objective. To assess peripheral blood (PB) and cerebrospinal fluid (CSF) immune reconstitution, serum biomarkers and inflammatory cytokines as well as pharmacokinetics at different time intervals.

1.2 Endpoints

[0134] 1.2.1. Primary Endpoint. The primary endpoint for the phase 1 Safety run-in part is to determine the safety, tolerability and treatment limiting toxicities of CK0803 as assessed by the incidence and severity of adverse events (AEs) and serious adverse events (SAEs) determined by the NCI-CTCAE Version 5.0. The primary endpoint for the phase 1b randomized control trial is to determine extended safety and evaluate the combined assessment of function and survival of subjects with ALS.

[0135] 1.2.2. Secondary Endpoint. The assessment of treatment response, and its duration will be assessed based on the following:

- Incidence of all cause AEs and SAEs
- Longitudinal processes of ALS functional score (ALSFRS-R) measured at baseline and at weeks 5, 8, 12 and/or 13, 16, 20, 24 and/or 25, 36 and 48 from first infusion and/or at end of treatment (EOT)
- Amyotrophic Lateral Sclerosis Specific Quality of Life - Revised (ALSSQOL-R), each measured at baseline and at weeks 5, 8, 12 and/or 13, 16, 20, 24 and/or 25, 36 and 48 from first infusion and/or at EOT
- Slow Vital Capacity (SVC), at baseline and at weeks 5, 13, 25, 36 and 48 from first infusion and/or at EOT
- Handheld dynamometer (HHD), at baseline and at weeks 5, 13, 25, 36 and 48 from first infusion and/or at EOT
- Neurofilament light (NfL) concentration in CSF at baseline and at weeks 5, 13, 25, 36 and 48 from first infusion and/or at EOT
- Neurofilament light (NfL) concentration in serum at baseline and at weeks 5, 13, 25, 36 and 48 from first infusion and/or at EOT
- Ventilation assistance-free survival (VAFS), at baseline and at weeks 5, 8, 12 and/or 13, 16, 20, 24 and/or 25, 36 and 48 from first infusion and/or at EOT, defined as the time to the earliest occurrence of 1 of the following events:

- Death
- Permanent ventilation (> 22 hours of mechanical ventilation [invasive or non-invasive] per day for > 21 consecutive days in the absence of a potentially acute reversible event)

- Overall survival

[0136] 1.2.3. Exploratory Endpoint. Correlative samples from PB and CSF will be collected during the conduct of this study as per the schedule described in **Tables 9A** and **9B** to study the exploratory endpoints as described below.

[0137] 1.2.3.1. Pharmacokinetics

[0138] 1.2.3.1.1. Persistence. We hypothesize that the infused CK0803 cells will home to the area of inflammation and undergo proliferation. The scheduled infusions will allow for continued replenishment on the healthy Treg cells which will then maintain a steady state concentration locally as well as systemically. In order to explore this hypothesis, we will analyze the correlative samples for the origin of the cell populations based on their HLA phenotype as well as their DNA materials as described in Section 6.4.

[0139] 1.2.3.2. Pharmacodynamics

[0140] 1.2.3.2.1. Inflammatory biomarkers. We hypothesize that the regular administration of the CK0803 infusions at scheduled intervals over a treatment period will allow for resolution of neuroinflammation in the ALS subjects. We propose to evaluate such an impact by serial measurement of the inflammatory cytokine levels in the PB and the CSF of the subjects in longitudinal samples over time as described in Section 6.5.1.

[0141] 1.2.3.2.2. Immunogenicity. We hypothesize that due to their derivation from naïve umbilical cord blood, the CK0803 cellular product will not incite an immune reaction and/ or development of allo-specific antibodies. In order to explore this hypothesis, we will examine donor specific antibodies in the PB samples over a period of time as described in Section 6.5.2.

[0142] 1.2.3.2.3. Immune reconstitution. We hypothesize that the infused CK0803 cells will restore the immune homeostasis towards normalization in subjects suffering from the chronic neuro-inflammation observed in patients with ALS. Therefore, we will study the impact of the infused cells on the different compartments of the immune system including their quantification as well as study their functionality as described in Section 6.5.3.

[0143] 1.2.3.3. ECAS. Edinburgh Cognitive and Behavioral ALS Screen (ECAS) baseline and at weeks 5, 8, 12 and/or 13, 16, 20, 24 and/or 25, 36 and 48 from first infusion and/or at EOT as per **Tables 9A** and **9B**.

2. STUDY DESCRIPTION

2.1. Overall Design.

[0144] This is a Phase I Safety run-in study and Phase Ib randomized, double blinded, placebo control trial of CK0803 in subjects with ALS.

[0145] 2.1.1. Safety Run-in. Infusion of CK0803 at dose level 0 of 100 million Treg cells will be explored in this trial. Initially three (3) subjects will be enrolled. The following staggering will be applied to the dosing of the first three subjects.

[0146] *Between doses:* The first three subjects will receive infusions as per the infusion schedule where the interval between each dose in the induction phase is 7 days. This will allow to capture the acute/subacute reaction. If no SAE, then the subject would be eligible to proceed to receive the additional doses.

[0147] *Between subjects:* Subject enrollment will be staggered such that the first subject will have to complete 21 days follow up after their first dose to enroll the second subject. The same staggering will be followed between the 2nd and 3rd patient enrolled. The safety evaluation will be performed by the data monitoring committee (DMC).

[0148] The next cohort of 3 subjects will enter the study on a rolling basis once the third subject has completed 28 days of treatment, and the DMC has given their approval to proceed. Tolerability of the treatment will be assessed based on treatment limiting toxicities (TLTs) occurring up to 28 days after the first infusion.

[0149] 2.1.1.1. Treatment Limiting Toxicity. Treatment-limiting toxicity (TLT) will be defined as the occurrence of any of the toxicities listed in **Table 2** occurring during or after first infusion of CK0803, Day 1 up to and including Day 28, except those with a clear alternative explanation (*e.g.*, disease progression) or transient ($\leq$ 72 hours) abnormal laboratory values without associated clinically significant signs or symptoms based on investigator determination. CK0803 infusion will be monitored as described in Section 4.1.1.

Table 2. Definition of Treatment-Limiting Toxicity

Toxicity	Definition
Non-hematologic	Any ≥ Grade 3 nonhematologic infusion toxicity as per CTCAE v5.0. EXCEPT for the following: Grade 3 cytokine release syndrome that resolves to ≤ Grade 1 within 72 hours with optimal symptomatic management Grade 3 infusion reaction that resolves to ≤ Grade 1 within 6 hours with optimal symptomatic management. Abnormal laboratory values without associated clinically significant signs or symptoms that are corrected to ≤ Grade 1 or baseline with appropriate management or supplementation within 72 hours of onset of treatment. Grade 3 or 4 nausea, vomiting, and diarrhea adequately controlled with medical therapy within 72 hours. Grade 3 rash in the absence of desquamation, with no mucosal involvement, that does not require systemic steroids and that resolves to Grade 1 in ≤ 14 days. Grade 3 hypertension that resolves in ≤ 7 days with optimal therapy. Grade 3 fatigue lasting ≤ 7 days.
Death	Regimen related death within 28 days
Other toxicities not meeting TLT criteria	While the rules for adjudicating TLTs in the context of treatment tolerability are specified above, an AE not listed above may be defined as a TLT after a consultation with the sponsor and investigators based on the emerging safety profile.

[0150] If ≤ 1 of these three (3) subjects experience any TLTs during the observation period of 28 days, then an additional three (3) subjects will be enrolled, and all 6 patients will be observed for at least 28 days. The overview of the decision tree is provided in **FIG. 2**.

[0151] If the number of subjects with TLT(s) specified in **FIG. 2** is reached, then the treatment will be deemed intolerable. It is possible that the Sponsor after consultation with Investigators and the DMC will consider the following change in dosing for the subsequent subjects: Decreasing the CK0803 dose by 50% (Dose level -1).

[0152] TLT occurring during the first 28 days of treatment will guide determination of the tolerability; however, subjects who may not have had a TLT but who had intolerable, lower grade persistent toxicity determined to be attributable to study drug will be considered in the determination of the overall tolerability of the treatment before expansion of the cohort to the additional 34 patients is initiated. Telephone conferences with study investigators will be

scheduled by the sponsor in order to review data and overall safety data, to agree on enrollment of additional cohorts of subjects and on how to adjudicate individual high-grade AEs as potentially treatment-limiting, and to guide other major study decisions. The final decision will be made by the Sponsor.

[0153] 2.1.1.2. Stopping rules. Accrual on the clinical trial will be suspended as below:

• if greater than one treatment limiting toxicity (TLT) as described in **Table 2** is reported in the first 3 subjects; or

• if greater than 2 TLTs are reported in the first 6 subjects of the clinical protocol

[0154] The study will be temporarily suspended for study drug administration, pending a safety investigation. The subject(s) will be observed for 28 days for evaluating the course and determining the tolerability of the TLT. The Data Monitoring Committee (DMC) will review the TLT and results from the investigation and make a determination regarding the suitability of potential dose reduction by 50%.

[0155] Formal safety analyses will be performed during the safety run-in part of the study after the 7 day follow up of the first 3 subjects. Clinical trial accrual will be suspended during this time.

[0156] 2.1.2. Randomized, double blind, placebo control trial. Upon determination of the tolerability of treatment in the safety run-in of the first 6 patients, an additional randomized comparative trial will be initiated, with up to two stages (stages 1 and 2), each stage of size 30 patients, with a safety rule applied after stage 1 that may terminate the trial early, for a maximum total sample size of 6+30+30 = 66 patients. The primary objective of this part of the Study will be combined assessment of function and survival (CAFS) evaluation (Berry et al., (2013) *Amyotroph Lateral Scler Frontotemporal Degener* 14(3): 162-168).

2.2. Overall Study Duration.

[0157] The study begins when the first subject signs the informed consent form (ICF). The duration of each period is described in **Table 3**. A subject is considered to have completed the study if he/she has completed all planned therapy and 28 days of follow up after the last dose.

Table 3. Study Period Duration and Timing

Study Period	Duration/ Timing
Screening Period	30 days prior to start of therapy

Treatment Period	Subjects receiving induction regimen of once every 7 days (+/-3) x 4 doses followed by consolidation of followed by once every 28 days (+/-3) x 5 doses
End of Treatment	28 days after the subject has completed all assigned doses of CK0803 and/or study termination as per Section 2.3
Follow Up Period	28 days after the last dose of CK0803 or until the start of a new therapy (whichever is earlier)
End of Study	Date of the last visit of the last subject in the study
Duration	It is estimated that an individual will participate for approximately 2 years
Survival Follow up	Up to 10 years after last infusion of the last subject

2.3. Study Termination

[0158] The sponsor may terminate the study if required by a regulatory decision or upon advice of the DMC (Section 3.6). If the study is terminated prematurely, the sponsor will notify the investigators, the Institutional Review Boards (IRBs), and the regulatory bodies of the decision and reason for termination of the study. The DMC will recommend termination of the study if warranted, as described in Section 5.1.

3. STUDY POPULATION

[0159] Deviations from eligibility criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability, and/or subject safety. Therefore, adherence to the criteria as specified in the Protocol is essential. Prospective approval of Protocol deviations to recruitment and enrollment criteria, also known as Protocol waivers or exemptions, are not permitted.

3.1. Inclusion Criteria

[0160] 1) Ability of the subject or his/her legally authorized representative (e.g., spouse) to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use confidential health information in accordance with national and local subject privacy regulations.

[0161] 2) Adult ALS subjects (≥18 years of age) with the diagnosis of ALS, meeting the possible, laboratory-supported probable, probable, or definite criteria for diagnosing ALS according to the Revised El Escorial Criteria for ALS (Brooks et al., (2000) *Amyotroph Lateral Scler Other Motor Neuron Disord* 1(5): 293-299).

[0162] 3) Subjects with disease onset ≤ 5 years.

[0163] 4) Upright (sitting position) Slow Vital Capacity (SVC) as adjusted for sex, age and height ≥ 50% predicted.

[0164] 5) Subjects must have documented ALSFRSR score of 36-45 at baseline.

[0165] 6) Subjects taking concomitant riluzole at study entry must be on a stable dose for ≥ 30 days prior to the first dose of study treatment (Day 1).

[0166] 7) Subjects taking concomitant riluzole must be willing to continue with the same dose regimen throughout the study, unless the Investigator determines that riluzole should be discontinued for medical reasons, in which case it may not be restarted during the study.

[0167] 8) Subjects taking concomitant edaravone at study entry must be on a stable dose for ≥ 30 days prior to the first dose of study treatment (Day 1).

[0168] 9) Subjects taking concomitant edaravone must be willing to continue with the same dose regimen throughout the study, unless the Investigator determines that edaravone should be discontinued for medical reasons, in which case it may not be restarted during the study. Edaravone may not be administered on dosing days of this study.

[0169] 10) Subjects taking concomitant albrioza at study entry must be on a stable dose for ≥ 30 days prior to the first dose of study treatment (Day 1).

[0170] 11) Subjects taking concomitant albrioza must be willing to continue with the same dose regimen throughout the study, unless the Investigator determines that albrioza should be discontinued for medical reasons, in which case it may not be restarted during the study.

[0171] 12) Medically able to undergo the study procedures, and to adhere to the visit schedule at the time of study entry, as determined by the Investigator.

[0172] 13) Screening values of coagulation parameters including platelet count, international normalized ratio (INR), prothrombin time (PT), and activated partial thromboplastin time (APTT) should be within normal ranges. Coagulation tests may be repeated once at the local laboratory if, in the opinion of the Investigator, values of the initial tests are out of range but not clinically significant. Subjects with non-clinically significant and stable out-of-range values may be eligible to enroll in the study at the discretion of the Investigator.

[0173] 14) All subjects of childbearing potential must agree to practice highly effective contraception during the study and be willing and able to continue contraception for 90 days after their last dose of study treatment.

3.2. Exclusion Criteria

[0174] 1) Uncontrolled infection, not responding to appropriate antimicrobial agents after seven days of therapy. The Protocol medical monitor is the final arbiter of eligibility.

[0175] 2) Antiplatelet or anticoagulant therapy within the 14 days prior to Day 1 or anticipated use during the study, including but not limited to daily, low-dose aspirin (defined as ≤ 150 mg/day), clopidogrel, dipyridamole, warfarin, dabigatran, rivaroxaban and apixaban.

[0176] 3) Clinically significant low platelet count (defined as < 100,000/mm3), coagulation tests (greater than 2 times upper limit of normal), or laboratory abnormalities that would render a subject unsuitable for inclusion.

[0177] 4) Unwillingness to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator.

[0178] 5) Have any other conditions, which, in the opinion of the Investigator would make the subject unsuitable for inclusion, or could interfere with the subject participating in or completing the study.

[0179] 6) Concurrent participation in any other interventional clinical study.

[0180] 7) Treatment with another investigational drug, biological agent, or device, including, but not limited to sodium phenylbutyrate, within 1 month of Screening, or 5 half-lives of investigational agent, whichever is longer.

[0181] 8) Treatment of cancer in the last 5 years (except in situ carcinoma of the cervix or basal cell carcinoma).

[0182] 9) Female subjects who are pregnant or currently breastfeeding.

[0183] 10) Other unspecified reasons that, in the opinion of the Investigator or Sponsor, make the subject unsuitable for enrolment.

3.3. Lifestyle Considerations

[0184] 3.3.1. Meals and Dietary Restrictions. No specific restrictions are required.

[0185] 3.3.2. Activity. Subjects will abstain from strenuous exercise for 6 hours before each blood collection for clinical laboratory tests. Subjects may participate in light recreational activities (e.g., watching television, reading) immediately following blood collection, laboratory test and/ or study treatment studies.

[0186] 3.3.3. Contraception Requirements. All male subjects and women of childbearing potential (WOCBP) must refrain from sperm/ egg donation and either be abstinent or use highly effective contraception from the time of signing the ICF until at least 90 days after their last dose of Study Drug.

[0187] For the purposes of this study, WOCBP are defined as any female who has experienced menarche, and who does not meet one of the following conditions:

[0188] • Post-menopausal: no menses for 12 months without an alternative medical cause. A high FSH level in the post-menopausal range may be used to confirm a post-menopausal state

in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

[0189] • 6 weeks after surgical bilateral oophorectomy with or without hysterectomy.

[0190] • Post-hysterectomy.

[0191] For the purposes of the study, highly effective contraception is defined as follows:

[0192] • For males: surgical sterilization (vasectomy with negative semen analysis at follow-up, or a surgically sterile non-pregnant female partner), or the non-pregnant female partner of WOCBP uses a highly effective contraceptive method (defined below).

[0193] • For females: surgical sterilization (e.g., hysterectomy, salpingectomy, bilateral oophorectomy or vasectomized male partner), hormonal contraception associated with inhibition of ovulation (combined estrogen and progestogen containing, or progestogen-only), intrauterine contraception device or intrauterine hormone-releasing system (IUS).

[0194] Male subjects with partners that are pregnant must use condoms as contraception to ensure that the fetus is not exposed to the Study Drug.

[0195] Note: Abstinence (i.e., refraining from heterosexual intercourse throughout the duration of study participation) is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial and withdrawal are not acceptable methods of contraception.

[0196] 3.3.4. Other Requirements. Subjects should be encouraged to maintain consistency throughout the study with respect to smoking, caffeine consumption and alcoholic beverage consumption.

3.4. Screen Failures

[0197] Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently entered in the study. Rescreening is permitted. Tests with results that fail eligibility requirements may be repeated once during screening. Additionally, a subject who fails screening may repeat the screening process one time if the investigator believes that there has been a change in eligibility status (e.g., recovery from an infection). Subjects who rescreen must reconsent and be assigned a new subject number.

3.5. Replacement of Subjects

[0198] Subjects may be replaced for any of the following reasons:

[0199] • Any subject who withdraws from treatment before the completion of the TLT observation period (i.e., the 28-day period after the first dose of CK0803) for any reason other

than a TLT (e.g., not evaluable for TLT), should be replaced to ensure a minimum number of evaluable subjects.

[0200] • Subjects who do not meet the eligibility requirements of the study may be replaced.

3.6. Data Monitoring Committee

[0201] The Sponsor and/or Sponsor's designee has established a Data Monitoring Committee (DMC), which will conduct safety and efficacy evaluations quarterly throughout the duration of the study and will be initiated before the first subject has been enrolled. In addition, evaluations will be conducted prior to the initiation of the expansion part and regularly afterwards. The DMC is an expert advisory group, made up of members independent of the Sponsor and/or designee that is responsible for evaluating cumulative safety data at regular intervals. The primary objective of the DMC is to monitor subject safety by reviewing the available clinical data at scheduled time points. The DMC may also conduct ad hoc meetings, as necessary. Following each meeting, the DMC will make a recommendation to Sponsor regarding the study. The details regarding the DMC processes and procedures are outlined in the DMC Charter, which will be finalized prior to the review of any data.

4. STUDY DRUG

[0202] The investigational agent, CK0803 (Allogeneic, Cryopreserved, Cord blood-derived T regulatory cells, expressing neurotropic markers) that is

• prepared from a single unit of umbilical cord blood (CBU) for each batch

• cryopreserved at a dose of 100 million Treg cells per dose

• thawed immediately before administration

• administered by intravenous infusion within 60 minutes of thawing

4.1. Study Drug Administered

[0203] **Table 4** presents the study treatment information.

Table 4. Study Treatment Information

Study drug name:	CK0803
Mechanism of action:	Anti-inflammatory effect exerted by: • Secretion of inhibitory cytokines including IL-10 to suppress inflammation • Metabolic starvation of cytotoxic T cells by depletion of IL-2 • Cell-to-cell regulation through the antigen presenting cells (microglia) • Direct cell kill by secretion of perforin/ granzyme B

Dosage formulation:	CK0803 consists of allogeneic cord blood-derived T-regulatory cells with a flow cytometry phenotype of • ≥ 60% $CD3^+CD4^+CD25^+$ T regulatory phenotype, of which at least 70% have high expression of CD11a and • ≤ 10% $CD4^-CD8^+$ T- cytotoxic/suppressor cells. The CK0803 final product is the cryopreserved form of the liquid cell suspension consisting of the CK0803 cells resuspended in Cryostor® cryoprotectant solution, with a final volume of approximately 20mL.
Unit dose strength(s) /dosage level(s):	100 million Treg cells in ~20 ml to be administered every 7 days (+/-3) days x 4 doses followed by once every 28 days (+/-3) x 5 doses for up to 9 total doses
Administration instructions:	The CK0803 product must be infused by gravity, intravenously ideally within thirty (30) minutes and definitely within one hour of thawing. Total time (60 minutes) includes bag rinse. Detailed instructions in Product Manual.
Packaging and labeling:	The study drug product is cryopreserved and provided in cryobags stored in vapor phase LN2 (< minus 150°C). The product label will include facility manufacturer's name, product name, and any other applicable regulatory requirements.
Storage:	CK0803 is stable when stored at ≤ -150°C vapor phase.
Status of treatment in participating countries:	Investigational

[0204] 4.1.1. Clinical monitoring plan. The investigational agent should be infused by gravity for a period of time not to exceed 60 minutes from the time of thawing (which includes rinsing of the bag). Per standard of care, patient will be pre-medicated with Benadryl 25 mg PO thirty (30) minutes and Paracetamol (Acetaminophen) 650 mg PO before infusion of investigational agent. Patient will receive pre- and post-infusion of 250 mL normal saline bolus over 1 hour. Weight will be measured on the day of infusion, at least 60 minutes prior to infusion. Vital signs including heart rate, blood pressure, temperature, respiratory rate, oxygen saturation will be measured at the following timepoints: pre-infusion, approx. midway through infusion, and 15, 30, 60, 90, 120, 150, 180 mins post-infusion start time. Each time point has a window of +/- 5mins. Infusion reactions including any adverse events and vital signs will be monitored during the infusion and up to 2 hours post-infusion start time.

4.2. Preparation, Handling, and Accountability

[0205] CK0803 will be received at the site in a transport container validated to maintain temperatures of ≤ minus 150°C. CK0803 will be thawed and infused by gravity within thirty (30) minutes and within one hour preferably of thawing. Total time (60 minutes) includes bag rinse. Detailed instructions will be provided in the Product Manual to each clinical site. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatments received and any discrepancies are reported and resolved before use of the study treatment. Only subjects enrolled in the study may receive study drug, and only authorized site staff may supply or administer study drug. All study treatment must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff. The investigator (or designee) is responsible for study drug accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records). Inventory and accountability records must be maintained and readily available for inspection by the study monitor and are open to inspection at any time by any applicable regulatory authorities. The investigator or designee must maintain records that document: Delivery of study drug to the study site; Inventory of study drug at the site; and Subject use of the study drug, including the cryobags from each supply dispensed.

[0206] The investigational product must be used only in accordance with the Protocol. The investigator will also maintain records adequately documenting that the subjects were provided the specified study drug. These records should include dates, quantities, and any available batch or serial numbers or unique code numbers assigned to the investigational product and study subjects. Completed accountability records will be archived by the site. The investigator or designee will be expected to collect and retain all used, unused, and partially used containers of study drug until verified by the study monitor (unless otherwise agreed to by the sponsor). At the conclusion of the study, the investigator or designee will oversee shipment of any remaining study drug back to the sponsor or its designee for destruction according to institutional SOPs. If local procedures mandate on-site destruction of the investigational supply, the site should (where local procedures allow) maintain the investigational supply until the study monitor inspects the accountability records in order to evaluate compliance and accuracy of accountability by the investigative site. At sites where the study drug is destroyed before monitor inspection, the monitors rely on documentation of destruction per the site SOP.

4.3. Measures to Minimize Bias: Randomization and Blinding

[0207] This is an open-label study; no comparisons will be made between subjects or against historical controls. Measurements of safety and efficacy are objective measurements, and only comparisons to pretreatment conditions will be mad.

4.4. Study Treatment Compliance

[0208] Compliance with all study-related treatments should be emphasized to the subject by the site personnel, and appropriate steps should be taken to optimize compliance during the study. Compliance with this protocol will be assessed by the sponsor based on the drug accountability documented by the site staff and monitored by the sponsor/designee. Compliance with study drug administration will be assessed by the sponsor based on study drug accountability and infusion records documented by the site staff and monitored by the sponsor/designee.

4.5. Dose Interruptions

[0209] Dose interruptions may occur for individual study subjects. The occurrence of TLTs and other toxicities (related or unrelated to study drug) will guide decisions for treatment interruptions and discontinuation for individual subjects.

[0210] 4.5.1. Management of Treatment-Limiting Toxicities or Other Urgent Situations. Investigators may employ any measures or concomitant medications necessary to optimally treat the subject after discussion with the sponsor (whenever possible).

[0211] 4.5.2. Follow-Up of Treatment-Limiting Toxicities. Any TLT should be monitored until it resolves to baseline or appears to have stabilized for a minimum of 72 hours. During follow-up, subjects should be seen as often as medically indicated to assure safety.

[0212] 4.5.3. Criteria and Procedures for Dose Interruptions. Safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the subject should continue or discontinue study treatment as shown in **Table 5**.

[0213] Treatment with CK0803 may be delayed up to 2 weeks (14 days) to allow for resolution of a toxicity. Subjects may resume treatment if no medical condition or other circumstance exists that, in the opinion of the investigator, would make the subject unsuitable for further participation in the study. The treating investigator should contact the sponsor to discuss the case of any subject whose treatment has been delayed for more than 14 days before restarting treatment with CK0803.

[0214] Individual decisions regarding dose interruptions should be made using clinical judgment and in consultation with the sponsor's medical monitor, taking into account relatedness of the AE to the study drug and the subject's underlying condition. AEs that have

a clear alternative explanation or transient (≤ 72 hours) abnormal laboratory values without associated clinically significant signs or symptoms may be exempt from dose-interruption rules.

Table 5. Guidelines for Interruption and Restarting of Study Drug

CTCAE v5.0 Grade/ Severity	Hold Treatment	Timing for Restarting Treatment	Dose Level for Restarting Study Drug	Treatment Discontinuation
1-2 (Mild-Moderate)	No	Continue treatment at the discretion of the investigator.	NA	NA
3 (Severe)	Yes	Toxicity resolves to Grade 0-1.	Restart at lower dose level	Toxicity does not resolve within 4 weeks (28 days) of last dose, except by approval of the medical monitor. OR Second occurrence of previously resolved Grade 3 AE.
4 (Life-threatening)	Yes	Permanent discontinuation, except by approval of the medical monitor. If continuing, toxicity must resolve to Grade 0-1.	Permanent discontinuation, except by approval of the medical monitor. If resolved, restart at lower dose level	Permanent discontinuation for any severe or life-threatening event, except by approval of the medical monitor.

4.6. Concomitant Medications

[0215] All concomitant medications and treatments (including over-the-counter or prescription medicines, vitamins, vaccines, and/or herbal supplements) must be recorded in the electronic case report form (eCRF). Any prior medication received up to 30 days before the first dose of study drug and 30 days after the last dose of study drug, or until the subject begins a new ALS therapy, whichever occurs first, will be recorded in the eCRF. Any addition, deletion, or change in the dose of these medications will also be recorded. Concomitant medications administered after 30 days after the last dose of study drug should be recorded for SAEs as defined in Section

8.2. Concomitant treatments/procedures that are required to manage a subject's medical condition during the study will also be recorded in the eCRF. The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.

[0216] 4.6.1. Steroid Use. Chronic steroid use is allowed where the dose is stable for 3 months.

[0217] 4.6.2. Transfusion. For subjects needing transfusions: minimum 48 hours should lapse from the end of blood or platelet transfusion to beginning of CK0803 product infusion.

[0218] 4.6.3. Other Therapies. Subjects taking concomitant SOC treatment for ALS should be on a stable dose as below:

• Riluzole must be on a stable dose for ≥ 30 days.

• Edaravone must be on a stable dose for ≥30 days.

• Albrioza must be on a stable dose for ≥30 days.

4.7. Treatment After the End of the Study

[0219] There is no recommendation on the treatment after the end of the study. The subject will continue treatment as per his/ her treating physician.

5. DISCONTINUATION OF STUDY TREATMENT AND SUBJECT WITHDRAWAL

5.1. Discontinuation of Study Drug

[0220] 5.1.1. Reasons for Discontinuation. Subjects must be discontinued from study drug for the following reasons:

[0221] 1. Consent is withdrawn. Note: Consent withdrawn means that the subject has explicitly indicated that he/she does not want to be followed any longer; in this case no further data, except data in the public domain, may be solicited from or collected on the subject. Subjects may choose to discontinue study treatment and remain in the study to be followed for progression and survival.

[0222] 2. Further participation would be injurious to the subject's health or well-being, in the investigator's medical judgment.

[0223] 3. The occurrence of unacceptable toxicity not caused by the underlying disease will require that the study treatment be permanently discontinued. Unacceptable toxicity is defined as follows: The occurrence of a study drug TLT that in the judgment of the investigator or the sponsor's medical monitor, compromises the subject's ability to continue study-specific procedures or is considered to not be in the subject's best interest.

[0224] 4. TLT requiring more than 2 dose interruptions.

[0225] 5. A persistent TLT requiring a delay of therapy for more than 6 weeks (42 days) unless a greater delay has been approved by the sponsor.

[0226] 6. The study is terminated by the sponsor.

[0227] 7. The study is terminated by the local health authority, institutional review board (IRB), or independent ethics committee (IEC).

[0228] A subject may be discontinued from study treatment as follows:

[0229] 1. If, during the course of the study, a subject is found not to have met eligibility criteria, the medical monitor, in collaboration with the investigator, will determine whether the subjects should be withdrawn from study treatment.

[0230] 2. If a subject is noncompliant with study procedures or study drug/treatment administration in the investigator's opinion, the sponsor should be consulted for instruction on handling the subject.

[0231] 5.1.2. Discontinuation Procedures. In the event that the decision is made to permanently discontinue the study drug, the EOT visit should be conducted. Reasonable efforts should be made to have the subject return for a follow-up visit. These visits are described in **Tables 9A** and **9B**. The last date of the last dose of study drug and the reason for discontinuation of study drug will be recorded in the eCRF.

[0232] If a subject is discontinued from study drug:

[0233] 1. The study monitor or sponsor must be notified.

[0234] 2. The reason(s) for discontinuation must be documented in the subject's medical record and the primary reason for discontinuation must be included in the eCRF.

[0235] 3. The EOT visit should be performed and date recorded.

[0236] 4. Subjects must be followed for safety until the time of the follow-up visit or until study drug–related toxicities resolve, return to baseline, or are deemed irreversible, whichever is longest.

[0237] If the subject discontinues study drug and actively withdraws consent for collection of follow-up data (safety follow-up or disease assessment), then no additional data collection should occur; however, subjects will have the option of withdrawing consent for study treatment but continuing in the follow-up period of the study for safety/efficacy assessments.

5.2. Subject Withdrawal from the Study

[0238] A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons. If a subject withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records. If the subject withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent. See **Tables**

9A and **9B** for data to be collected at the time of study withdrawal and follow-up and for any further evaluations that need to be completed.

5.3. Lost to Follow-Up

[0239] A subject will be considered lost to follow-up if he/she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site. The following actions must be taken if a subject fails to return to the clinic for a required study visit:

• The site must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.

• Before a subject is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.

• Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the study.

6. STUDY ASSESSMENTS AND PROCEDURES

6.1. Administrative and General Procedures

[0240] 6.1.1. Informed Consent Process. The investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorized representative and answer all questions regarding the study.

a) Informed consent must be obtained before any study-related procedures are conducted, unless otherwise specified by the Protocol.

b) Informed consent must be obtained using the IRB-approved version in a language that is native and understandable to the subject. A template will be provided by the sponsor or its designee. The sponsor or its designee must review and acknowledge the site-specific changes to the ICF template. The ICF must include a statement that the sponsor or its designee and regulatory authorities have direct access to subject records.

c) The informed consent form (ICF) must contain all required elements and describe the nature, scope, and possible consequences of the study in a form understandable to the study subject.

[0241] Subjects must be informed that their participation is voluntary. Subjects or their legally authorized representative will be required to sign a statement of informed consent that meets the applicable requirements and regulations for the country in which the study is being conducted as well as the IRB or study center.

[0242] The subject must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection laws. The level of disclosure must also be explained to the subject.

[0243] The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

[0244] The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

[0245] Subjects must provide consent to the most current version of the ICF during their participation in the study.

[0246] A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.

[0247] Subjects who are rescreened are required to sign a new ICF with a new subject number.

[0248] 6.1.2. Screening Procedures. Screening is the interval between signing the ICF and the day the subject is enrolled in the study (Cycle 1 Day 1). Screening may not exceed 30 days. Assessments that are required to demonstrate eligibility may be performed over the course of 1 or more days during the screening process. Procedures conducted as part of the subject's routine clinical management (e.g., blood count, imaging study) and obtained before signing of the ICF may be used for screening or baseline purposes provided the procedure meets the Protocol-defined criteria and has been performed in the timeframe of the study (i.e., within 30 days of Cycle 1 Day 1). For subjects who are enrolled in the study, information associated with eligibility requirements must be entered into the appropriate eCRF pages.

[0249] Results from the screening visit evaluations will be reviewed to confirm eligibility before enrollment or the administration of study treatment. Tests with results that fail eligibility requirements may be repeated once during screening if the investigator believes the results to be in error. For screening assessments that are repeated, the most recent available result before treatment initiation will be used to determine eligibility. Treatment should start as soon as possible but within 30 days after the date of enrollment.

[0250] 6.1.3. Demography and Medical History.

[0251] 6.1.3.1. Demographics and General Medical History. Demographic data and general medical history will be collected at screening by the investigator or qualified designee and will include year of birth/age, race, ethnicity, medical and surgical history, and current illnesses.

Medical history will include relevant medical or surgical treatment within the last 5 years that are considered to be clinically significant by the investigator.

[0252] 6.1.3.2. Disease Characteristics and Treatment History. A disease-targeted medical and treatment history will be collected at screening. Details regarding the subject's disease, including date of diagnosis, age, initial and current ALSFRS-R score, relevant disease characteristics, and prior treatments will be recorded.

6.2. Study Assessments.

[0253] 6.2.1. Adverse Events. Adverse events will be monitored from the time the subject signs the ICF until at least 28 days after the last dose of study treatment or until the start of new ALS therapy, whichever occurs first. The definition, reporting, and recording requirements for AEs are described in Section 8.

[0254] 6.2.2. Physical and Neurological Examinations. Physical examinations must be performed by a medically qualified individual, such as a licensed physician, physician's assistant, or an advanced registered nurse practitioner, as local law permits. Full physical and neurological exams will be given at Screening; at subsequent visits, abbreviated physical and full neurological exams are given to assess changes from Screening. Height should be measured in all subjects at Screening.

[0255] Abnormalities identified after the first dose of study treatment constitute an AE if they are considered clinically meaningful, induce clinical signs or symptoms, require concomitant therapy, or require changes in study treatment. Investigators should pay special attention to clinical signs related to previous serious illnesses. At the screening visit, a comprehensive physical examination should be conducted. The comprehensive physical examination will include height and body weight, and assessment(s) of the following organ or body systems: skin; head, eyes, ears, nose, and throat; thyroid; lungs; cardiovascular system; abdomen; extremities; and lymph nodes; as well as a detailed neurological examination.

[0256] Neurological examinations include, but are not limited to assessment of mental status, level of consciousness, sensory function, motor function, cranial nerve function and reflexes. In addition to the administration schedule in **Tables 9A** and **9B** neurological exams should be conducted if clinically indicated, such as in the event of an AE suggestive of cognitive or motor decline. During the study, subjects will be assessed by the investigator or medically qualified designee per institutional standard of care. These assessments should be an evaluation as indicated by subject symptoms, AEs, or other findings and documented on the AE eCRF.

[0257] 6.2.3. Vital Signs Measurement. Abnormal vital sign results identified after the first dose of study treatment constitute an AE if they are considered clinically meaningful, induce

clinical signs or symptoms, require concomitant therapy, or require changes in study treatment. Vital signs are to be measured at visits indicated in **Tables 9A** and **9B**. Vital sign measurements (to be taken before blood collection for laboratory tests), include blood pressure, pulse, respiratory rate, pulse oximetry, and body temperature. Blood pressure and pulse will be taken with the subject in the recumbent, semi-recumbent, or sitting position after 5 minutes of rest.

[0258] 6.2.3.1. Body Weight and Height (or Length). Height (or length, if subject is unable to stand) will be assessed in all subjects as per **Tables 9A** and **9B**. For measurements of body weight, the same weighing scales should be used to weigh a given subject throughout the study. Scales should be calibrated and reliable; scales should be zeroed just prior to use. Weight will be assessed in all subjects as per **Tables 9A** and **9B**.

[0259] 6.2.4. Laboratory Assessments. See Tables 9A and 9B for the timing and frequency of clinical laboratory tests. A certified laboratory local to the investigative site will perform all clinical laboratory assessments for safety as per standard of care. The investigative site will enter the laboratory results and laboratory normal ranges into the eCRF. Additional testing may be required by the sponsor based on emerging safety data. Additional tests may also be performed if clinically indicated.

[0260] Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition. All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 24 hours after the last dose of study treatment should be repeated at a minimum of every 7 days until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor. Screening laboratory assessments must be performed within 30 days before Cycle 1 Day 1. If performed more than 30 days before Cycle 1 Day 1, then the tests must be repeated and eligibility confirmed before study treatment administration on Cycle 1 Day 1. Laboratory sample collection on Cycle 1 Day 1 must be performed before study treatment administration. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours before study treatment administration (within the 3-day study window), and results should be reviewed by the investigator or qualified designee and found to be acceptable before a new cycle of treatment is initiated.

[0261] 6.2.4.1. Pregnancy Testing. A serum pregnancy test will be required for all women of childbearing potential during screening. Urine pregnancy tests will be performed locally as outlined in **Tables 9A** and **9B**, as medically indicated (e.g., in case of loss of menstrual cycle, when pregnancy is suspected), or per country-specific requirement (note that country-required

urine pregnancy testing will be outlined and communicated to investigational sites under separate cover). If a urine pregnancy test is positive, the results should be confirmed with a serum pregnancy test. If the serum pregnancy test is negative after a urine test was positive, the investigator will assess the potential benefit/risk to the subject and determine whether it is in the subject's best interest to resume study treatment and continue participation in the study.

6.3. Efficacy Assessments

[0262] 6.3.1. Subject-Reported Outcomes.

[0263] 6.3.1.1. ALSFRS-R Score. The symptom burden in subjects with ALS will be assessed using the provided ALSFRS-R Score form.

[0264] 6.3.1.2. Remote Contact Assessments. Changes in concomitant medications, AEs, and ancillary procedures will be recorded during the monitoring remote contacts. Remote contact visits may be performed via telephone or video.

[0265] 6.3.2. Functional Outcome Assessments. The assessments listed in the sections below will be performed at study visits or in the subject's home according to **Tables 9A** and **9B**. The order of assessments will be detailed in the Study Assessment manual and should be followed at all visits; however, efforts should be made to complete the ALSFRS-R and SVC first followed by the remaining clinical measures, and before blood draws or physical exams. For assessments that require an evaluator (clinician, physical or occupational therapist, etc.) or caregiver, efforts should be made to ensure the same evaluator performs these assessments throughout the study, for each individual subject. When appropriate, some measures may be completed at home prior to the clinic visit within the study visit window, or by remote contact such as telephone or video interview (refer to **Tables 9A** and **9B**). Appropriate instructions will be provided to the study staff and caregiver with detailed instructions on when to complete.

[0266] 6.3.2.1. Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R). Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) measures disease severity and has been demonstrated to predict survival. The self-reported loss of function scale measures 4 functional domains, bulbar function, gross motor skills, fine motor skills, and respiratory. The assessment contains 12 questions scored from 0 to 4 and takes approximately 10 minutes to complete. A total possible score of 48 indicates the highest level of function. At each study site, the same qualified and trained study site staff member will consistently perform the ALSFRS-R for a subject. A qualified and trained backup ALSFRS-R rater will be identified in case the primary rater is unavailable. The assessment may be performed in-clinic or remotely (refer to **Tables 9A** and **9B**).

[0267] 6.3.2.2. Combined Assessment of Function and Survival (CAFS). CAFS ranks patients' clinical outcomes based on survival time and change in the ALS Functional Rating Scale–Revised (ALSFRS-R) score (Berry et al., (2013) *Amyotroph Lateral Scler Frontotemporal Degener* 14(3): 162-168). Each patient's outcome is compared to every other patient's outcome, assigned a score, and the summed scores are ranked. The mean rank score for each treatment group can then be calculated. A higher mean CAFS score indicates a better group outcome. CAFS ranks each subject according to their outcome, with the worst outcome assigned to the subject who dies first in the study and the best outcome assigned to the subject who survives with the least functional decline. Based on the Finkelstein and Schoenfeld methodology (Finkelstein and Schoenfeld (1999) *Stat Med* 18(11): 1341-1354), the CAFS can be viewed as an analysis of ALSFRS-R that adjusts for mortality.

[0268] 6.3.2.3. Handheld Dynamometery (HHD). Handheld dynamometry will be used to evaluate muscle strength, a determinant of function and survival in ALS. The assessment tests isometric strength of multiple muscles using standard subject positioning. An evaluator will examine muscle groups in both upper and lower extremities when assessments are done in the clinic. HHDs assessing only grip strength will be done remotely, in the subject's home, at specified visits (refer to **Tables 9A** and **9B**).

[0269] 6.3.2.4. Vital Capacity. Vital capacity will be measured by means of a Slow Vital Capacity (SVC) test and will be measured in-clinic (refer to **Tables 9A** and **9B**). The vital capacity will be used to assess the strength of the ventilatory muscles. Subjects should use snorkels for the in-clinic SVC assessment, unless it is medically necessary for the subject to use another variation of the snorkel mouthpiece as determined by the Investigator; however, the chosen method should remain consistent throughout the duration of the study. The results of the in-clinic SVC will be reviewed by a central reader.

[0270] 6.3.2.5. Edinburgh Cognitive and Behavioral ALS Screen (ECAS). The ECAS, a disease specific assessment used to evaluate cognitive and behavioral impairments, comprises 2 sections. The first section, to be completed by the subject, includes 15 individual tasks split into 5 subdomains (language, verbal fluency, executive, memory and visuospatial). The total score is a sum of the individual task sub scores. A second section is an interview regarding changes in the subject's behavior and is to be completed by the subject's caregiver. The assessment may take 15–20 minutes to complete. ECAS will be measured in-clinic (refer to **Tables 9A** and **9B**).

[0271] 6.3.2.6. Amyotrophic Lateral Sclerosis Specific Quality of Life Instrument-Revised (ALSSQOL-R). The ALSSQOL-R is a disease specific 50-item assessment that

measures quality of life (QoL). Each item is rated by the subject on a scale of 0 to 10, with 0 being the least desirable situation and 10 being the most desirable. A Single-item QOL score, an Average Total QOL score, and 6 domain scores: 1) Negative Emotion; 2) Interaction with People and the Environment; 3) Intimacy; 4) Religiosity; 5) Physical Symptoms; and 6) Bulbar Function. The assessment may take approximately 15-20 minutes to complete. ALSSQOL-R will be measured in-clinic (refer to Tables 9A and 9B).

6.4. Pharmacokinetic Assessments

[0272] Pharmacokinetic parameters of CK0803 will be evaluated in this study by examining the T cell compartment as described in the laboratory manual. Whole blood samples will be collected for the measurement of CK0803 as specified in the **Tables 9A** and **9B**. A maximum of 6 samples may be collected at additional timepoints during the study if warranted and agreed upon between the investigator and the sponsor. Samples collected for analyses of CK0803 concentrations may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study. The actual date and time (24-hour clock time) of each sample will be recorded.

[0273] 6.4.1. Blood Sample Collection. Timing of blood PK assessments is outlined in **Tables 9A** and **9B**. After the pre-dose PK sample is drawn, subjects will begin the study treatment. Predose is defined as within 24 hours before administration of study treatment. Adjustments to the timing of blood sampling may be made based on emerging PK data.

6.5. Pharmacodynamic and Translational Assessments

[0274] The evaluation of pharmacodynamics will be included in the study as per the schedule described in the **Tables 9A** and **9B**.

[0275] 6.5.1. Biomarkers. Collection of samples for other biomarker research is also part of this study. The following samples for biomarker research are required and will be collected from all subjects in this study as specified in **Tables 9A** and **9B**: Peripheral blood and CSF.

[0276] Samples will be tested for Exploratory Objective to evaluate their association with the observed clinical responses to study treatment. The biomarker research will include inflammatory cytokine analysis performed on the serum and plasma. Samples may be stored for a maximum of 10 years (or according to local regulations) after the last subject's last visit for the study at a facility selected by the sponsor to enable further analysis of biomarker responses to study treatment.

[0277] 6.5.2. Immunogenicity Assessments. Donor Specific Antibodies to the study drug will be evaluated in serum samples collected from all subjects according to **Tables 9A** and **9B**. Additionally, serum samples should also be collected at the final visit from subjects who

discontinued study drug or were withdrawn from the study. These samples will be tested by the sponsor or sponsor's designee. Serum samples will be screened for antibodies against the allogeneic cord blood unit utilized for the generation of the CK0803 product and the titer of confirmed positive samples will be reported. Other analyses may be performed to verify the stability of antibodies to study drug and/or further characterize the immunogenicity of study drug. The detection and characterization of antibodies to study drug will be performed using a validated assay method as described in laboratory manual. Samples may be stored for a maximum of 10 years (or according to local regulations) after the last subject's last visit for the study at a facility selected by the sponsor to enable further analysis of immune responses to study treatment.

[0278] 6.5.3. Immune Reconstitution. Collection of whole blood lymphocyte samples for examining the impact of CK0803 on the immune reconstitution is also part of this study. The following samples for whole blood lymphocyte analysis are required and will be collected from all subjects in this study as specified in **Tables 9A** and **9B**. Peripheral blood and CSF samples will be tested for Exploratory Objective to evaluate their association with the observed clinical responses to study treatment. Immune reconstitution studies will include the composition and functional analysis of the T-cell, B-cell, NK cells and other elements of the immune system as well as single-cell RNA and T cell receptor (TCR) sequencing (scRNA/TCR-seq) on immune cells isolated from CSF.

6.6. Unscheduled Visits

[0279] Unscheduled virtual or telephone visits may take place at the study physician's discretion throughout the course of this study.

6.7. End of Treatment and/or Early Termination.

[0280] End of Treatment (EOT) visit will occur 28 days after the last dose of therapy. In subjects who complete or discontinue therapy for any reason, EOT visit will occur within 28 days (+/-7 d) of last dose. Assessment will occur per the EOT schedule of assessments **Tables 9A** and **9B**. Subjects who terminate study during treatment will have an EOT visit and assessment within 28 days (+/- 14 days). Subjects who wish to terminate study during follow up will have the next scheduled assessment within 28 days. The reason for study discontinuation or termination will be documented.

6.8. Follow-Up

[0281] Subjects will follow up on day+28 post last infusion of CK0803 for assessment of clinical response, correlative assays as well as safety evaluations. Follow-Up and EOT may fall on the same day.

[0282] 6.8.1. Safety Follow-Up. The safety follow-up period will coincide with EOT visit and is defined at day +28 after the last infusion of CK0803. Adverse events and SAEs must be reported up until

• at least 28 days after the last dose of study drug or the start of a new ALS therapy or

• until toxicities resolve, return to baseline, or are deemed irreversible, whichever is longer.

[0283] Reasonable efforts should be made to have the subject return for the follow-up visit and report any AEs that may occur during this period. If the subject cannot return to the site for the safety follow-up visit (e.g., lives far away, then the subject should be contacted by telephone for assessment of AEs and SAEs. Sites should be instructed to document this contact in the source document. If a subject is scheduled to begin a new ALS therapy before the end of the 28-day safety follow-up period, the safety follow-up visit should be performed before a new ALS therapy is started. Once a new ALS therapy has been initiated, the subject will move into the survival follow-up period.

[0284] 6.8.2. Post-Treatment Disease Follow-Up. Subjects who discontinue study treatment for a reason other than disease progression will move into the disease status follow-up period. Every effort should be made to collect information regarding disease status until: Withdrawal of consent; Start of new ALS therapy; Disease progression; Death; or End of the study. For subjects having entered the post-treatment follow-up period of the study, the site will use continuing subject records to supply data on subsequent treatment regimens, ALS disease assessments (if discontinued treatment for a reason other than progression), and life status if applicable in the eCRF. For subjects who do not intend to return to the study investigator for their ongoing care, follow-up should be maintained by phone contact, subject records, and public records/databases at intervals of no longer than 6 months.

[0285] 6.8.3. Survival Follow up. Once a subject has received the last dose of study drug and follow up, has confirmed disease progression, or starts a new ALS therapy, the subject moves into the survival follow-up period. The subject may be contacted during this period by telephone, email, or visit to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first. For subjects having entered the survival follow-up period of the study, the site will use continuing subject records to supply data on subsequent treatment regimens, ALSFRS-R score (if discontinued treatment for a reason other than progression), and overall survival in the eCRF for up to 10 years. For subjects who do not intend to return to the study investigator for their ongoing care, follow-up will be maintained by phone contact, subject records, and public records/databases. After the final primary

analysis is performed, the follow-up interval for subsequent ALS treatments and survival may be reduced.

7. STATISTICS

7.1. Goals

[0286] The primary goal for the Phase 1 safety run-in is to establish the safety of multiple infusions of CK0803 in ALS patients and the primary goal of the phase 1b randomized, double blind, placebo control trial is to collect extended safety data and evaluate the combined assessment of function and survival over the 6-month time period during which treatment with CK0803 Treg cells is administered.

7.2. Safety Run-in

[0287] 7.2.1. Sample Size for Safety Run-in Phase. A minimum of 6 subjects will be treated based on the 3+3 design (Storer (2001) *Stat Med* 20(16): 2399-2408).

[0288] 7.2.2. Populations for Analysis for Safety Run-in. The populations for analysis are provided in **Table 6**.

Table 6. Populations for Analysis

Population	Description
Full Analysis Set (FAS)	All subjects enrolled in the study who received at least 1 dose of study treatment. The FAS will be used for the summary of demographics, baseline characteristics, subject disposition, and analyses of all efficacy data.
Safety Set	All subjects enrolled in the study who took at least one dose of study drug. All safety analyses will be conducted using the safety population. At least 1 laboratory or vital sign measurement obtained subsequent to at least 1 dose of study treatment is required for inclusion in the analysis of each specific parameter. To assess the change from baseline, a baseline measurement is also required.
TLT evaluable	The TLT evaluable population will include all subjects in the FAS population that meet the following criteria: • Subjects who are observed for at least the first treatment cycle (i.e., 28 days), • Subjects who do not meet the eligibility requirements of the study

[0289] 7.2.3. Analysis Endpoints for Safety Run-in. The primary and key secondary endpoints and analysis populations are provided in **Table 7.**

Table 7. Primary and Secondary Endpoints and Analysis Populations

Endpoint	Analysis Population
Primary Endpoints	
• TLT • AE • Tolerability	• TLT evaluable • Safety Set • Safety Set
Key Secondary Endpoints	
• ALSFRS-R Score • ALSSQOL-R • SVC response • HHD response • Neurofilament light (NfL) concentration in CSF and serum • Ventilation assistance-free survival (VAFS) • Overall survival (OS)	FAS

[0290] 7.2.4. Level of Significance for Safety Run-in. Not applicable. No formal hypotheses tests will be performed and only summary statistics including 95% CIs will be provided for all efficacy endpoints that are only considered as secondary and/or exploratory in this protocol.

[0291] 7.2.4.1. Statistical Analyses for Safety Run-in. Descriptive summaries for continuous variables will include but not be limited to the number of observations, mean, standard deviation, median, minimum, and maximum. Descriptive summaries for categorical variables will include the number and percentage of subjects in each category. The statistical analyses for the primary endpoints and key secondary endpoints are outlined below. The full statistical analysis will be detailed in the statistical analysis plan (SAP).

7.3. Randomized Control Trial (RCT)

[0292] 7.3.1. Sample Size for Expansion Cohort. After completion of the safety run-in of 6 patients (28 days post-infusion for the 6th subject enrolled), the RCT will be initiated with up to two stages (stages 1 and 2), each stage of size 30 patients, with a safety rule applied after stage 1 that may terminate the trial early, for a maximum total sample size of 6+30+30 = 66 patients.

[0293] 7.3.1.1. Stage 1. A maximum of 30 patients will be treated in stage 1, with an anticipated accrual rate of three to five patients per month. These 30 patients will be randomized between CK0803 and placebo in a 2:1 ratio, with the randomization restricted so that exactly 20 patients receive CK0803 and 10 patients receive placebo.

[0294] 7.3.1.2. Safety Stopping Rule After the Randomized Stage 1. There are two types of safety signals of concern. The first are severe life-threatening adverse events that are rare among ALS patients. Any such event would be thoroughly investigated and two such events that were sufficiently similar as to indicate that they were the result of treatment would halt the trial. With 26 treated patients (6 patients from the safety run-in and 20 patients (treated with CK0803) from the RCT) we will have a 93% chance of seeing at least one of any event that occurs 10% of the time on treatment. Note that the initial safety run in has a 90% chance of seeing at least one of any event that occurs 1/3 of the time. The second type of safety signal is a side effect that makes the treatment intolerable to patients. We will consider a treatment tolerable if the proportion of side effects that lead a patient to stop treatment is less than 40% with 80% confidence. With 26 subjects this would occur if 7 or fewer subjects fail to complete the study. With 26 subjects we will have more than an 87% chance of declaring a dosage tolerable if the true treatment failure rate is 20%. In addition, we will use the following Bayesian rule to determine a *side effect of concern*. Denote the probabilities of an AE during the first 28 days of treatment by p(CK0803) and p(placebo) for the two treatment arms in stage 1. Assuming non-informative beta(.3, .7) priors for these probabilities, based on the stage 1 data, the trial will be stopped early for safety if Pr[p(CK0803) > p(placebo) | stage 1 data] > .90. This inequality says that the probability of an AE with CK0803 is likely to be larger than an AE with placebo, based on the stage 1 data. For example, if an AE has a frequency of 30% on treatment and 10% on placebo the probability would be roughly 92% and the side effect would be one of concern.[7] The DSMB will carefully review any side effects of concern, will consider that they are most likely due to treatment and will stop the study if the existence and frequency of these side effects would negatively impact the value of this treatment.

[0295] 7.3.1.3. Stage 2. A maximum of 30 additional patients will be treated in stage 2. These 30 additional patients will be randomized between CK0803 and placebo in a 2:1 ratio, with the randomization restricted so that exactly 20 patients receive CK0803 and 10 patients receive placebo.

[0296] 7.3.1.4. Total Sample Size. The total sample size of 60 patients randomized in stages 1 and 2 combined will ensure that if, for example, 5 of the 40 = 20+20 patients treated with

CK0803 experience a TLT then, assuming that the probability p(CK0803) of TLT follows a beta(.30, .70) prior, a posterior 95% credible interval for p(CK0803) would be [.05, .25].

[0297] 7.3.2. Outcomes and Covariates for RCT.

[0298] 7.3.2.1. Primary Outcomes. The primary outcome will be combined assessment of survival and function (CAFS) (Berry et al., (2013) *Amyotroph Lateral Scler Frontotemporal Degener* 14(3): 162-168). Treatment-related AEs and SAEs will be evaluated as a part of extended safety.

[0299] Secondary Outcomes for RCT Cohort: Secondary outcomes will include the longitudinal processes ALS functional score (ALSFRS-R) and Amyotrophic Lateral Sclerosis Specific Quality of Life - Revised (ALSSQOL-R), each measured at baseline and then monthly over 6 months of follow up and analyzed as a function of the predetermined covariates.

[0300] The details of secondary outcomes for the RCT cohort:

• Incidence of all cause AEs and SAEs.

• ALS functional score (ALSFRS-R), at baseline and at weeks t = 5, 8, 12, 16, 20, 24, 36, 48 and at EOT.

• Amyotrophic Lateral Sclerosis Specific Quality of Life - Revised (ALSSQOL-R), at baseline and at weeks t = 4, 8, 12, 16, 20, 24, 36, 48 and at EOT.

• Slow Vital Capacity (SVC), at baseline, at weeks t = 5, 8, 12, 16, 20, 24, 36, 48 and at EOT.

• Handheld dynamometer (HHD), at baseline, and at weeks 5, 13, 25, 36 and 48 from first infusion and/or at EOT.

• Ventilation assistance-free survival (VAFS), at baseline, and at weeks 5, 8, 12 and/or 13, 16, 20, 24 and/or 25, 36 and 48 from first infusion and/or at EOT.

• Neurofilament light (NfL) concentration in CSF and serum, at baseline, and at weeks 5, 13, 25, 36 and 48 from first infusion and/or at EOT.

• Overall survival (OS) time at the time of last follow up.

• Dropout and treatment discontinuation times and reasons.

[0301] 7.3.2.2. Subject Covariates for RCT. Baseline patient covariates will include:

• *Delta* ALSFRS-R defined as the baseline ALSFRS0-R score divided by the prior duration of disease.

• Bulbar onset.

• Probable verses definite El-Escorial classification.

[0302] 7.3.3. Populations for Analysis for RCT Cohort. Full Analysis Set (FAS) as defined in **Table 6**.

[0303] 7.3.4. Statistical Models and Data Analyses for RCT Cohort.

[0304] **7.3.4.1. Primary Outcome.** Primary outcome will be CAFS rank as described in (Berry et al., (2013) *Amyotroph Lateral Scler Frontotemporal Degener* 14(3): 162-168). The rank for patient I will be defined as follows: We compare patient I with each patient, say J, in the data set, and give a score of x=1 if I has a better outcome than J as follows: First we find the minimum of the follow up times(up to 6 months) of patient I and J, call that time T, all comparisons are made up to time T. If neither patient drops out then T will be 6 months. If either patient dies at or before T then x=1 if patient I lives longer than patient J. If neither I nor J die before or at time T then we calculate the change in ALSFRS-R between time T and baseline (ALSFS_R(T)-ALSFRS-R(0) and x=1 if the change from baseline for patient I is greater than that of patient J. We set x=0 otherwise. Note that given the way we have defined change, a larger change is better. The score for patient I is then the sum of the values of x for all the patients in the data set. In simple terms the score for patient I is their rank in the data set in terms of this composite measure of ALSFRS-R and survival. In all cases the initiation of permanent assisted ventilation with a tracheotomy will be considered equivalent to death.

[0305] **7.3.4.2. Secondary Outcome.** The secondary outcomes will include the following longitudinal processes.

- ALS functional score (ALSFRS-R) measured at baseline and at weeks 5, 8, 12 and/or 13, 16, 20, 24 and/or 25, 36 and 48 from first infusion and/or at EOT Change from Baseline in ALSFRS-R score (in-clinic)
- ALS Quality of Life (ALSQOL) measured at baseline and at weeks 5, 8, 12 and/or 13, 16, 20, 24 and/or 25, 36 and 48 from first infusion and/or at EOT
- Slow Vital Capacity (SVC), at baseline and at weeks 5, 13, 25, 36 and 48 from first infusion and/or at EOT
- Handheld dynamometer (HHD), at baseline and at weeks 5, 13, 25, 36 and 48 from first infusion and/or at EOT
- Neurofilament light (NfL) concentration in CSF at baseline and at weeks 5, 13, 25, 36 and 48 from first infusion and/or at EOT
- Neurofilament light (NfL) concentration in serum at baseline and at weeks 5, 13, 25, 36 and 48 from first infusion and/or at EOT

[0306] Secondary outcome will also include:

• Ventilation assistance-free survival (VAFS), defined as the time to the earliest occurrence of the following events: Death or Permanent assisted ventilation.

• Incidence of all cause AEs and SAEs.

[0307] 7.3.4.3. Summary Statistics. A table of summary statistics by treatment arm will be constructed for the outcome variables, AEs and SAEs, and the covariates. Counts and percentages will be used to summarize binary and categorical variables, and numbers of missing values. Means, medians, standard deviations, and ranges will be used to summarize numerical valued variables.

[0308] 7.3.4.4. Analysis of CAFS. The primary analysis will be analysed as described in (Finkelstein and Schoenfeld (1999) *Stat Med* 18(11): 1341-1354; Berry et al., (2013) *Amyotroph Lateral Scler Frontotemporal Degener* 14(3): 162-168) with the CAFS ranking compared between treatment groups.

[0309] 7.3.4.5. Analysis of Longitudinal Outcomes. The secondary analysis including the longitudinal outcomes of ALSFRS-R, ALSQOL, SVC, HHD, NfL. The secondary outcomes will not be used to generate p-values but rather will be analyzed to develop models for determining the sample size and optimal design for future studies. In order to do this a parametric model will be used. These analyses will use the model described in (Vonesh et al., (2006) *Stat Med* 25(1): 143-163). This model assumes that each patient's longitudinal outcome has a random linear trajectory and then determines the difference in the average of these trajectories between treatment groups. In addition, the model assumes that the hazard of death or permanent assisted ventilation is a linear function of the random slope of the patients' trajectory as well as of treatment. Thus, the model measures the direct effect of treatment on mortality and the effect that is mediated through the longitudinal outcome. This analysis will account for prognostic covariates by assuming that these covariates affect the rate of decline in the longitudinal outcome. The variables considered will be Delta-ALSFRS-R defined as 48 minus the current value of ALSFRS-R divided by the time since first symptoms and bulbar involvement, and probable verses definite El-Escorial classification.

[0310] The primary use of the measurements made after treatment ends is to determine whether the effect of treatment persists. This will be done using a hocky-stick model where we assume two trajectories one described above and the second starting at the time of treatment discontinuation. Using this model, we will determine whether the treatment effect diminishes once treatment is stopped.

[0311] 7.3.4.6. Analyses of Time to death or permanent assisted ventilation.

[0312] a) Kaplan Meier plots (Kaplan and Meier (1958) *J Amer Statist Assoc* 53) by treatment arm will be constructed.

b) AEs and SAEs will be tabulated by treatment group.

c) A plot of each longitudinal variably over time, including individual paths and an overall mean path, will be constructed.

7.4. Dropouts

[0313] Dropouts will be handled as described in the original CAFS paper (Berry et al., (2013) *Amyotroph Lateral Scler Frontotemporal Degener* 14(3): 162-168). That is in comparing two patients with unequal follow up, the comparison will be made at the minimum of both of their follow up times.

7.5. Overall Survival

[0314] The uncorrected distribution of OS time will be estimated by the Kaplan-Meier method (Kaplan and Meier (1958) *J Amer Statist Assoc* 53).

7.6. Exploratory Analysis

[0315] Serum and plasma biomarkers and inflammatory cytokines as well as immune reconstitution at different time intervals will be analyzed and presented as summary statistics.

7.7. Safety Analysis

[0316] 7.7.1. Adverse Events. Severity of AEs will be based on the NCI CTCAE v5 using Grades 1 through 5. The subset of AEs considered by the investigator to have a relationship to study drug will be considered to be treatment-related AEs. If the investigator does not specify the relationship of the AE to study drug, then the AE will be considered treatment-related. The incidence of AEs and treatment-related AEs will be tabulated. Periodic safety stopping rules will be applied throughout the study.

[0317] TLT evaluable subjects will be summarized by disease type and dose level. The TLT incidence rate along with a 95% confidence interval based on exact binomial distribution will be provided for each dose level using the TLT evaluable set. The subjects with TLTs and the type of TLT will be listed by disease type and dose level.

[0318] The tolerability of study treatment is assessed by summarizing the number of subjects with treatment dose interruptions, dose reductions and treatment discontinuation due to AE. The reason for dose interruption, dose reductions and treatment discontinuation will be listed by subject and summarized.

[0319] 7.7.2. Clinical Laboratory Tests. Laboratory test values outside the normal range will be assessed for severity based on the normal ranges for the clinical reference laboratory. The incidence of abnormal laboratory values and shift tables relative to baseline will be tabulated. Laboratory data will be classified into Grades 1 through 5 using CTCAE v5. The following summaries will be produced for the laboratory data: Number and percentage of subjects with worst post-baseline CTCAE grade (regardless of baseline value). Each subject will be counted

only for the worst grade observed post-baseline. Shift tables from baseline to the worst post-baseline value using CTCAE grade. For laboratory parameters where CTCAE grades are not defined, shift tables to the worst post baseline value using the low/normal/high classifications based on laboratory reference ranges.

[0320] 7.7.3. Vital Signs. Descriptive statistics and mean change from baseline will be determined for vital signs (blood pressure, pulse, respiratory rate, and body temperature) at each assessment time. Vital sign results will be reviewed for clinically notable abnormalities and subjects exhibiting clinically notable vital sign abnormalities will be listed. A value will be considered an "alert" value if it is outside the established range and shows a > 25% change from baseline.

7.8. Interim Analysis

[0321] Interim analysis will be performed after the day 28 follow up of the 30th patient treated in the RCT.

8. ADVERSE EVENTS: DEFINITIONS AND PROCEDURES FOR RECORDING, EVALUATING, FOLLOW-UP, AND REPORTING

8.1. Definition of Adverse Event

[0322] Adverse Event Definition:

• An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not it is considered drug-related.

• An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

[0323] Additional Guidance for Events Meeting the Adverse Event Definition:

[0324] • Any safety assessments (e.g., vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease) are to be reported as an AE.

[0325] • Abnormal laboratory test results are to be reported as an AE if they are considered clinically meaningful, induce clinical signs or symptoms, require concomitant therapy, or require changes in study treatment.

[0326] • Exacerbation of a chronic or intermittent pre-existing condition/disease, including either an increase in the frequency and/or intensity of the condition, is to be reported as an AE.

[0327] • New conditions detected or diagnosed after the start of study drug administration are to be reported as an AE.

[0328] • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction are to be reported as an AE.

[0329] • Signs and/or symptoms from dosing errors of a study drug/treatment or a concomitant medication are to be reported as an AE.

[0330] • "Lack of efficacy," "disease progression," or "failure of expected pharmacological action" will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments.

[0331] • A condition that leads to a medical or surgical procedure (e.g., endoscopy, appendectomy) will be reported as an AE if it occurs after obtaining informed consent. If the condition is present before entering the study, then it should be captured as medical history.

[0332] • Pre-existing diseases or conditions with expected fluctuations in signs or symptoms should be reported as an AE only if the investigator judges the fluctuation to have worsened more than expected during study participation.

8.2. Definition of Serious Adverse Event

[0333] If an event is not an AE per the definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

[0334] A serious adverse event is defined as any untoward medical occurrence that:

[0335] • Results in death.

[0336] • Is life-threatening.

[0337] The term "life-threatening" in the definition of "serious" refers to an adverse drug experience that places the subject, in the opinion of the initial reporter, at immediate risk of death from the adverse experience as it occurs. This does not include an adverse drug experience that, had it occurred in a more severe form, might have caused death.

[0338] • Requires inpatient hospitalization or prolongation of existing hospitalization.

[0339] In general, hospitalization signifies that the subject has been detained (involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious.

[0340] Hospitalization for elective treatment or planned surgery (e.g., stent replacement, hip surgery) is not considered an SAE.

[0341] Hospitalization for medical interventions in which no unfavorable medical occurrence occurred (i.e., elective procedures or routine medical visits) are not considered SAEs.

[0342] • Results in persistent or significant disability/incapacity.

[0343] • The term "disability" means a substantial disruption of a person's ability to conduct normal life functions.

[0344] • This definition is not intended to include experiences of relatively minor medical significance, such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle), that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

[0345] • Is a congenital anomaly/birth defect.

[0346] • Is an important medical event.

[0347] An important medical event is an event that may not result in death, be immediately life-threatening, or require hospitalization but may be considered serious when, based on appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the above definition. Examples of such events include intensive treatment in an emergency department or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

8.3. Recording and Follow-Up of Adverse Events and/or Serious Adverse Events

[0348] Adverse Event and Serious Adverse Event Recording

[0349] • An AE/SAE that begins or worsens after informed consent is signed should be recorded on the Adverse Event Form in the eCRF. Conditions that were present at the time informed consent was given should be recorded on the Medical History Form in the eCRF.

[0350] • When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostic reports) related to the event.

[0351] • The investigator (or delegate) will then record all relevant AE/SAE information in the eCRF.

[0352] • It is not acceptable for the investigator to send photocopies of the subject's medical records in lieu of completing the Adverse Event Form in the eCRF.

[0353] • There may be rare instances when copies of medical records for certain cases are requested. In this case, all subject identifiers, with the exception of the subject number, will be redacted by the site staff on the copies of the medical records before submission. These records

can be submitted to Sponsor Pharmacovigilance by email/fax per the contact information listed in the Study Reference Manual or as per SAE completing guidelines.

[0354] • The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE/SAE.

[0355] To the extent possible, each AE/SAE should be evaluated to determine the following:

[0356] • The severity grade (CTCAE v5.0 Grade 1 to 5). See below for further instructions on the assessment of intensity.

[0357] • Whether there is at least a reasonable possibility that the AE is related to the study drug/treatment: suspected (yes) or not suspected (no). See below for further instructions on the assessment of causality.

[0358] • The start and end dates, unless unresolved at the final safety follow-up visit.

[0359] • The action taken with regard to study drug/treatment as a result of the AE/SAE(s).

[0360] • The event outcome (e.g., not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown).

[0361] • The seriousness, as per the SAE definition provided in Section 8.2.

[0362] • The action taken with regard to the event. Note: If an AE is treated with a concomitant medication or nondrug therapy, this action should be recorded on the Adverse Event Form and the treatment should be specified on the appropriate eCRF (e.g., Prior/Concomitant Medications, Procedures and Non-Drug Therapy).

[0363] Assessment of Intensity

[0364] The severity of AEs will be assessed using CTCAE v5.0 Grades 1 through 5. If an event is not classified by CTCAE, the severity of the AE will be graded according to the scale below to estimate the grade of severity.

[0365] The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; treatment not indicated.

Grade 2: Moderate; minimal, local, or noninvasive treatment indicated; limiting age-appropriate activities of daily living.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.

Grade 4: Life-threatening consequences; urgent treatment indicated.

Grade 5: Fatal.

[0366] Events not included in the CTCAE chart will be scored as follows:

[0367] Grade 1: Mild: discomfort present with no disruption of daily activity, no treatment required beyond prophylaxis.

[0368] Grade 2: Moderate: discomfort present with some disruption of daily activity, require treatment.

[0369] Grade 3: Severe: discomfort that interrupts normal daily activity, not responding to first line treatment.

[0370] Grade 4: Life Threatening: discomfort that represents immediate risk of death.

[0371] Grade 5: Death.

[0372] Grading of cytokine release syndrome (CRS): Described with T cell therapies but not expected due to the suppressive nature of the Tregs and not yet seen.

[0373] Grade 1: Mild reaction; infusion interruption not indicated; intervention not indicated.

[0374] Grade 2: Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for </= 24 hrs.

[0375] Grade 3: Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion; recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates.

[0376] Grade 4: Life-threatening consequences; pressor or ventilator support indicated.

[0377] Grade 5: Death.

[0378] Assessment of Causality

[0379] The investigator is obligated to assess the relationship between study drug/treatment and each occurrence of each AE/SAE.

[0380] A "reasonable possibility" of a relationship conveys that there are medical facts, evidence, and/or arguments to suggest a causal relationship, rather than that a relationship cannot be ruled out. The investigator will use clinical judgment to determine the possibility of a relationship. The investigator will also consult the reference safety information (RSI) in the IB or Product Information for study drug/treatment, or marketed products, respectively, in making his/her assessment. Alternative causes, such as underlying or concurrent disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study drug/treatment administration, will be considered and investigated. For each AE/SAE,

the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

[0381] With regard to assessing causality of SAEs:

[0382] There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report. However, the causality assessment is one of the criteria used when determining regulatory reporting requirements. Therefore, it is very important that the investigator always make an assessment of causality based on the available information for every event before the initial transmission of the SAE.

[0383] The investigator may change his/her opinion of causality in light of follow-up information and submit the updated causality assessment.

[0384] Follow-Up of Adverse Events and Serious Adverse Events

[0385] The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

[0386] • Once an AE is detected, it should be followed in the AE eCRFs until it has resolved or until it is judged to be permanent; assessment should be made at each visit (or more frequently if necessary) of any changes in severity, the suspected relationship to the study drug/treatment, the interventions required to treat the event, and the outcome.

[0387] • If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide the sponsor with a copy of any postmortem findings, including histopathology.

[0388] Updated SAE information will be recorded in the originally completed eCRF and reported to Sponsor Pharmacovigilance (either via email/fax if paper SAE form is used or in the SAE EDC CRF) until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up.

[0389] Any updated SAE data (including SAEs being downgraded to nonserious) will be submitted to the sponsor (or designee) within 24 hours of receipt of the information.

[0390] 8.4. Reporting of Serious Adverse Events

[0391] Regardless of suspected causality (e.g., relationship to study drug), all SAEs occurring after the subject has signed the ICF through the last safety visit or at least 28 days after the last dose of study drug/treatment or until the subject starts a new ALS therapy must be reported to the sponsor (or designee) within 24 hours of learning of its occurrence unless otherwise

specified by the Protocol. The investigator will submit any updated SAE data to the sponsor (or designee) within 24 hours of it being available.

[0392] Investigators are not obligated to actively seek SAE information after the safety follow-up visit or 28 days after the last dose of study drug (whichever is later). If the investigator learns of any SAE, including death, at any time as per schedule of assessments and he/she considers the event to be reasonably related to the study drug/treatment or study participation, then the investigator must notify the sponsor (or designee) within 24 hours of becoming aware of the event.

[0393] After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs (as defined in Section 8.2) will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up. Prompt notification by the investigator to the sponsor regarding an SAE is essential so that legal obligations and ethical responsibilities toward the safety of subjects and the safety of a study drug/treatment under clinical investigation are met.

[0394] If the SAE is not documented in the RSI of the IB for the study drug (new occurrence) and is thought to be related to the study drug, the sponsor or its designee may urgently require further information from the investigator for expedited reporting to health authorities. The sponsor or its designee may need to issue an Investigator Notification to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected unexpected serious adverse reactions (SUSAR) will be collected and reported to the competent authorities and relevant ethics committees in accordance with the national regulatory requirements in participating countries. The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study drug/treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and investigators.

[0395] Investigator safety reports must be prepared for SUSAR according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary. An investigator who receives an investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the IRB, if appropriate, according to local requirements.

[0396] Serious Adverse Event Reporting

[0397] Information about all SAEs is collected and recorded on the Adverse Event Form in the eCRF.

[0398] The investigator must report within 24 hours of learning of its occurrence any SAE via the EDC system (primary method) or by completing the Serious Adverse Event Report Form in English (only if the EDC system is not available).

[0399] In circumstances where the EDC system is not accessible for reporting SAE information (initial and/or follow-up SAE information) to the sponsor within 24 hours. Once the EDC system is functional, the SAE report should be retrospectively added to the EDC system and follow-up should be completed through the EDC. The original copy of the Serious Adverse Event Report Form and the email or facsimile confirmation sheet must be kept at the study site.

[0400] Follow-up information is also recorded in the eCRF and transmitted to Sponsor Pharmacovigilance via the EDC system. The follow-up report should include information that was not provided previously, such as the outcome of the event, treatment provided, action taken with study drug/treatment because of the SAE (e.g., dose reduced, interrupted, or discontinued), or subject disposition (e.g., continued or withdrew from study participation). Each recurrence, complication, or progression of the original event should be reported as follow-up to that event, regardless of when it occurs.

[0401] Information about all SAEs is collected and recorded on the Adverse Event Form in the eCRF.

[0402] The investigator must report within 24 hours of learning of its occurrence any SAE by completing the Serious Adverse Event Report Form in English.

[0403] Follow-up information is also recorded and transmitted to Sponsor Pharmacovigilance on an amended or new Serious Adverse Event Report Form, with an indication that it is follow-up to the previously reported SAE and the date of the original report. The follow-up report should include information that was not provided on the previous Serious Adverse Event Report Form, such as the outcome of the event (e.g., resolved or ongoing), treatment provided, action taken with study drug/treatment because of the SAE (e.g., dose reduced, interrupted, or discontinued), or subject disposition (e.g., continued or withdrew from study participation). Each recurrence, complication, or progression of the original event should be reported as a follow-up to that event, regardless of when it occurs.

Example 2. *In vitro* Characterization of CK0803 CB Tregs

[0404] CK0803 (Cryopreserved, Allogeneic, Cord blood-derived T regulatory cells that express neurotropic homing markers) is designed to enhance the ability of Tregs to home to the CNS and therefore exert their potential beneficial effect on neuro-inflammation. In vitro studies

on cord blood (CB) derived Tregs that express neurotropic homing markers are presented below.

CK0803 Dual Selection Process

[0405] A sequential two-step negative depletion and selection process is utilized to generate the CK0803 cells (**FIG. 3**). A single CBU from a public bank was thawed and washed, followed by negative depletion of CD8+ T cells and then followed by a positive enrichment for CD25+ cells using immunomagnetic methods, and cells were cultured continuously in the presence of CD3/28 beads in 1:1 cell: bead ratio and IL-2 at 1000IU/ml, replenished every 48 hours for a total of 14 ± 2 days.

[0406] Harvested cells at the end of the culture were examined for their phenotype using cell surface and intracellular markers. As shown in **FIG. 4**, the dual-selected $CD4^+CD25^+CD11a^+$ CB Treg cells (CK0803) expressed the core Treg signature with a high intracellular expression of Helios that correlates with the demethylation of TSDR (Kim et al., (2015) *Science* 350(6258): 334-339) and FOXP3, a bona fide marker for Tregs (Ohkura et al., (2013) *Immunity* 38(3): 414-423).

[0407] In vitro functional characterization of the purified Treg cell population as a result of the dual selection process, expressing neurotropic markers including CD11a, is presented below, including (a) Suppressive function, (b) IL-10 secretion, (c) Homing to ICAM-1 in a transwell migration assay, (d) Treg cell subsets in CK0803 and control population and (e) Differential expression of CXCR3 in CK0803 Treg cells vs. control Tregs.

Suppressive Function of CK0803 cells

[0408] In order to examine the ability of the CK0803 to be able to resolve inflammation, $CD4^+CD25^-$ conventional T cells (Tcons) were stained with CellTraceViolet (CTV) dye (Thermo Fisher Scientific, Waltham, MA) as per manufacturer's instructions. CTV-labeled Tcons were co-cultured with different ratios of unlabeled CK0803 cells and then activated with a 1 CTV-labeled Tcon cell: 1 CD3/CD28 bead ratio. Proliferation of CTV-labeled Tcons was assessed by LSR Fortessa Cell Analyzer after 4 days of culture. Percentage suppression was calculated: 100 x (1 – percentage of proliferating CTV-diluting Tcons in the presence of CK0803 cells/percentage of proliferating CTV-diluting Tcons when cultured alone). As shown in **FIG. 5** (n=3; mean ± SEM), CK0803 cells are able to suppress the proliferating Tcon cells as a function of their total concentration: 95% suppression at Treg: Tcon ratio of 4:1; 90.7% suppression at Treg: Tcon ratio of 2:1 and 69.7% suppression at Treg: Tcon ratio of 1:1.

Suppressor Cytokine Secretion of the CK0803 Treg Cells

[0409] We examined the impact of the CK0803 Treg cell concentration and duration of proliferation on the secretion of suppressor cytokine IL-10. CK0803 Treg cells were seeded at $1x10^6$ cells, $2x10^6$ cells or $4x10^6$ cells per well in 6-well plate and were stimulated with 1x106 Human T-Activator CD3/CD28 beads in X-VIVO™ 15 medium supplemented with 10% FBS, 2 mM L-glutamine, 1% Penicillin-Streptomycin and 1,000 IU/ml IL-2. After 3 days or 6 days, cell culture supernatants were collected for cytokine analysis. Production level of soluble human IL-10 in the cell culture supernatants were assessed using Human IL-10 ELISA kit (#BMS215-2, Thermo Fisher Scientific) according to manufacturer's instruction. As shown in **FIG. 6**, a cell concentration dependent increase in the IL-10 secretion was observed on days 3 and day 6, where the cells seeded at $4x10^6$ cells/well secreted significantly higher IL-10 as compared to the cells seeded at $1x10^6$ cells/well on day 3 ($p=0.0176$, paired t-test and day 6 ($p=0.0152$, paired t-test). Also, as shown **FIG. 6**, the secretion of the IL-10 was higher on day 3 compared to day 6 of the culture duration ($p=0.0013$).

***In Vitro* Migration of CK0803 Cells**

[0410] In order to examine the impact of the CD11a expression on the trafficking of the enriched CK0803 cells to the neural tissue, a transwell migration assay that measures the chemotactic capability of cells toward a chemo-attractant was utilized. The CK0803 cells were plated at a concentration of $1x10^6$ cells/ml in the top chamber of a 0.5 micron transwell. The chemoattractant ICAM-1, a ligand for CD11a, implicated in homing of cells to the site of neuroinflammation (Edwards et al., (1995) *J Biol Chem* 270(21): 12635-12640), was added to the lower chamber at a concentration of 400 ng/ml or 800 ng/ml or 1600 ng/ml (**FIG. 7A**). The cells were then allowed to migrate across the trans-well membrane and the lower chamber was examined for cell number as well as photomicrographs were taken at 1.0 hour. As shown in **FIG. 7B**, increasing concentration of ICAM-1 led to increased degree of migration of the CK0803 cells.

Treg Cell Subsets in CK0803

[0411] In order to identify the differences in the CK0803 and compare the cells to the control CB Tregs, we sought to examine different Treg subsets. Lymphocytes were gated according to their size and granularity. Doublets were excluded and live CD3+ T cells gated, where the Treg cells were identified as $CD4^+CD25^{hi}$; each subset is described in **Table 8**. Data were collected on an Aurora Cytek.

Table 8. Treg cell subsets

T cell Population	Phenotype	Function
Total Tregs	$CD3^+CD4^+CD25^+$	
Memory Tregs	$CD4^+CD25^+CD45RA^-$	
Th17.1 Treg	$CCR6^+CCR4^+CXCR3^+$	$CXCR3^+$ Treg cells that also co-express CCR4 and CXCR3 acquire the trafficking properties of pathogenic TH17.1 cells that produce high amounts of the TH17-related cytokines interleukin-17 (IL-17) and IL-22 in addition to interferon-γ
Th1 Treg	$CCR6^-CCR4^-CXCR3^+$	Tregs that express CXCR3 and acquire trafficking properties of pathogenic $CXCR3^+$ TH1 cells as defined by CCR6 negative and CCR4 negative, allowing Treg localization and control of excessive TH1 responses at sites of inflammation
Th2 Treg	$CCR6^-CCR4^-CXCR3^+$	Compared with $CCR4^-$ Tregs, $CCR4^+$ Tregs exhibited increased IL-10 and IL-35 expression, and enhanced functionality in suppressing CD8+ T cells
Th17 Treg	$CCR6^+CCR4^+CXCR3^-$	Co-expression of CCR6 and CCR4 is important in migration of Treg cells to the inflammatory tissues

[0412] A significantly higher percentage of total Tregs were identified in the CK0803 cells (n=5) as compared to the control Treg population (n=3) (paired t-test, p=0.0013; **FIG. 8**). The differences in the other Treg subsets in the CK0803 vs. control Treg cell population are shown in **FIG. 9A – FIG. 9D**. A significantly higher percentage of $CD3^+/CD4^+CD25^{hi}$ cells was observed in CK0803 as compared to the Treg control: 92.24% vs. 77.50% (n=3, t-test, P=0.0013). CK0803 Treg cells are pure and more homogenous as compared to Treg control. Therefore, such a purified Treg cell population should be able to exert an efficient and sustained anti-inflammatory effect at the target site resulting in interruption of the injury-inflammation loop leading to the possibility of motor neuron regeneration with the potential translation into clinical improvement.

CXCR3 Expression in CK0803 vs. Control Tregs

[0413] Chemokine CXCL-10 has been shown to act as a strong chemoattractant for T-cells (Klein (2004) *J Cell Biochem* 92(2): 213-222; Trifilo and Lane (2004) *Virology* 327(1): 8-15) and plays an important role in the T-cell mediated inflammation in the brain (Fife et al., (2001)

J Neurosci Res 66(4): 705-714; Dufour et al., (2002) *J Immunol* 168(7): 3195-3204). CXCL10 is expressed by neurons, glia, and stromal cells in a number of different CNS diseases including ALS (Israelsson et al., (2010) *Eur J Neurosci* 31(5): 852-863). CXCL10 together with CXCL9 and CXCL11 binds to CXCR3, which is predominantly expressed on activated T-cells memory T cells and natural killer cells (Yamamoto et al., (2000) *J Leukoc Biol* 68(4): 568-574; Muller et al., (2010) *Neuropathol Appl Neurobiol* 36(5): 368-387), where similar to its ligands, expression of CXCR3 in these cells is dominantly driven by IFN-g (Nakajima et al., (2002) *Eur J Immunol* 32(6): 1792-1801).

[0414] To understand the migratory potential towards the areas of neuroinflammation in ALS patients, we examined the differential expression of CXCR3 on the CK0803 Tregs vs. control. As shown in **FIG. 10**, a significantly higher cell surface expression of the CK0803 Treg cells (n=4) was demonstrated compared to the control(n=3) (p=0.004, 2-sided t-tail test).

Example 3. Preclinical In Vivo Characterization of CB Tregs

[0415] Exemplary cryopreserved Cord blood (CB) derived Tregs were evaluated for anti-inflammatory functions in graft-versus-host disease (GVHD) and xenogeneic systemic lupus erythematosus (SLE) mouse models as described below.

CB Treg Expansion

[0416] Cryopreserved human CB units were thawed and washed in CliniMACS buffer (Miltenyi Biotec, Bergish Gladbach, Germany) containing 0.5% HSA (Baxter Healthcare, Westlake Village, CA) to yield CB mononuclear cells (MNC) (Parmar et al. 2014). CB mononuclear cells (MNC) were then subjected to CD25+ cell enrichment using magnetic activated cell sorting (MACS) according to manufacturer's instructions (Miltenyi Biotec, Bergish Gladbach, Germany). Positively selected cells were co-cultured with CD3/CD28 co-expressing commercial beads in a 1 cell: 3 bead ratio (Parmar et al. 2006).

[0417] The average number of CD25+ cells isolated from one cord blood was 0.78×10^6; after 2 weeks expansion, up to 400×10^6 Tregs could be obtained. The purity of the expanded Tregs was > 94%. Expanded Tregs are capable of specifically inhibiting an allogeneic mixed lymphocyte reaction (MLR).

Evaluating Anti-inflammatory Function of CB Tregs in Graft-versus-host Disease (GVHD) Mouse Model

[0418] The ability of CB Tregs to reduce systemic inflammation was evaluated in GVHD mouse models.

[0419] NOD/SCID IL-2Rγnull (NSG) mice (Jackson Laboratory, Bar Harbor, ME) received sublethal whole body irradiation (300 cGy from a ^{137}Cs source delivered over 1 minute by a J. L. Shepherd and Associates Mark I-25 Irradiator, San Fernando, CA) 1 day prior to intravenous infusion of human PBMCs (day -1). In order to study the effect of CB Tregs in the xenogeneic model, mice received an additional injection of $1x10^7$ *ex vivo* expanded CB-derived Tregs shortly after irradiation on day -1. On day 0, mice received PBMCs at a dose of $1x10^7$. At least 10 mice were randomized to each group. Mice were evaluated every other day for weight, appearance and survival. Although several mice were followed past 30 days in a survival study, the majority of mice were euthanized between 14 to 21 days post-transplant.

[0420] Mice that received CB Treg infusions showed overall healthier physical parameters, such as fur and weight retention, compared to mice that were not infused with the CB Tregs, which showed fur loss and weight loss.

[0421] GVHD prognosis was also assessed in the mice using the Ferrara GVHD score (also known as the Mount Sinai Acute GVHD International Consortium algorithm probability (MAP)). The Ferrara GVHD score is determined based on serum concentrations of two known biomarkers (suppressor of tumorigenesis (ST2) and regenerating islet-derived 3α (REG3 α)) for treatment response and non-relapse mortality in acute GVHD, with lower scores indicating better prognosis. As shown in **FIG. 11A**, mice that received CB Treg infusion showed lower GVHD scores compared to those mice that only received human PBMCs, indicating increased chances of survival. Supporting the predicted better prognosis of the mice receiving CB Treg infusions, these mice showed over 60% survival rate after 50 days. In contrast, mice infused with human PBMC only without CB Tregs showed about 10% survival rate at 30 days after beginning of the study.

[0422] Systemic inflammation in mice that received and did not receive CB Treg infusion was assessed by evaluating tissue histology and measuring serum levels of inflammatory cytokines.

[0423] Formalin-fixed tissues (small intestine, liver, lung, spleen, and bone marrow) were embedded in paraffin and sections stained with H&E. **FIG. 11B** shows representative histological samples from mice that received Treg infusions (PBMC+Treg, right panels) and mice that did not receive Treg infusions (PBMC only, left panels). As opposed to mice recipients of CB Treg infusions, which showed normal histology, microscopic sections of lung from recipients of PBMC alone show areas of evidence of GVHD in form of apoptotic bodies, lymphocytic infiltration and loss of architecture in small intestine, liver and lung. Bone marrow aplasia and lymphocytic infiltration into the spleen was present.

[0424] Measurements of circulating serum cytokines at day 14 showed a consistent decrease in IL-6, IFN- γ, IP-10, IL-5, MIP-1β, TNF α levels in the Treg and PBMC recipients compared to the PBMC only group (n=5 mice/gp; mean ± SEM.) as shown in **FIG. 11C** (the unit of y-axis is pg/ml).

Biodistribution of CB Tregs in Xenogeneic GVHD Model

[0425] Tregs were labeled using a retroviral vector that expressed Firefly luciferase and eGFP (eGFP-FFLuc). This vector was developed by the Center for Cell and Gene Therapy (Baylor College of Medicine, Houston, TX). To produce a retroviral supernatant for transduction, HEK 293T cells were co-transfected with the retroviral vector eGFP-FFLuc and Peg-Pam-e (containing the sequence for the MoMLV gag-pol) and RDF plasmids (encoding for the RD114 envelope). Retroviral supernatant was collected at 48 and 72 hours after transfection, filtered (0.45μm) and stored at -80°C until required. Prior to transfection, $CD25^+$ cells were enriched from CB by MACS and activated by culture at $1x10^6$ cells/ml in X-Vivo medium containing 200 IU/ml IL-2 and CD3/28 co-expressing Dynal® beads at a 3 bead to 1 cell ratio. Culture at 37°C was performed for 3 days. For transduction, Tregs were plated at a concentration of $5x10^5$ cells/ml in thawed retroviral supernatant in Retronectin-coated 24-well tissue culture plates and continually cultured for 11 days by using the same procedure as described previously (*see* Parmar, Simrit et al. Cytotherapy vol. 16,1 (2014): 90-100). As shown in **FIG. 18**, on day 0 when mice were imaged at 30 minutes post injection of the transfected CB Treg cells, trafficking to the lungs was visualized.

Evaluating Anti-inflammatory Function of CB Tregs in Xenogeneic Systemic Lupus Erythematosus (SLE) Mouse Model

[0426] The ability of CB Tregs to reduce systemic inflammation was evaluated in xenogeneic SLE mouse models.

[0427] PBMC derived from SLE patients were injected into BALB-$Rag2^{-/-}$$IL2Rgc^{-/-}$mice (DKO), which lack T, B and NK cells, to induce SLE. Four to five weeks old female and male mice were used for engraftment of human SLE PBMC at a dose of 4 $x10^6$/mouse, followed 4 weeks later by cryopreserved CB Treg cells at a weekly dose of $1x10^7$ cells x 4 injections. This model was utilized to examine the effect of multiple doses of CB Tregs on the systemic inflammation.

[0428] Assessing the physical health of the mice after treatment, control mice (recipients of SLE-PBMC) showed widespread loss of fur and skin inflammation as compared to the treatment mice (recipients of SLE-PBMC and multiple injections of CB Treg cells), which showed healthy fur. **FIG. 12A** shows representative histology sections of skin from control and

treated mice. Microscopic histopathological analysis showed extensive tissue necrosis as evidenced on the skin biopsy of the control mice (Left panel), whereas preservation of the hair follicles and tissue architecture was observed in the treatment mice (Right panel), indicating a healthy skin with no inflammatory injuries. The difference in generalized skin inflammation was accompanied by a decrease in circulating pathogenic $CD8^+$ T cells in peripheral blood and different organs, as shown in **FIG. 12B.**

[0429] Levels of CD8+ T cells in peripheral blood, spleen, lung, and liver were assessed. As shown in **FIG. 12B**, mice from the treatment group showed lower levels of CD8+ T cells in all tissues compared to the control mice, in accordance with the reduced skin inflammation observed in the mice treated with CB Treg cells.

[0430] The distribution of CB Treg cells in the peripheral blood was also analyzed and the results are shown in **FIG. 12C.** Serial blood draws were performed on the mice in both the cohort of control group treated w SLE-PBMC alone and those treated with multiple injections of cryopreserved CB Treg cells. Phenotypic analysis of the cells was performed via surface staining with anti-human specific antibodies, including: CD4, CD8, CD25, CD127 and CD45 (BD Biosciences, San Jose, CA). Anti-mouse CD45 antibody (BD Biosciences, San Jose, CA) was used as negative control in the xenogeneic mouse model. Events were acquired using a FACSCalibur flow cytometer (BD Biosciences) and data analysis was performed using CellQuest™ Pro software (BD Biosciences). The left panel of **FIG. 12C** shows the transient increase in the circulating $CD4^+$ cells that coincides with the $CD4^+$ CB Treg cell injections. The right panel of **FIG. 12C** shows a corresponding sustained decrease in the $CD8^+$ effector T cells.

Example 4. Effect of HLA matching in CB Treg function

[0431] Exemplary cryopreserved cord blood (CB) derived Tregs were assessed for their ability to suppress proliferating conventional (CD4+CD25-) T cells (Tcon) when HLA was fully or partially matched, or mismatched.

[0432] For fully HLA-matched experiments, Tcons and Tregs were isolated from a single cord blood unit (CBU) and selected accordingly. For partially matched HLA experiments, Tcons and Tregs were isolated from two different CBUs. Although a true haploidentical combination is between parent and child or between half-matched siblings, we made the assumption that cells derived from two different cord blood units may offer a similar degree of mismatch due to the permissive nature of the CB HLA, which is hypothesized to be due to the increased tolerance of cord blood lymphocytes induced by the state of pregnancy, in which the infant and the mother tolerate one another despite an HLA mismatch between them. For fully mismatched

HLA experiments, Tcons were generated from adult peripheral blood, and Tregs were generated from unrelated CBU. Tcons derived from adult PB are competent, aggressive and highly proliferative due to their exposure to multiple antigens as part of aging as well as a large component of memory T cells that can be triggered easily by the engagement their T cell receptor (TCR) by the CD3/28 beads.

[0433] Table A shows a comparison of the Tcon suppression effectiveness according to the three HLA matching conditions described above. As shown by the data, the ability of Tregs to suppress Tcons was independent of the HLA matching between the target cells and the Tregs.

Table A. Percent suppression of Tcon proliferation by CB Tregs with full, partial, or mismatch of HLA type

	Full HLA match (N=2)	**Partial HLA match (N-2)**	**HLA mismatch (N=3)**
Percent suppression of Tcon proliferation	97.1%	97.25%	95.5%

[0434] While the UCB Tregs tested in this experiment were not CK0803 (described in Example 2 above), they still represent HLA-matched or mismatched cord blood-derived Treg cells that are expected to function the same as CK0803. Without wishing to be bound by theory, these data suggest that CB Tregs show no differential suppression based on HLA mismatch, and, therefore, CB Tregs can be effective in the treatment of inflammation-associated diseases, such as ALS, regardless of HLA matching in subjects to be treated.

Example 5. Clinical safety of CB Treg infusions

[0435] Exemplary cryopreserved cord blood (CB) derived Tregs were assessed for their safety in subjects with moderate to severe COVID-19 patients who were intubated and mechanically ventilated due to lung tissue damage from severe inflammatory response. Exemplary CB Tregs were administered to the subjects at a fixed dose of 100 million or 300 million cells.

[0436] FIG. 13 shows a survival rate curve of the subjects who were administered placebo (1), 100 million CB Tregs (2), or 300 million CB Tregs (3) and evaluated for about a year. The posterior probability of beneficial effect of the exemplary CB Treg at 100 million cells versus placebo was 89.7% for S28 and 98.6% for overall survival in a Bayesian regression analyses, including covariates of age, gender, race, vasopressors, oxygenation and duration of intubation. As shown in **FIG. 13**, the exemplary CB Treg at 100 million cell dose led to a longer overall survival compared to both placebo and CK0802 at 300 million cells dose.

[0437] Without wishing to be bound by theory, based on this data, a fixed cell dose of 100 million Treg cells for CK0803 would be reasonable to be examined.

Example 5. ***In Vitro CK0803 Migration Assay***

[0438] CK0803 (described in Example 2) mobility was evaluated in a transwell migration assay in the presence of three different chemoattractant (ICAM-1 at 1 µg/ml, CXCL-10 at 50 ng/ml, and/or CCL21 at 50 ng/ml). Three different cocktails of known Tcell chemoattracts were prepared: (1) CXCL-10 alone, (2) ICAM-1 and CXCL-10, and (3) ICAM-1, CXCL-10, and CCL21. Methods described in Example 2 and **FIG. 7A** were followed. The chemoattractant cocktails were added to the media at 1X, 2X, or 4X, and cells were incubated for 30-60 minutes. The average number of cells migrated (from 3 triplicates) and the average migration index (calculated as the number of migrated cells in bottom chamber of treatment group divided by the number of migrated cells in bottom chamber of negative control) are tabulated in **Table B**.

Table B. Average cell migration and migration index in the presence of different chemoattractants

	CXCL10		ICAM-1 and CXCL10		ICAM-1, CXCL10, and CCL21	
Cocktail dose	**Average cells migrated (count/µl)**	**Average migration index**	**Average cells migrated (count/µl)**	**Average migration index**	**Average cells migrated (count/µl)**	**Average migration index**
Control	16		9		9	
1 X	59	3.7	68	7.5	77	9.2
2 X	58	3.6	70	7.8	81	9.7
4 X	69	4.3	100	11.1	112	13.5

[0439] CK0803 cells cultured in ICAM-1, CXCL-10, and CCL21 showed the highest amount of migration compared to the other two cocktails, followed by the combination of ICAM-1 and CXCL10. In all cocktails added to the media, no difference was observed between the 1 X and 2X concentrations, while 4 X doses showed an increase in migration in all cocktails tested.

[0440] Without wishing to be bound by theory, the results demonstrate that CK0803 homing can be increased to sites with more than one T cell chemoattractant, such as ICAM-1, CXCL-10, and CCL21. As a non-limiting example, diseases associated with inflammation that activate T cells with ICAM-1, CXCL-10, and/or CCL21, could be targeted and suppressed by CB Tregs described herein, such as CK0803.

Example 6. Stability Of CK0803 Cells After Freeze-thaw Cycle

[0441] Stability of CK0803 (described in Example 2) was evaluated after a freeze-thaw cycle. Three different batches of CK0803 (Batch #1, #2, and #3) were frozen and then thawed for evaluation at 0 hour, 4 hours, and 24 hours post-thaw. The different stability parameters were compared to each sample before freezing (pre-freeze).

[0442] To determine cell survival, the percentage of total nucleated cells (TNC) was calculated by counting the number of thawed cells stained with 7-amino-actinomycin D (7-AAD). The percentage of TNC, average of all three lots, and standard deviation (SD) are tabulated in **Table C**.

Table C. Percentage of total nucleated cells after freeze-thaw cycle from recovered CK0803 batches

Batch #	Sampling Timepoints Pre-Freeze and Post-Thaw Hold Time			
	Pre-Freeze	Post-Thaw	Post-Thaw	Post-Thaw
		T=0	T=4 hr	T=24 hr
1	92	72	71	63
2	93	84	81	78
3	93	76	68	60
Average	93	77	73	67
SD	0	5	6	8

[0443] The purity of Tregs with the desired phenotype (CD3+CD4+CD25+) relative to all cells in each batch sample was measured by flow cytometry. The percentage recovery of those Tregs was then calculated based on measurements done pre-freeze. The percentage of Tregs in the three batches before freezing and post-thaw are recorded in **Table D**, and the percentage recovery based on pre-freeze values are recorded in **Table E**.

Table D. Percentage of viable Tregs with CD3+CD4+CD25+ phenotype from recovered CK0803 batches

Batch #	Sampling Timepoints Pre-Freeze and Post-Thaw Hold Time			
	Pre-Freeze	Post-Thaw	Post-Thaw	Post-Thaw
		T=0	T=4 hr	T=24 hr
1	93.7	93.4	92.5	90.6
2	96.1	95.8	95.9	94.0
3	97.2	97.2	96.5	97.1
Average	95.7	95.5	95.0	93.9
SD	1.4	1.6	1.8	2.7

Table E. Percentage recovery of viable Tregs with CD3+CD4+CD25+ phenotype from recovered CK0803 batches

Batch #	Sampling Timepoints Pre-Freeze and Post-Thaw Hold Time			
	Pre-Freeze	Post-Thaw T=0	Post-Thaw T=4 hr	Post-Thaw T=24 hr
1	N/A	71	70	60
2	N/A	82	79	81
3	N/A	70	73	52
Average	N/A	74	74	64
SD	N/A	5.6	3.5	12.0

[0444] Next, the phenotype of CK0803 was further assessed by measuring the number of cells that are CD11a high (**Table F**), FoxP3 high (**Table G**), and HELIOS high (**Table H**) from the population of Tregs that are CD3+CD4+CD25+. All cells in the samples were found to be CD11a high. The number of FoxP3 high and HELIOS high cells increased after 0 and 4 hours from thawing the CK0803 batches, but decreased to comparable levels to pre-freeze after 24 hours.

Table F. Percentage of CD11a high Tregs from recovered batches of CK0803

Batch #	Sampling Timepoints Pre-Freeze and Post-Thaw Hold Time			
	Pre-Freeze	Post-Thaw T=0	Post-Thaw T=4 hr	Post-Thaw T=24 hr
1	100.0	100.0	100.0	100.0
2	100.0	100.0	100.0	100.0
3	100.0	100.0	100.0	100.0
Average	100.0	100.0	100.0	100.0
SD	0.0	0.0	0.0	0.0

Table G. Percentage of FoxP3 high Tregs from recovered batches of CK0803

Batch #	Sampling Timepoints Pre-Freeze and Post-Thaw Hold Time			
	Pre-Freeze	Post-Thaw T=0	Post-Thaw T=4 hr	Post-Thaw T=24 hr
1	86.3	93.2	93.5	91.3
2	68.6	95.3	88.5	68.6
3	96.4	97.4	96.8	89.6
Average	83.8	95.3	92.9	83.2
SD	11.5	1.7	3.4	10.3

Table H. Percentage of HELIOS high Tregs from recovered batches of CK0803

Batch #	Sampling Timepoints Pre-Freeze and Post-Thaw Hold Time			
	Pre-Freeze	Post-Thaw T=0	Post-Thaw T=4 hr	Post-Thaw T=24 hr
1	85.6	94.4	94.2	93.3
2	68.8	95.8	91.2	68.8
3	96.6	97.9	97.8	88.1
Average	83.7	96.1	94.4	83.4
SD	11.4	1.4	2.7	10.5

[0445] To assess whether the thawed CK0803 batches maintained their function, suppression activity was determined as described in Example 2 (*see*, "Suppressive Function of CK0803 Cells"). The percentage suppression of Tcon cells is recorded in **Table I**. Variability was observed in studies with 1:4, 1:2, and 1:1 ratios across the different batches, but the averages of all ratios were comparable to those with non-freeze-thawed samples of CK0803 (see, Example 2 and **FIG. 5**).

Table I. Percentage of Tcon suppression by Tregs from recovered batches of CK0803

Batch #	Treg:Tcon ratio				
	1:4	1:2	1:1	2:1	4:1
1	19.7	59.1	89.5	97.6	98.6
2	3.9	26.7	73.2	92.9	96.9
3	3.4	9.1	50.4	87.6	94.8
Average	9.0	31.6	71.0	92.7	96.8
SD	7.6	20.7	16.0	4.1	1.6

[0446] Without wishing to be bound by theory, these data indicate that CK0803 is stable and maintains function after undergoing a freeze-thaw cycle, supporting the feasibility of manufacturing and distribution of CK0803.

Example 7. Preliminary Clinical data for Treatment of ALS Using CK0803

Compassionate Use of CK0803 for Treatment of ALS

[0447] In a non-limiting example, CK0803 (described in Example 2) was administered to six subjects with ALS under compassionate use. The demographic information of the patients is shown in **Table J**.

[0448] All patients received a fixed, intravenous dose of 100 million cells that were not HLA matched. **FIG. 14A and FIG. 14B** outline the CB Treg therapy treatment plan for all patients (**FIG. 14A**) and the optional maintenance treatment plan (**FIG. 14B**). The treatment plan started with weekly infusions for induction of the cell therapy and was then proceeded with monthly infusions for consolidating (**FIG. 14A**).

Table J. Demographic information of six subjects with ALS administered with CB Tregs

Patient number	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**
Type of ALS	Gene negative, spinal onset	Gene negative, spinal onset	Gene negative, spinal onset	Gene-negative, bulbar-onset	Familial ALS associated with a mutation in the TARDBP gene	Gene negative, spinal onset
Dominance*	UMN	UMN	LMN, Bulbar	Bulbar	UMN, Head drop	LMN
Gender	Female	Female	Female	Female	Female	Male
Race	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Age, years	27	32	57	66	40	64
Riluzole or Edaravone	Yes	No	Yes	Yes	Yes	Yes
Riluzole	Yes	No	Yes	Yes	Yes	Yes
Edaravone	No	No	No	No	No	No
Both	No	No	No	No	No	No
Pre-baseline ALSFRS-R Slope	4.2	1.1	0.4	1.0	2.0	1.8

*Note: "UMN" refers to "upper motor neuron;" "LMN" refers to "lower motor neuron."

[0449] The number of infused doses and duration of the treatment for each patient is recorded in **Table K**. Patient #5 received a revised diagnosis and patient #5 started a secondary therapy during the treatment plan, so they were not included in the evaluation of the safety and efficacy of CK0803.

Table K. Number of CK0803 doses infused and duration of treatment

Patient #	1	2	3	4	5	6
Doses infused	22	12	10	10	6	20
Duration of treatment (months)	18	9	6	6	3	19

[0450] The number of infused doses and duration of the treatment for each patient is recorded in **Table K**. Patient #5 received a revised diagnosis and patient #5 started a secondary therapy during the treatment plan, so they were not included in the evaluation of the safety and efficacy of CK0803. During the treatment plan, patients did not need to be hospitalized. In addition, no interventional therapies such as immune suppression or IL-2 administration were needed.

[0451] FIGs. 15A-D show the recorded ALS Functional Rating Scale (ALSFRSR) scores of the four evaluable patients during the treatment period. ALSFRS is a questionnaires that measures motor/physical function over time based on 12 aspects: speech, salivation, swallowing, handwriting, cutting food, climbing stairs, turning in bed, walking, dressing and hygiene, dyspnea, orthopnea, and breathing insufficiency. Higher ALFSRS scores indicate increased motor function retention. Horizontal bars within each of the graphs indicate the period in which each of the patients were in the treatment period, and the number of CK0803 doses received (N) is indicated. Patients administered with CK0803 showed slower decrease in ALFSRS scores compared to the times before (**FIGs. 15A** and **15B**) and after (**FIG. 15C**) being administered with CK0803. Patient #6 showed steadiness in ALFSRS score during the second treatment period after a break from the first treatment period (**FIG. 15D**).

[0452] Without wishing to be bound by theory, these preliminary clinical results suggest that CK0803 treatment in subjects with ALS can slow down progression of the disease.

Case Study of Allogeneic CK0803 for Treatment of Fast-progressing ALS

[0453] In another non-limiting example, CK0803 (described in Example 2) was administered to a 26-year-old female subject with fast-progressing ALS.

[0454] FIG. 16A shows a graph plotting the recorded ALFSRS scores over a period of 15 months. Month 0 (marked with a solid vertical line) was the beginning of the CK0803 treatment, with each infusion marked with a vertical arrow. Before the treatment, the subject showed an over 20-point decrease in ALFSRS score in 3 months. After starting treatment with

CK0803 infusions, the subject showed a 5-point decrease in a period of 12 months, correlating with a slowdown in ALS progression.

[0455] Neurofilament concentration was measured in the cerebrospinal fluid (CSF) of subject as a marker of neuro-axonal damage. **FIG. 16B** shows the concentration of neurofilaments from the beginning of CK0803 administration (month 0, marked with a solid vertical line). CK0803 doses are marked with vertical arrows along the x-axis. Infusions of CK0803 correlate with a decrease in neurofilament levels in the CSF.

[0456] Immune cell population distribution in CSF and peripheral blood was assessed as markers for inflammation and cytotoxicity by single-cell RNA sequencing. In the CSF, a decrease in macrophage population was observed. In the peripheral blood, a decrease in CD4+ and NK cells was observed. The populations of cytotoxic, inflammatory , and Treg populations were found proportionally stable over the course of the treatment.

[0457] Without wishing to be bound by theory, these results indicate that CK0803 infusions can slow down ALS progression and decrease neuronal damage, which can be attributed to the decrease in cells associated with immune responses and inflammation, such as macrophages, CD4+ cells, and NK cells.

Case Study I of Allogeneic CK0803 for Treatment of Lung-dominant ALS

[0458] In another non-limiting example, CK0803 (described in Example 2) was administered to a subject with lung-dominant ALS.

[0459] ALSFRSR scores and forced vital capacity of the subject were recorded before and during the CK0803 treatment schedule. Forced vital capacity indicates lung function by measuring the maximum amount of air that a subject can exhale following a deep inhale. **FIGs. 17A** and **17B** show the recorded ALSFRSR scores (**FIG. 17A**) and forced vital capacity (FVC; **FIG. 17B**) of the subject from 9 months before the start (month 0) of CK0803 treatment and 16.2 months after. The vertical arrows indicate the CK0803 infusions, and each horizontal bar at the base of the arrows indicates two different dosing schedules. Before CK0803 infusions, the subject showed progressive decline in both ALSFRSR and FVC (see from -9.0 months to 0 months in **FIGs. 17A** and **17B**). The ALSFRSR score of the subject stabilized at about 31 points after starting the treatment, and a steady improvement in FVC can be observed after 4.5 months from the start of the therapy.

[0460] The presence of peripheral blood (PB) Tregs in the subject was measured after beginning the CK0803 therapy. **FIG. 17C** shows the percentage of PB Tregs measured in the PB in a weekly basis over a period of 261 days. PB Treg amount was steady from the beginning

of the therapy until Day 157, and then an increase was observed. The increase in PB Tregs correlated with a decrease in the concentration of neurofilaments (**FIG. 17D**) and an improvement in the ALSFRSR score (**FIG. 17E**).

[0461] Without wishing to be bound by theory, these results indicate that CK0803 infusions can prevent progression ALS and treat the disease, as observed by the increased FVC and ALSFRSR scores.

[0462] In a non-limiting manner, the data in this Example preliminarily show that CK0803-based cell therapies are safe in humans for the slowing down progression of and treating ALS that manifests in different forms. The therapeutic efficacy is correlated with decreases in activated immune cells and reduction in biomarkers for neurological damage, such as neurofilaments, suggesting that CK0803 has to potential to reduce inflammatory responses and associated damage, as shown by the embodiments of the present disclosure.

Example 8 Clinical Safety and Preliminary Efficacy of Regulatory T cells for ALS

[0463] The following is a non-limiting example describing results from a clinical trial in which six participants having ALS received infusions of a fixed dose ($100x10^6$ cells) of umbilical cord blood (UCB)-derived, allogeneic, non-HLA matched, cryopreserved Tregs (TREG).

[0464] Briefly, participants with ALS received infusions of a fixed dose ($100x10^6$ cells) of umbilical cord blood (UCB)-derived, allogeneic, non-HLA matched, cryopreserved Tregs (TREG); administered as 4 weekly infusions followed by 6 monthly infusions. No lymphodepletion, immunosuppression, or interleukin-2 was administered. The primary outcome was dose limiting toxicity including infusion reaction within 24 hours (NCI-CTCAE V4.0) and/or regimen related death, or grade 3 or 4 cytokine release syndrome within 14 days post infusion. Clinical response was measured by the ALS-Functional Rating Scale-Revised (ALSFRS-R). Exploratory analysis measured serum and plasma neurofilament light (NfL) and inflammatory biomarkers.

[0465] Six participants with a median age of 48.5 years (range 27-66 years); and baseline ALSFRS-R score of 31.5 (range 23-43) were treated with 11 (6-22) TREG infusions, in an ambulatory setting. No dose-limiting toxicity was observed. In participants with sufficient datapoints (n=4), the mean ALSFRS-R slope of decline was -1.66±1.03 points/month before treatment, -0.41±0.45/month during treatment and -0.60±0.59/month post-treatment. Multiple biomarkers including NfL and inflammatory markers MIP-1δ, CTACK, and GROα, exhibited different relationships with ALSFRS-R score between participants.

Participants and Methods

Allogeneic umbilical cord blood T regulatory cell product

[0466] Cryopreserved, allogeneic, umbilical cord blood Treg cell products (TREG) were manufactured as described previously (*see* Kadia TM, et al. Phase 1 Study of CK0801 in Treatment of Bone Marrow Failure Syndromes. *NEJM Evid* 2024;3(6):EVIDoa2300362; Gladstone DE, et al. Randomized, double-blinded, placebo-controlled trial of allogeneic cord blood T-regulatory cells for treatment of COVID-19 ARDS. *Blood Adv 2023*;7(13):3075-3079; and Gladstone DE et al. Regulatory T Cells for Treating Patients With COVID-19 and Acute Respiratory Distress Syndrome: Two Case Reports. *Ann Intern Med* 2020;173(10):852-853).

Study Design

[0467] Participants with ALS were treated with multiple infusions of TREG, for whom unique clinical protocols were approved by the FDA under individual, compassionate-use IND applications. Participants were identified by the principal investigator based on disease severity and degree of progression. The treatment plan included an Induction Phase of four weekly infusions followed by a Maintenance Phase of six monthly infusions. TREG were administered at a fixed dose of 100 million Treg cells per infusion, regardless of participant weight. Additional treatment(s) with six monthly infusions were allowed for participants at the treating physician's discretion and after FDA approval. No HLA matching was performed between the TREG product and the recipient. No IL-2, lymphodepletion or immune suppression was administered.

[0468] The primary objective was to examine safety of infusion of multiple doses of cryopreserved, non-HLA matched TREG products. Close clinical monitoring was performed including measurement of vital signs: pre-infusion, 15- , 30- , 60- , 120- minutes after start of infusion, and then per standard clinical practice. Dose-limiting toxicity was defined as severe (grade 3 or 4) infusion reaction/toxicity within 24 hours (NCI-CTCAE V4.0), regimen-related death within 14 days, or severe (grade 3 or 4) cytokine release syndrome within 14 days.

[0469] Clinical assessment, physical exam, neurological exam, motor strength and ALSFRS-R scoring was performed at baseline (day -10 +/- 4 days), day of infusion on Day 0, 7, 14, 21 (prior to infusion) and at Week 4, 8, 12, 16, 20 and 24 after first infusion. Additional assessments were performed at 9 and 12 months. There was no pre-established statistical hypothesis.

Correlative Assays

[0470] CSF was collected at screening and Week 4, 8, 12, 16 and 24 after first infusion. Plasma was collected at screening, on Day 0, 7, 14, 21 (prior to infusion) and at Week 4, 8, 12, 16, 20

and 24 after first infusion. For participant 1 (TREG_1), who received an additional 12 infusions, plasma was collected prior to each infusion and at 6 months after final infusion; CSF was collected at screening and at Month 1, 3, 6, 9, 12, 15 and 22. Neurofilament light chain (NfL) concentration was measured using the Simoa™ NF-Light® kit (Quanterix #103186, PBL Assay Science, Piscataway, NJ, USA). Inflammatory biomarkers were measured using the Human Cytokine Discovery Assay kit (Eve Technologies, Calgary, Alberta, Canada) (Table M, Table N). When duplicate measurements were available, the most recent was used to limit inter-sample variability. Measurement of peripheral blood and CSF Tregs and $CD8^+$ T cells was performed by multiparametric flow cytometry.

Statistical Analysis Plan

[0471] No formal statistical analysis plan was implemented at the time of conduct of the single participant INDs. All analysis on research samples are post-hoc.

Data Analysis

[0472] *Safety Data Analysis*

[0473] Safety data were collected longitudinally for each participant and included measurements of peripheral blood white blood cell (WBC) count (10^3 cells/µl), CSF protein concentration (mg/dL), and CSF total nucleated cell count (cells/µl) (Table O, FIG. 22). Data outside the normal range were flagged as outliers.

Missing Data

[0474] For correlation analyses, given the small sample size, measurements were collapsed for each 3-month interval, taking the mean where there are multiple values in the interval.

ALSFRS-R, NfL and Biomarker Analysis

[0475] ALSFRS-R, a 12-item measurement of functional capability comprising gross motor, fine motor, bulbar and respiratory domains, was used to measure functional status longitudinally. For data analysis and correlation with clinical outcomes, four evaluable participants were included (TREG_1, TREG_2, TREG_3, and TREG_6). Evaluable participants were defined as those who had at least three consecutive ALSFRS-R score measurements, which excluded TREG_5; TREG_4 was also excluded from this analysis due to a mixed neuroinflammatory disease diagnosis. ALSFRS-R scores were analyzed across three treatment phases: pre-treatment (before first infusion), on-treatment (during regular infusions), and post-treatment (after last infusion). Linear regression models were fitted to calculate the slope of ALSFRS-R change for each phase. The slope coefficient represents the rate of change in ALSFRS-R scores per month during that treatment window. NfL and biomarker concentrations were analyzed and visualized using raw values.

Correlative Data Analysis

[0476] To address sparse data points and enable comparisons between ALSFRS-R scores and biomarker levels, a 3-month window approach was employed for correlation analyses. Measurements were grouped into 3-month intervals relative to the first infusion date by converting days since first infusion to months (dividing by 30 and rounding down to the nearest integer using the floor function). Measures in each interval were averaged for both ALSFRS-R scores and biomarker concentrations. Averaged values were used to compute Spearman correlations between ALSFRS-R scores and biomarker concentrations for each participant and fluid type, requiring a minimum three comparable measurements for each analysis group. This approach was used only for correlations. All visualizations present raw, unadjusted data.

Biomarker Prioritization

[0477] An exploratory approach was implemented to identify biomarkers with consistent median correlations with ALSFRS-R in CSF and plasma. Correlations were retained if at least one confidence interval excluded 0 in either fluid and calculated median correlations across participants for each marker. For FIG. 20A and FIG. 20B heatmaps, biomarkers with at least one non-zero-spanning confidence interval in both fluids were prioritized. FIGs. 21A-21D highlights biomarkers with consistent median correlation directions across fluids and a confidence interval that excludes 0 in either fluid for a single participant. Top biomarkers for FIGs. 21A-21D were determined based on highest absolute coefficients consistent across fluids.

Statistical Considerations

[0478] Due to the exploratory nature and sample size, no formal statistical hypothesis testing was performed. Correlation analyses were not adjusted for multiple comparisons. Confidence interval widths indicate precision but are not used for hypothesis testing.

Results

Participant Characteristics

[0479] Six participants with a diagnosis of ALS (female=5), median (range) age of 48.5 years (27-66 years) and disease duration of 10 months (6.1-45.6 months) with a starting ALSFRS-R score of 31 (23-43), received TREG cell therapy (Table L). Four had gene-negative, spinal-onset ALS, one had gene-negative, bulbar-onset ALS, and one had familial ALS associated with a *TARDBP* mutation. Median values of baseline CSF and plasma NfL levels were 5,498.8 pg/mL and 53.6 pg/mL, respectively. All participants received prior riluzole and continued on treatment as per the recommendation of their physician.

Safety

[0480] A median of 11 (6-22) TREG doses were administered. The TREG product was thawed and infused over 15-20 minutes in the ambulatory setting. No infusion reactions or instances of cytokine release syndrome were observed. All participants were able to go home the day of treatment. A total of 19 adverse events (AEs) were recorded in 3 participants, the most common being headache, occurring 6 times in 1 participant, possibly related to the excipient, dimethyl sulfoxide, used for cryopreservation of Treg cells (Table P, Table O, FIG. 22). There were no grade 3 or 4 AEs or infusion reactions. Additional safety measures including blood WBC count, CSF protein concentration, and CSF total nucleated cell count are shown in FIG. 22. A transient increase in peripheral blood Tregs was observed in available samples from one participant (TREG_6) post-treatment (FIG. 23, top left panel). For TREG_6, ALSFRS-R, average ALS quality of life (ALSQoL) (range 0-10, with higher scores indicating better quality of life), forced vital capacity (range 0-100%, with higher scores indicating better function), and total Edinburgh Cognitive and Behavioural ALS Screen (ECAS) (range 0-136, with higher scores indicating better function) scores were also collected (FIG. 23); slight increases in ALSFRS-R, ALSQoL and ECAS scores were observed during treatment. An apparent increase in CSF Tregs was observed post-TREG infusion in available samples from two participants (FIGs. 24A-24B).

ALSFRS-R scores and NfL Concentration

[0481] Analysis of ALSFRS-R scores in four evaluable participants revealed varying patterns of functional decline across treatment phases (Table Q, FIG. 19A). A temporary slowing of the rate of ALSFRS-R decline was observed during the on-treatment period compared to the pre-treatment period was observed for three out of four patients (TREG_1, TREG_2, TREG_6) (FIG. 19A). The decline of TREG_1 slowed from -3.04 points/month before treatment to -0.61 points/month on treatment, the decline of TREG_2 slowed from -1.50 points/month to -0.13 points/month, and TREG_6 stabilized/improved from -1.58 points/month to +0.04 points/month. In contrast, TREG_3 exhibited an accelerated decline rate across phases: -0.55, -0.95, and -1.36 points/month for pre-, on-, and post-treatment periods, respectively. Post-treatment, ALSFRS-R score decline was still reduced compared to pre-treatment for 3 of 4 participants (TREG_1, TREG_ 2, TREG_6). The mean (± standard deviation) ALSFRS-R slope of decline across all participants improved from -1.66 ± 1.03 points/month before treatment to -0.41 ± 0.45 points/month during treatment, followed by -0.61 ± 0.59 points/month post-treatment (FIG. 19B). The mean (± standard deviation) ALSFRS-R slope of decline

improved from -1.66 ± 1.03 points/month before treatment to -0.41 ± 0.45 points/month during treatment and –0.60 ± 0.59 points/month post-treatment (FIG. 19B).

[0482] NfL concentration, a marker for neuronal injury and neurodegeneration, was measured in participant CSF and plasma. A moderate decrease in CSF NfL concentration was observed for 2 of 4 evaluable participants during treatment (TREG_1, TREG_2). For TREG_1, baseline CSF NfL was 3,189 pg/mL which decreased to 2,090 pg/mL after 11.4 months of treatment. For TREG_2, baseline CSF NfL concentration was 3,061 pg/mL, which decreased to 2,035 pg/mL after 5.5 months of treatment. In plasma, NfL concentration decreased in 2 out of 4 evaluable participants (TREG_1, TREG_6). For TREG_1, baseline plasma NfL was 20.87 pg/mL, which increased to 21.12 pg/mL at 6 months and decreased to 15.06 pg/mL at 12 months post-treatment. For TREG_6, baseline plasma NfL concentration was 109 pg/mL, which decreased to 85 pg/mL after 3.5 months of treatment.

Correlation of ALSFRS-R and NfL levels

[0483] To investigate the relationship between ALSFRS-R scores and NfL concentration over time, correlation analyses were performed between ALSFRS-R score and NfL concentration in CSF and plasma (Table R). Correlations varied among participants and fluids (FIG. 19C-D). In CSF, correlations ranged from strongly negative (TREG_6, rho = -0.98) to moderately positive (TREG_2, rho = 0.79). Plasma correlations showed less variability, ranging from weakly negative (TREG_2, rho = -0.27) to weakly positive (TREG_6, rho = 0.04). Overall, we observed a poor mean correlation in CSF (Spearman's rho = -0.01) and a weak negative mean correlation in plasma (Spearman's rho = -0.11).

Correlation of ALSFRS-R with inflammatory biomarkers

[0484] To investigate the relationship between TREG cell infusion, inflammation, and ALSFRS-R scores, correlation analyses were performed between ALSFRS-R scores and inflammatory markers in CSF and plasma (Table S6). The aim was to identify biomarkers with consistency between CSF and plasma, which could be potentially monitored less invasively through blood draws while still reflecting the neuroinflammatory processes typically assessed in CSF. Strength and direction of correlations varied among biomarkers and participants (FIG. 20). TNF superfamily member 10 (TRAIL), cutaneous T cell-attracting chemokine (CTACK), and Macrophage inflammatory protein-1 delta (MIP-1δ) showed negative correlations with ALSFRS-R, while C-X-C Motif Chemokine Ligand 1 (GROα) and C-X-C Motif Chemokine Ligand 5 (ENA-78) exhibited positive correlations in plasma (FIG. 20A). A different pattern

was observed in CSF (FIG. 20B). Eotaxin, Osteocalcin, and DKK1 correlated positively with ALSFRS-R scores, while sVEGFR2, Osteopontin, and Resistin showed negative correlations.

[0485] To further explore biomarkers with consistent correlations across CSF and plasma, median correlations were examined between ALSFRS-R and each marker across participants, including markers with at least one correlation confidence interval excluding 0 in either CSF or plasma (FIG. 21A). MIP-1δ and CTACK demonstrated negative median correlations with ALSFRS-R in both fluids, while GROα showed positive median correlations. Temporal analysis (FIGs. 21B-21D) revealed that MIP-1δ maintained consistently negative median correlations across most participants in both fluids. CTACK exhibited similar negative trends, particularly in CSF, while GROα demonstrated positive correlations, especially pronounced in CSF for several participants.

Summary

[0486] The clinical trial described in this example investigated the effect of multiple infusions of a fixed dose of 100 $x10^6$ cryopreserved, off-the-shelf, non-HLA matched, allogeneic, umbilical cord blood Treg cells (TREG) for the treatment of ALS. All infusions were administered safely, in an ambulatory setting, for up to 22 infusions without the need for immunosuppression, lymphodepletion, or IL-2 supplementation. Infusions were well tolerated without any grade 3 or 4 AEs. Transient increases in peripheral blood Tregs post-infusion observed in TREG_6, as well as trends toward an increase in CSF Tregs in TREG_4 and TREG_5, may represent a groundswell effect of both cell and cytokine mixture that results in inducible Tregs. Without wishing to be bound by theory, increases in peripheral blood Tregs have been shown to correlate with clinical improvement in participants with bone marrow failure syndromes who received umbilical cord blood Tregs. In xenogeneic models, improvement in phenotype and function in response to multiple injections of umbilical cord blood Tregs correlated with the increase in Tregs in circulation and tissues.

[0487] The decrease observed in ALSFRS-R decline during the on-treatment phase in 3 out of 4 evaluable participants indicates that the Treg cells of the present disclosure can be used to effectively treat ALS.

[0488] The correlation between NfL levels and ALSFRS-R scores was variable between participants and fluids, where a weak negative correlation was observed overall in CSF (Spearman's rho = -0.027) and plasma (Spearman's rho = -0.178) was observed. Without wishing to be bound by theory, disease heterogeneity might contribute to this variability, as participants exhibited different types of onset and disease stage at treatment initiation. It is possible that the degeneration of upper versus lower motor neurons might be different in our

participants and may reflect the differences in the NfL concentration changes. Specifically, TREG_6 had an atypical disease presentation that primarily included respiratory muscle involvement.

[0489] The exploratory biomarker analysis identified several markers that showed consistent correlation directions with ALSFRS-R scores across CSF and plasma. Without wishing to be bound by theory, these findings are consistent with previous studies implicating MIP-1δ in neuroinflammatory processes and disease progression in other conditions. Interestingly, GROα (CXCL1) showed a positive correlation with ALSFRS-R scores in our study. CXCL1 belongs to the ELR+ subclass of CXC chemokines, which have significant neutrophil chemotactic and activating characteristics. It primarily acts through the CXCR1 and CXCR2 receptors, potentially influencing intracellular signaling pathways and calcium mobilization in ALS. Cutaneous T cell-attracting chemokine (CTACK), shown to selectively attract CLA^+ memory T cells[48], has been implicated in increased risk of Alzheimer's disease. Underlying disease heterogeneity may contribute to the varied response as measured by ALSFRS-R, NfL concentration, and inflammatory biomarker concentrations observed across the 4 evaluable participants.

Table L. Patient Characteristics

Participant ID	**TREG_1**	**TREG_2**	**TREG_3**	**TREG_4**	**TREG_5**	**TREG_6**
Inheritance	Gene negative	Gene negative	Gene negative	Gene-negative	Familial, TDP-43 variant p.S379P	Gene-negative
Dominance	UMN	UMN	LMN, bulbar	Bulbar	UMN, Head drop	LMN
Gender	Female	Female	Female	Female	Female	Male
Race	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Year of Diagnosis	Dec-19	May-20	Aug-17	Jan-21	Oct-20	Oct-21
Age at Treatment Initiation	27	32	57	66	40	64
Riluzole, Edaravone or TUDCA	Y	N	Y	Y	Y	Y
Riluzole	Y	N	Y	Y	Y	Y
Edaravone	N	N	N	N	N	N
TUDCA	N	Y	N	N	N	Y
Baseline ALSFRS-R	23	36	30	43	26	32
Plasma NfL (pg/mL)	22.8	17.6	53.5	69.9	53.72	55
CSF NfL (pg/mL)	3189.05	3061.2	5587.5	10629.5	7070.7	5410
UCB Treg Product	CK0801	CK0801	CK0802	CK0802	CK0802	CK0802
No. administrations	22	12	10	10	6	10

ALSFRS-R: Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; LMN: lower motor neuron; NfL: Neurofilament; Treg: T regulatory cell; TUDCA: Tauroursodeoxycholic acid; UCB: umbilical cord blood; UMN: upper motor neuron.

Table M

Adiponectin/Acrp30	Granzyme B
ALCAM/CD166	IFN-gamma
BDNF	IL-1 beta/IL-1F2
CCL2/JE/MCP-1	IL-4
CCL3/MIP-1 alpha	IL-6
CCL4/MIP-1 beta	ICAM-1/CD54
CCL7/MCP-3/MARC	IL-8/CXCL8
CCL11/Eotaxin	IL-10
CCL23/MPIF-1	IL-17/IL-17A
CCL24/Eotaxin-2/MPIF-2	IL-6R alpha
CCL25/TECK	MMP-2
CCL26/Eotaxin-3	MMP-13
CD27/TNFRSF7	Oncostatin M/OSM
CD40/TNFRSF5	Osteopontin/OPN
CD40 Ligand/TNFSF5	E-Selectin/CD62E
CX3CL1/Fractalkine	TNF-alpha
CXCL6/GCP-2	TNF RII/TNFRSF1B
FGF basic/FGF2/bFGF	TRAIL/TNFSF10
Follistatin-like 1/FSTL1	TRANCE/TNFSF11/RANKL
GM-CSF	VEGF

Table N

6CKine	I-309	IL-1α	IL-7	MIP-1δ
BCA-1	IFN-α2	IL-1β	IL-8	PDGF-AA
CTACK	IFNγ	IL-2	IL-9	PDGF-AB/BB
EGF	IL-10	IL-20	IP-10	RANTES
ENA-78	IL-12p40	IL-21	LIF	sCD40L
Eotaxin	IL-12p70	IL-22	M-CSF	SCF
Eotaxin-2	IL-13	IL-23	MCP-1	SDF-1α+β
Eotaxin-3	IL-15	IL-27	MCP-2	TARC
FGF-2	IL-16	IL-28A	MCP-3	TGFα
FLT-3L	IL-17A	IL-3	MCP-4	TNFα
Fractalkine	IL-17E/IL-25	IL-33	MDC	TNFβ
G-CSF	IL-17F	IL-4	MIG/CXCL9	TPO
GM-CSF	IL-18	IL-5	MIP-1α	TRAIL
GROα	IL-1RA	IL-6	MIP-1β	TSLP
				VEGF-A

Table O

Participant	Measure	min_value	max_value	Median value	SD	No. of measurements	No. Out of range	Normal reference value
TREG_1	CSF Cells (cells/µl)	0	1	0	0.47	14	0	0-5
TREG_1	CSF Protein (mg/dL)	14	20	18	2.11	14	2	15-45
TREG_1	WBC (10^3 cells/µl)	6.15	8.28	6.88	0.71	14	0	3.12-8.44
TREG_2	CSF Cells (cells/µl)	0	1	0	0.52	6	0	0-5
TREG_2	CSF Protein (mg/dL)	28	35	28	3.61	6	0	15-45
TREG_2	WBC (10^3 cells/µl)	6.04	8.66	7.73	1.19	6	2	3.12-8.44
TREG_3	CSF Cells (cells/µl)	0	2	0	1.03	6	0	0-5
TREG_3	CSF Protein (mg/dL)	30	33	30	1.55	6	0	15-45
TREG_3	WBC (10^3 cells/µl)	4.2	6.07	5.19	0.84	6	0	3.12-8.44
TREG_6	CSF Cells (cells/µl)	1	2	2	0.52	6	0	0-5
TREG_6	CSF Protein (mg/dL)	49	74	55	11.67	6	6	15-45

TREG_6	WBC (10^3 cells/µl)	6.05	9.01	6.57	1.13	10	2	3.12-8.44

Table P

Participant	**AE**	**Occurrences**	**Severity**	**SAE**	**Related to Study Drug**	**Related to Infusion Procedure**
TREG_1	Headache	N=6	Mild	N	Possible	Possible
	Joint Pain	N=2	Mild	N	Unrelated	Unrelated
	Chest Tightness and Head Warmth	N=5	Mild	N	Possible	Possible
	Syncope	N=1	Moderate	N	Possible	Possible
	Hand Swelling	N=1	Mild	N	Unrelated	Possible
TREG_2	Post-LP Headache	N=1	Mild	N	Unrelated	Unrelated
	Vomiting	N=1	Mild	N	Unrelated	Unrelated
	Fall	N=1	Mild	N	Unrelated	Unrelated
TREG_6	Mild Injury of Bruising to Ribs	N=1	Mild	N	Unrelated	Unrelated

Table Q. ALSFRS-R slope of decline across treatment phases

Treatment Phase	Range (days)	ALSFRS-R Slope (points/month)	Median ALSFRS-R Score	Standard Error	Number of Measurements
TREG_1					
Pre-treatment	-5 to 0 months	-3.04	28	4.10	3
On-treatment	0 to 15 months	-0.61	17.5	1.37	8
Post-treatment	15 to 27 months	0.04	16	1.00	3
TREG_2					
Pre-treatment	-7 to 0 months	-1.50	42	2.91	3
On-treatment	0 to 9 months	-0.13	35	0.24	5
Post-treatment	9 to 19 months	-0.39	33.5	1.08	4
TREG_3					
Pre-treatment	-4 to 0 months	-0.55	30	0.67	3
On-treatment	0 to 4 months	-0.95	29	1.20	3
Post-treatment	4 to 17 months	-1.36	21.5	3.93	4
TREG_6					
Pre-treatment	-9 to 0 months	-1.56	39	7.00	2
On-treatment	0 to 6 months	0.04	31	0.63	9
Post-treatment	6 to 12 months	-0.70	33	0.91	4

ALSFRS-R slopes of decline (points/month) for each participant and treatment phase. The treatment interval (pre-treatment, on-treatment, post-treatment), corresponding interval range in months ALSFRS-R slope, and median ALSFRS-R score are shown for each participant with two or more datapoints during pre- and post-treatment phases. Positive slopes indicate improvement in ALSFRS-R score, while negative values represent decline.

Table R. Details of ALSFS-R scores and TREG infusion dates

Participant	Days since 1st infusion	Infusion number	ALSFRS-R Score
TREG_1	-141	1	37
TREG_1	-65	1	28
TREG_1	-2	1	23
TREG_1	28	4	23
TREG_1	84	6	21
TREG_1	137	8	18
TREG_1	165	9	17
TREG_1	262	12	14
TREG_1	346	15	13
TREG_1	458	19	16
TREG_1	593	22	13
TREG_1	810	22	16
TREG_2	-205	1	46
TREG_2	-121	1	42
TREG_2	-4	1	36
TREG_2	24	4	36
TREG_2	133	8	35
TREG_2	167	9	35
TREG_2	264	11	35
TREG_2	355	12	33
TREG_2	474	12	34
TREG_2	572	12	30
TREG_3	-121	1	32
TREG_3	-37	1	30
TREG_3	-3	1	30
TREG_3	32	4	29
TREG_3	124	7	26
TREG_3	243	10	25
TREG_3	334	10	18

TREG_3	523	10	9
TREG_4	-58	1	46
TREG_4	-8	1	43
TREG_4	25	4	40
TREG_4	80	6	35
TREG_4	181	9	18
TREG_5	-16	1	31
TREG_5	41	4	26
TREG_6	-270	1	46
TREG_6	-1	1	32
TREG_6	27	4	30
TREG_6	36	4	34
TREG_6	76	5	30
TREG_6	105	7	30
TREG_6	133	8	31
TREG_6	134	8	31
TREG_6	156	9	30
TREG_6	168	9	35
TREG_6	260	10	34
TREG_6	302	10	31
TREG_6	324	10	32
TREG_6	331	10	31
TREG_6	338	10	31
TREG_6	346	10	31

Table 9A. Schedule of Activities for All Subjects

STUDY PERIOD	Screening/ Baseline	Treatment & Follow-Up Period										
STUDY WEEK	-4 to -1	1	2	3	4	5	8	12	13	16	20	24
STUDY DAY*	-30 to -1	1	8	15	22	29	50	78	85	106	134	162
VISIT WINDOW (DAYS)	-	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3
VISIT TYPE[1]	CL	CL, T[4]	CL, T[4]	CL, T[4]	CL, T[4]	CL, FU	CL, T[4]	CL, T[4]	CL, FU	CL, T[4]	CL, T[4]	CL, T[4]
Study Treatment Administration[2,3]		X	X	X	X		X	X		X	X	X
Lumbar Puncture for CSF Collection[3]	X					X			X			
Informed Consent	X											
Inclusion/Exclusion	X											
Medical History	X											

Prior/ Concomitant Meds/ Procedures[4]	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events[4]		X	X	X	X	X	X	X	X	X	X	X
Body Weight & Height[5]	X				X				X			
Pregnancy Test[6]	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs (BP, HR, RR, Body Temp, O2)	X	X[a]	X[a]	X[a]	X	X[b]	X[b]	X[b]	X	X[b]	X[b]	X[b]
Physical & Neurological Exam[7]	X	X	X	X	X	X	X	X	X	X	X	X
Blood Draw for Safety Labs[8]	X	X[c]	X[c]	X[c]	X	X[c]	X[c]	X[c]	X	X[c]	X[c]	X[c]
Blood Draw for PK Analysis[9]		X[c,d]	X	X		X	X			X	X	X
Blood Draw for Biomarker/ Correlative Analysis[9, 14]	X	X[c]	X[c]	X[c]	X	X[c]	X[c]	X	X[c]	X[c]	X[c]	X[c]
Urine Safety Labs[10]	X	X[c]	X[c]	X[c]	X	X[c]	X[c]	X[c]	X	X[c]	X[c]	X[c]
CSF Safety Labs[11]	X				X				X			
CSF Biomarker/Correlative Analysis[12]	X				X				X			
CAFS												
ALSFRS-R[13]	X	X	X	X	X[e]	X	X	X	X	X	X	X[e]

ALS QOL	X	X	X	X	Xe	Xe	X	X	X	X	X	Xe
VAFS13	X	X	X	X	Xe	Xe	X	X	X	X	X	Xe
SVC	X					X			X			
HHD	X					X			X			
ECAS	X					X			X			

Table 9B. Schedule of Activities for All Subjects

STUDY PERIOD	**Post-treatment Follow-Up Period**			**EOT****	
STUDY WEEK	**25**	**36**	**48**	*4 weeks after last infusion**	
STUDY DAY*	**169**	**253**	**337**	*28 days after last infusion*	
VISIT WINDOW (DAYS)	**+/-3**	**+/- 7**	**+/- 7**	**+/- 7**	

VISIT TYPE[1]	CL, FU	CL, FU	CL, FU	CL, FU
Study Treatment Administration[2,3]				
Lumbar Puncture for CSF Collection[3]	X	X	X	X
Informed Consent				
Inclusion/ Exclusion				
Medical History				
Prior/ Concomitant Meds/ Procedures[4]	X	X	X	X
Adverse Events[4]	X	X	X	X
Body Weight & Height[5]	X	X	X	X
Pregnancy Test[6]	X	X	X	X
Vital Signs (BP, HR, RR, Body Temp, O2)	X	X	X	X
Physical & Neurological Exam[7]	X	X	X	X
Blood Draw for Safety Labs[8]	X	X	X	X
Blood Draw for PK Analysis[9]		X	X	X
Blood Draw for Biomarker/ Correlative Analysis[9], 14	X	X	X	X

Urine Safety Labs[10]	X	X	X	X
CSF Safety Labs[11]	X	X	X	X
CSF Biomarker/Correlative Analysis[12]	X	X	X	X
CAFS		X		X
ALSFRS-R[13]	X[e]	X	X	X
ALS QOL	X[e]	X	X	X
VAFS[13]	X[e]	X	X	X
SVC	X	X	X	X
HHD	X	X	X	X
ECAS	X	X	X	X

*Visit days are calculated relative to Study Day 1 (i.e. all study visit days are anchored off Study Day 1)

** EOT to occur at 28 days after the infusion of last dose of CK0803

1 CL = in-person clinic visit; T = study treatment infusion; FU = follow-up visit with lumbar puncture but no study treatment infusion

2 On Study Treatment Administration days, subjects may be discharged from the Study Clinic no sooner than 3 hours after Study Treatment Administration (Infusion) during the Safety Period (Days 1, 8, 15 and 22) and no sooner than 1 hour after Study Treatment Administration during the Expansion Period (Days 50, 78, 106, 134, and 162) and may stay in the clinic longer for further safety monitoring if determined by the Investigator

3 Study Treatment Administration should never occur on the same day as a Lumbar Puncture for CSF Collection

4 Adverse Events and ConMeds/Ancillary Procedures will be collected during a phone visit 1-2 days following each T visit (24-48 hours post-infusion start time).

[5] Height only collected at Screening visit. Weight collected at Screening and all FU visits.
[6] Urine or Serum pregnancy test done for all people of child-bearing potential at Screening visit, each FU visit and morning of each T visit (a negative pregnancy result should be confirmed by study staff prior to start of infusion; a Urine hCG cassette/kit done bedside is allowed).
[7] Full neurological and physical exams done at Screen and each FU visit. A full neurological and abbreviated physical exam done at each T visit pre-infusion.
[8] Blood safety labs include: Coagulation panel (aPTT, PT/INR), Basic Metabolic Panel, Hepatic Panel, Hematology (sent to hospital's local lab)
[9] Blood biomarkers/correlatives include: Plasma, serum, PBMCs, mononuclear cells, donor specific antibody (refer to Lab Manual for sample collection and processing details)
[10] Urine safety labs include: Urinalysis, Urine Total Protein (Urine safety labs sent to hospital's local lab)
[11] CSF safety labs include: Protein, Glucose, Cell Count (sent to hospital's local lab)
[12] CSF Biomarkers include: CSF pellet for cell analysis, archiving supernatant for biomarker assays, cytokine analysis, single-cell RNA and T cell receptor (TCR) sequencing (scRNA/TCR-seq) (refer to Lab Manual for sample collection and processing details)
[13] ALSFRS-R and VAFS may be completed over the phone within 7 days of timepoint if needed
[14] Donor-specific antibody is collected at every visit except Days 8, 15 and 22. On T visits Donor-specific antibody is collected up to 120 minutes prior to study drug infusion.
[a] Vital signs collected at the following timepoints: pre-infusion, approx. midway through infusion, and 15, 30, 60, 90, 120, 150, 180 mins post-infusion start time. Each time point has a window of +/- 5mins.
[b] Vital signs collected at the following timepoints: pre-infusion, approx. midway through infusion, and 15, 30, 60 mins post-infusion start time. Each time point has a window of +/- 5mins.
[c] Collected up to 120 minutes prior to study drug infusion
[d] Collected immediately after study drug infusion (≤10 mins after infusion stop time)
[e] ALSFRS-R score, VAFS and ALS SQOL-R can be measured on weeks 12 or 13 or both time points; and weeks 24 or 25 or both time points

CLAIMS

What is claimed is:

1. A method for treating amyotrophic lateral sclerosis (ALS) in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:

≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and

≤ 10% $CD4^-CD8^+$;

wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses;

wherein each dose is about 1 x 10^8 Treg cells.

2. A method for ameliorating a symptom of amyotrophic lateral sclerosis (ALS) in a subject in need thereof, the method comprising administering intravenously to the subject cryopreserved, allogeneic, cord blood derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:

≥ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and

≤ 10% $CD4^-CD8^+$;

wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses;

wherein each dose is about 1 x 10^8 Treg cells.

3. The method of claim 1 or claim 2, wherein the Treg cells are administered once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses.

4. The method of claim 2 or claim 3, wherein the symptom of ALS is weakness, fatigue, paralysis, decline of skeletal muscle function, or decline of respiratory function.

5. The method of any one of claims 1-4, wherein the subject has the following:

(a) ALS onset ≤ 5 years;

(b) upright Slow Vital Capacity (SVC) as adjusted for sex, age and height ≥ 50% predicted; and

(c) ALSFRSR (Revised ALS Functional Rating Scale) score of 36-45 at baseline.

6. The method of any one of claims 1-5, wherein the method further comprises administering to the subject one or more of:

(i) riluzole;

(ii) edaravone; and

(iii) sodium phenylbutyrate and taurursodiol.

7. The method of any one of claims 1-6, wherein the method further comprises measuring:

(a) the presence and/or levels of a set of cytokines; and/or

(b) the presence and/or levels of neurofilament light chain and/or neurofilament heavy chain,

in the peripheral blood and/or cerebrospinal fluid (CSF) of the subject.

8. The method of claim 7, wherein the measuring is performed at one or more of: baseline, 5 weeks after the first dose of Treg cells, 13 weeks after the first dose of Treg cells, 25 weeks after the first dose of Treg cells, 36 weeks after the first dose of Treg cells, 48 weeks after the first dose of Treg cells, and 4 weeks after last dose of Treg cells.

9. The method of claim 7 or 8, wherein the set of cytokines comprises: 6CKine, BCA-1, CTACK, EGF, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, FGF-2, Flt3L, Fractalkine, G-CSF, GM-CSF, GROα, I-309, IFNα2, IFNγ, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-

17E/IL-25, IL-17F, IL-18, IL-20, IL-21, IL-22, IL-23, IL-27, IL-28, IL-33, IP-10, LIF, MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, MDC, MIG, MIP-1α, MIP-1β, MIP-1δ, PDGF-AA, PDGF-AB/BB, RANTES, sCD40L, SCF, SDF-1α+β, TARC, TGFα, TNFα, TNFβ, TPO, TRAIL, TSLP, and VEGF-A.

10. The method of claim 7 or 8, wherein the set of cytokines comprises: TGFb1, TGFb2, and TGFb3.

11. The method of claim 7 or 8, wherein the set of cytokines comprises: BDNF, Cathepsin D, MPO, NCAM, PAI-1 (total), PDGF-AA, PDGF-AB/BB, RANTES, sICAM-1, and sVCAM-1.

12. The method of claim 7 or 8, wherein the set of cytokines comprises: sCD30, sEGFR, sgp130, sIL-1RI, sIL-1RII, sIL-2RA, sIL-4R, sIL-6R, sRAGE, sTNFRI, sTNFRII, sVEGFR1, sVEGFR2, and sVEGFR3.

13. The method of claim 7 or 8, wherein the set of cytokines comprises: HCKP1-16-10 - BTLA, CD27, CD28, CD40, CD80, CD86, CTLA4, GITR, GITRL, HVEM, ICOS, LAG3, PD-1, PD-L1, TIM-3, and TLR-2.

14. The method of claim 7 or 8, wherein the set of cytokines comprises: Granzyme A, Granzyme B, Perforin, sFas, and sFas Ligand.

15. The method of claim 7 or 8, wherein the set of cytokines comprises: MMP-1 (Collagenase 1), MMP-2 (Gelatinase A), MMP-3 (Stromelysin 1), MMP-7 (Matrilysin), MMP-8 (Collagenase 2), MMP-9 (Gelatinase B), MMP-10 (Matrix Metalloproteinase 10), MMP-12 (Macrophage Metalloelastase), MMP-13 (Collagenase 3), TIMP-1, TIMP-2, TIMP-3, and TIMP-4.

16. The method of claim 7 or 8, wherein the set of cytokines comprises: ACTH, DKK-1, FGF-23, IL-1β, IL-6, Insulin, Leptin, PTH, OC, OPG, OPN, SOST, and TNFα.

17. Cryopreserved, allogeneic, cord blood derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:

$\geq$ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and

$\leq$ 10% $CD4^-CD8^+$;

for use in treating a subject having amyotrophic lateral sclerosis (ALS), wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses;

wherein each dose is about 1 x 10^8 Treg cells.

18. Cryopreserved, allogeneic, cord blood derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:

$\geq$ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and

$\leq$ 10% $CD4^-CD8^+$;

for use in ameliorating a symptom of amyotrophic lateral sclerosis (ALS) in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses;

wherein each dose is about 1 x 10^8 Treg cells.

19. The cryopreserved, allogeneic, cord blood derived Treg cells for use of claim 17 or claim 18, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses.

20. The cryopreserved, allogeneic, cord blood derived Treg cells for use of claim 18 or claim 19, wherein the symptom of ALS is weakness, fatigue, paralysis, decline of skeletal muscle function, or decline of respiratory function.

21. A pharmaceutical combination comprising:

(a) cryopreserved, allogeneic, cord blood derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:

$\geq$ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and

$\leq$ 10% $CD4^-CD8^+$; and

(b) one or more of:

(i) riluzole;

(ii) edaravone; and

(iii) sodium phenylbutyrate and taurursodiol;

for use in treating a subject having amyotrophic lateral sclerosis (ALS), wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses;

wherein each dose is about 1×10^8 Treg cells.

22. A pharmaceutical combination comprising:

(a) cryopreserved, allogeneic, cord blood derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:

$\geq$ 60% $CD3^+CD4^+CD25^+$, wherein at least 70% of the $CD3^+CD4^+CD25^+$ Treg cells are $CD11a^+$; and

$\leq$ 10% $CD4^-CD8^+$; and

(b) one or more of:

(i) riluzole;

(ii) edaravone; and

(iii) sodium phenylbutyrate and taurursodiol;

for use in ameliorating a symptom of amyotrophic lateral sclerosis (ALS) in a subject, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for at least about 5 doses;

wherein each dose is about 1×10^8 Treg cells.

23. The pharmaceutical combination of claim 21 or claim 22, wherein the Treg cells are to be administered to the subject once every 7 days ± 3 days for 4 doses, and then administered once every 28 days ± 3 days for about 5 doses to about 22 doses.

24. The pharmaceutical combination of claim 22 or claim 23, wherein the symptom of ALS is weakness, fatigue, paralysis, decline of skeletal muscle function, or decline of respiratory function.