

[0001] This application claims benefit of United States Provisional Patent Application No. 63/612,240 filed on December 19, 2023. That application is incorporated by reference as if fully rewritten herein.

FIELD

[0002] The present application relates to methods and compositions for the treatment of Alzheimer's disease in subjects in need thereof. Some embodiments are drawn to compositions comprising a therapeutically effective amount of allogeneic mesenchymal stem cells (MSCs), which are used to alleviate the symptoms of Alzheimer's disease. Other embodiments are drawn to methods of treatment wherein subjects suffering from symptoms of Alzheimer's disease are administered compositions comprising a therapeutically effective amount of MSCs. The effectiveness of these treatments is evaluated through measuring specific biomarkers in subjects after administration of compositions comprising MSCs, examining changes in their brain activity or morphology, and determining if their cognitive functioning or quality of life has improved after treatment.

BACKGROUND

[0003] Alzheimer's disease (AD) involves complex pathology and encompasses diverse mechanisms in addition to β -amyloid deposition and neurofibrillary tangles. There is growing recognition that a pro-inflammatory state contributes to the ensuing dementia. In this regard, proinflammatory cytokines are abundant in the vicinity of amyloid deposits and neurofibrillary tangles, and an association exists between systemic inflammation and β -amyloid accumulation. AD is further characterized by impaired neurovasculature that contributes to adverse outcomes. The resulting compromise of the blood-brain barrier (BBB) can impair exchange across the endothelium leading to inefficient clearance and accumulation of A β P in the brain.

[0004] Due to the complex nature of AD progression, the use of biomarkers to predict AD onset and progression remains challenging. Though the concentration of β -amyloid deposits and neurofibrillary tangles can be used to diagnose or predict the onset of AD, there are individuals that have been shown to possess a significant amount of amyloid deposits and neurofibrillary tangles at autopsy, which would qualify them for a diagnosis of AD, despite never showing a history of dementia. Currently approved treatments for AD (Rivastigmine, Donepezil, Memantine, Galantamine, Tacrine) have only marginal benefits that are largely

symptomatic; and no approved therapies can effectively stop, reverse, or prevent AD. The consistent failure of initially-promising lead compounds have resulted in no new AD drugs approved in over a decade. Recent among these are the failures of the anti-amyloid monoclonal antibody, solanezumab (Ely Lilly) and aducanumab (Biogen/Eisai), which were found ineffective in mild or moderate stage AD, and for mild cognitive impairment (MCI). A common theme among these failures is the targeting of a single pathological feature of AD.

[0005] Addressing these neuropathological features of AD simultaneously could offer therapeutic advantages and lead to novel treatment strategies. Medicinal signaling cells (MSCs, also known as mesenchymal stem cells) are multipotent cells (*in vitro*) with pleiotropic mechanisms of action (MOAs), including anti-inflammatory properties, ability to improve vascular function, and promotion of intrinsic tissue repair and regeneration. MSCs traffic to sites of inflammation and damage, and thus could target sites of neuroinflammation in AD. MSCs can also regulate host stem cell niches through paracrine activity and heterocellular coupling to promote intrinsic repair and regeneration. Finally, MSCs are immunoevasive/immunoprivileged, permitting allogeneic use, and have an acceptable safety profile in clinical trials. These immunoprivileged/immunoevasive properties allow MSCs to have the potential to be an “off-the-shelf” therapy that is readily available and accessible to broad patient populations due to their undetectable levels of major histocompatibility complex class II (MHC-II) molecules and low levels of MHC-I.

[0006] There are some preclinical data supporting efficacy of MSCs in AD. In animal models, MSCs cross the BBB, promote neurogenesis, inhibit β -amyloid deposition and promote clearance, reduce apoptosis, promote hippocampal neurogenesis, improve dendritic morphology, and improve behavioral and spatial memory performance. These beneficial effects were associated with decreased inflammation, increased $A\beta$ -degrading factors and $A\beta$ clearance, decreased hyperphosphorylated tau, and elevated alternatively activated microglial markers. These benefits appear, at least in part, due to $A\beta$ -induced MSC release of chemoattractants that recruit alternative microglia into the brain to reduce $A\beta$ deposition. MSCs have been reported to be effective in young AD-model mice prior to $A\beta$ accumulations, leading to significant decreases in cerebral $A\beta$ deposition, and a significant increase in expression of pre-synaptic proteins. Impressively, these effects were sustained for at least 2 months, and suggest MSCs could potentially be effective as an interventional therapeutic in prodromal AD.

[0007] Accordingly, the application seeks to not only provide methods of treatment for AD wherein the methods comprise the use of compositions containing MSCs, but this application

also seeks to provide methods that can evaluate their efficacy in the treatment of AD and alleviation of AD symptoms in subjects in need thereof.

SUMMARY OF THE INVENTION

[0008] An objective of the present application is to provide biomarkers indicating the efficacy of alleviating the symptoms of Alzheimer's disease (AD) or treating Alzheimer's disease (AD) or inhibiting AD disease progression, in a subject suffering from symptoms of AD, wherein one or more biomarkers are measured in the subject before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs). These biomarkers may be the change in the size in areas of a patient's brain, such as whole brain, lateral ventricles, gray matter, hippocampus, temporal cortex, medial temporal cortex, hippocampus, thalamus, white matter, cingulate cortex, and/or frontal cortex. Other biomarkers may be changes in diffusivity in the cingulate cortex, and/or changes in one or more of a whole blood, blood plasma, or blood serum biomarker or biomarkers, such as but not limited to levels of soluble TIE2 (sTIE2), eotaxin 1, eotaxin 2, or eotaxin 3.

[0009] In some embodiments, the biomarker is an MRI biomarker.

[0010] In some embodiments, the method comprises measuring the cognitive function of the subject suffering from symptoms of AD before and after administration of the composition comprising allogeneic MSCs.

[0011] In some embodiments, the method comprises assessing the quality of life of the subject suffering from symptoms of AD before and after administration of the composition comprising allogeneic MSCs.

[0012] In some embodiments, the MRI biomarker comprises a volume measurement of whole brain, lateral ventricles, gray matter, hippocampus, temporal cortex, medial temporal cortex, hippocampus, thalamus, white matter, cingulate cortex, frontal cortex, or combinations thereof.

[0013] In some embodiments, the MRI biomarker may be a decrease in the volume of whole brain, gray matter, temporal cortex, medial temporal cortex, hippocampus, frontal cortex, or thalamus. In preferred embodiments, the decrease in the volume of whole brain, gray matter, temporal cortex, medial temporal cortex, hippocampus, frontal cortex, or thalamus, is reduced after administration of allogeneic MSCs to a subject, indicative of efficacy. The decrease in volume can be from 1% to 5%, 5% to 10%, 10% to 50%, or greater than 50%.

[0014] In some embodiments, the MRI biomarker may be an increase in the volume of lateral ventricles, white matter, or cingulate cortex. In preferred embodiments, the increase in

the volume of lateral ventricles, white matter, or cingulate cortex, is reduced after administration of allogenic MSCs to a subject, indicative of efficacy. The volume reduction can be from 1% to 5%, 5% to 10%, 10% to 50%, or greater than 50%.

[00015] In some embodiments, the MRI biomarker may be a mean diffusivity or free water measurement via diffusion tensor imaging (DTI) or similar technology in the cingulate cortex. In preferred embodiments, the mean diffusivity or free water measurement via diffusion tensor imaging (DTI) or similar technology in the cingulate cortex is reduced after administration of allogenic MSCs to a subject, indicative of efficacy.

[00016] In some embodiments, the biomarker may be a one or more of a whole blood, blood plasma, or blood serum biomarker or biomarkers. In some embodiments the blood serum or plasma biomarker is the level of soluble TIE2 (sTIE2). In preferred embodiments, the level of sTIE2 in the blood serum or plasma is reduced after administration of allogenic MSCs to a subject.

[00017] In some embodiments, the composition comprising allogeneic mesenchymal stem cells (MSCs) is administered for alleviating the symptoms of AD, treating AD, and/or inhibiting AD disease progression, comprises 25×10^6 MSCs.

[00018] In other embodiments, the composition comprising allogeneic mesenchymal stem cells (MSCs), is administered for alleviating the symptoms of AD, treating AD, and/or inhibiting AD disease progression, comprises 100×10^6 MSCs.

[00019] In some embodiments, the composition comprising allogeneic mesenchymal stem cells (MSCs), is administered for alleviating the symptoms of AD, treating AD, and/or inhibiting AD disease progression, is administered to a subject by intravenous or intra-arterial infusion.

[00020] In some embodiments, the composition comprising allogeneic mesenchymal stem cells (MSCs), is administered for alleviating the symptoms of AD, treating AD, and/or inhibiting AD disease progression, is administered as one or more of a single dose, a monthly dose, and a repeated interval dose.

[00021] The allogenic MSCs may be LOMECEL-B™ cells, which is a Longeveron formulation of allogenic human mesenchymal stem cells. Further uses and preparation of useful stem cells, including LOMECEL-B™ brand mesenchymal cells, may be found in the following United States Patent Application Publications, all of which are incorporated by reference herein: US20190038742A1; US20190290698A1; and US20200129558A1.

BRIEF DESCRIPTION OF DRAWINGS

[00022] FIG. 1 depicts a consort study design diagram summarizing disposition of patients in the phase 2a double-blind, randomized, placebo-controlled 45-week trial to evaluate the effects of Lomecel-B on Alzheimer's Disease.

[00023] FIG. 2A depicts change in CADS scores at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ◆ MSC (100M $\times$ 4).

[00024] FIG. 2B depicts change in MoCA total scores at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ◆ MSC (100M $\times$ 4).

[00025] FIG. 2C depicts change in MMSE2 total scores at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ◆ MSC (100M $\times$ 4).

[00026] FIG. 2D depicts change in CDR-SB total scores at 0 (baseline), 4, 8, 12, 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ◆ MSC (100M $\times$ 4).

[00027] FIG. 2E depicts change in ADAS-cog13 total scores at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ◆ MSC (100M $\times$ 4).

[00028] FIG. 2F depicts change in ADCS-ADL scores at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ◆ MSC (100M $\times$ 4).

[00029] FIG. 2G depicts change in QoL-AD (Caregiver) scores at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ◆ MSC (100M $\times$ 4).

[00030] FIG. 2H depicts change in QoL-AD (Study Subject) scores at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ◆ MSC (100M $\times$ 4).

[00031] FIG. 2I depicts change in ADRQL scores at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ◆ MSC (100M $\times$ 4).

[00032] FIG. 3A depicts MR images of brain volume changes from patients in Group 1 (Placebo (Dose $\times$ 4)) vs Group 4 (MSC (100M $\times$ 4)) at week 39. Whole brain volume steadily

decreased by approx. 1.2% in the placebo group during the 39-week trial period, while treatment groups showed statistically significant slowing of whole brain atrophy.

[00033] FIG. 3B depicts change in whole brain volume at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00034] FIG. 3C depicts change in gray matter volume (left) at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00035] FIG. 3D depicts change in lateral ventricle volume (bilateral) at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00036] FIG. 3E depicts change in hippocampal volume (bilateral) at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00037] FIG. 3F depicts change in temporal cortex volume (left) at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00038] FIG. 3G depicts change in medial temporal cortex volume (left) at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00039] FIG. 3H depicts change in mean diffusivity (MD) in cingulate cortex (bilateral) at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00040] FIG. 4A depicts improvement in bilateral hippocampal volume correlated significantly with improvement in MMSE-2 scores.

[00041] FIG. 4B depicts improvement in whole brain volume correlated significantly with improvement in MMSE-2 scores.

[00042] FIG. 4C depicts improvement in bilateral lateral ventricle volume correlated significantly with improvement in MMSE-2 scores.

[00043] FIG. 4D depicts Least squares mean change from baseline in TIE2 at 0 (baseline), 4, 8, 12, 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00044] FIG. 5A depicts change in thalamus (left) at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00045] FIG. 5B depicts change in thalamus (right) at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00046] FIG. 5C depicts change in frontal cortex (left) at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00047] FIG. 5D depicts change in frontal cortex (right) at 0 (baseline), 16, 26, and 39 weeks, for four patient groups: ● Placebo (Dose $\times$ 4); ▲ MSC (25M $\times$ 1); ■ MSC (25M $\times$ 4); ♦ MSC (100M $\times$ 4).

[00048] FIG. 6A depicts MR image of cingulate cortex.

[00049] FIG. 6B depicts MR image of medial temporal cortex.

[00050] FIG. 6C depicts MR image of cingulate cortex.

[00051] FIG. 6D depicts MR image of medial temporal cortex.

[00052] FIG. 7A depicts linear decrease in placebo, whole brain shrinkage.

[00053] FIG. 7B depicts statistically significant improvement in Lomecel-B relative to placebo.

[00054] FIG. 8A depicts volume in lateral ventricles (left), where enlargement correlates to decreased cerebral volume.

[00055] FIG. 8B depicts volume in lateral ventricles (right), where enlargement correlates to decreased cerebral volume.

[00056] FIG. 8C depicts lateral ventricles left mean change from baseline.

[00057] FIG. 8D depicts lateral ventricles right mean change from baseline.

[00058] FIG. 9A depicts grey matter (left) ratio mean change from baseline, with statistically significant improvement in pooled Lomecel-b group related to placebo at week 39.

[00059] FIG. 9B depicts grey matter (right) ratio mean change from baseline.

[00060] FIG. 10A depicts mean change from baseline in left medial temporal cortex.

[00061] FIG. 10A depicts mean change from baseline in right medial temporal cortex.

[00062] FIG. 11A depicts change in left gray matter volumetry.

[00063] FIG. 11B depicts change in right gray matter volumetry.

[00064] FIG. 12A depicts change in left medial temporal cortex volumetry, where treatment arms are numerically superior at weeks 26 and 39.

- [00065] FIG. 12B depicts change in right medial temporal cortex volumetry, where treatment arms are numerically superior at weeks 26 and 39.
- [00066] FIG. 13A depicts left volumetry for hippocampus, where all treatment arms are numerically superior at week 39.
- [00067] FIG. 13B depicts right volumetry for hippocampus, where all treatment arms are numerically superior at week 39.
- [00068] FIG. 13C depicts left hippocampus volume mean change from baseline, where treatment arms are numerically superior at weeks 26 and 39.
- [00069] FIG. 13D depicts right hippocampus volume mean change from baseline, where treatment arms are numerically superior at weeks 26 and 39.
- [00070] FIG. 14A depicts thalamus left ratio mean change from baseline.
- [00071] FIG. 14B depicts thalamus right ratio mean change from baseline.
- [00072] FIG. 14C depicts thalamus left volumetry.
- [00073] FIG. 14D depicts thalamus right volumetry.
- [00074] FIG. 15A depicts white matter (bilateral) mean diffusivity.
- [00075] FIG. 15B depicts white matter (bilateral) axial diffusivity.
- [00076] FIG. 15C depicts white matter (bilateral) FA.
- [00077] FIG. 15D depicts white matter (bilateral) RD.
- [00078] FIG. 16A depicts cingulate cortex (bilateral) mean diffusivity.
- [00079] FIG. 16B depicts cingulate cortex (bilateral) axial diffusivity.
- [00080] FIG. 16C depicts cingulate cortex (bilateral) FA.
- [00081] FIG. 16D depicts cingulate cortex (bilateral) RD.
- [00082] FIG. 17A depicts whole corpus callosum (bilateral) mean diffusivity.
- [00083] FIG. 17B depicts whole corpus callosum (bilateral) axial diffusivity.
- [00084] FIG. 17C depicts whole corpus callosum (bilateral) FA.
- [00085] FIG. 17D depicts whole corpus callosum (bilateral) RD.
- [00086] FIG. 18A depicts white matter (bilateral) FA.
- [00087] FIG. 18B depicts white matter (bilateral) RD.
- [00088] FIG. 19 depicts whole cortex volume change from baseline.
- [00089] FIG. 20 depicts hypometabolic signature change from baseline.
- [00090] FIG. 21 depicts frontal cortex volume change from baseline.
- [00091] FIG. 22 depicts parietal cortex volume change from baseline.
- [00092] FIG. 23 depicts temporal cortex (bilateral) volume change from baseline.
- [00093] FIG. 24 depicts occipital cortex (bilateral) volume change from baseline.

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- [00094] FIG. 25 depicts cingulate cortex volume change from baseline.
- [00095] FIG. 26 depicts medial temporal cortex volume change from baseline.
- [00096] FIG. 27 depicts thalamus (bilateral) volume change from baseline.
- [00097] FIG. 28 depicts striatum (bilateral) volume change from baseline.
- [00098] FIG. 29 depicts whole cortex arterial spin labeling (ASL) change from baseline.
- [00099] FIG. 30 depicts medial temporal cortex arterial spin labeling (ASL) change from baseline.
- [00100] FIG. 31 depicts Eotaxin-1 concentration change from baseline data.
- [00101] FIG. 32 depicts Eotaxin-2 concentration change from baseline data.
- [00102] FIG. 33 depicts raw mean Eotaxin-3 concentration data.
- [00103] FIG. 34 depicts Eotaxin-3 concentration change from baseline data.
- [00104] FIG. 35 depicts Eotaxin-3 concentrations for Trial Groups 1-4.
- [00105] FIG. 36 depicts GIP (active) change from baseline data.
- [00106] FIG. 37 depicts GIP (active) for Trial Groups 1-4.
- [00107] FIG. 38 depicts raw mean GIP concentration.
- [00108] FIG. 39 depicts GIP concentration change from baseline data.
- [00109] FIG. 40 depicts GIPF concentration change from baseline data.
- [00110] FIG. 41 depicts TIE2 concentration change from baseline data.
- [00111] FIG. 42 depicts TIE2 concentrations for Trial Groups 1-4.

DETAILED DESCRIPTION

[00112] AD is a progressive disease for which there is no current cure. The FDA has only recently (2021-2024) approved a small number of antibody therapeutics (Cummings, J., et al. *BioDrugs* 38, 5-22 (2024)) designed to treat AD by removing β -amyloid peptide ($A\beta$ or $A\beta P$), including aducanumab, lecanemab, and donanemab, which have demonstrated some efficacy at reducing cognitive and functional decline in patients with early AD. Studies found that aducanumab carried a 35% risk of Alzheimer's related imaging abnormalities (ARIA), specifically ARIA-edema (ARIA-E) and 19% risk of ARIA-hemorrhage (ARIA-H) (Doran, S.J. & Sawyer, R.P. *Front Neurosci* 18, 1326784 (2024)) and was discontinued in 2024. Lecanemab resulted in a 27% improvement in Clinical Dementia Rating scale sum of boxes (CDR-SB) at 18 months (Swanson, C.J., et al., *Alzheimers Res Ther* 13, 80 (2021)), but was associated with a 21.5% risk of ARIA (Honig, L.S., et al. *Alzheimers Dement* (N Y) 9, e12377 (2023)) and the potential for progression of brain atrophy (Alves, F., et al. *Neurology* 100,

e2114-e2124 (2023); Couzin-Frankel, J. *Science* 380, 19 (2023)). Lecanemab also resulted in a 26.4% rate of infusion-related reactions in patients. Donanemab carried a 36.8% risk of ARIA, with drug-induced inflammation and brain bleeding also reported (Sims, J.R., et al. *JAMA* 330, 512-527 (2023)). Aside from these limited examples, most treatments for AD address symptoms, but do not alter disease progression, in part because the development and onset of AD is still not fully understood, and this uncertainty has been the cause of many disputes within the field. Indeed, though a majority of researchers in the field of AD development and treatment acknowledge that A β P accumulation plays a part in the progression of the disease, it is still unknown if A β P accumulation is the cause of AD or if it is merely a result of other cellular pathways becoming dysregulated as a result of aging.

[000113] Additionally, while A β P accumulation and tau-mediated formation of neurofibrillary tangles remain as defining pathological features of AD, non-amyloid and non-tau contributions to AD have been identified that may result in cerebrovascular degradation and a strong neuroinflammatory component contributing to neuronal death and brain atrophy (Schwab, C. & McGeer, P.L. *J Alzheimers Dis* 13, 359-369 (2008); Scheffer, S., et al. *Arterioscler Thromb Vasc Biol* 41, 1265-1283 (2021)).

[000114] Brain atrophy in AD involves multiple brain regions and begins relatively early in disease progression, affecting broad areas of the occipital, parietal, frontal, and temporal lobes, as well as the hippocampus, up to 8 years prior to AD diagnosis (Scahill, R.I., et al. *Proc Natl Acad Sci U S A* 99, 4703-4707 (2002); Traini, E., et al. *J Alzheimers Dis* 76, 317-329 (2020); Apostolova, L.G., et al. *Arch Neurol* 63, 693-699 (2006); Jia, J., et al. *N Engl J Med* 390, 712-722 (2024)). MRI imaging has revealed progressive atrophy at 39 weeks by volumetric MRI, affecting multiple brain regions and whole brain volume, accompanied by increased ventricular size (Fig. 3). During disease progression, biomarkers of brain atrophy may include decreases in the volume of whole brain, gray matter, temporal cortex, medial temporal cortex, hippocampus, frontal cortex, and/or thalamus, while volume increases may also be observed in the lateral ventricles, white matter, or cingulate cortex. Additionally, mean diffusivity or free water measurement in the cingulate cortex may increase with disease progression. These biomarkers can be assessed quantitatively, and over time during disease progression, using Magnetic Resonance Imaging (MRI).

[000115] Another important biomarker related to AD progression is soluble TIE2 (sTIE2). Tyrosine kinase with immunoglobulin and epidermal growth factor homology domains (TIE2), the cognate receptor for angiopoietins 1 and 2, is expressed by endothelial cells, activates pro-angiogenic and anti-inflammatory downstream signaling pathways, and can

be degraded into a soluble form (sTIE2) that is released into the bloodstream. The concentration of sTIE2 was recently found to increase in the blood serum in AD patients indicating cell-surface receptor shedding and therefore inactivation that would reduce anti-inflammatory activity.

[000116] We have surprisingly discovered that the use of a composition comprising allogeneic mesenchymal stem cells (MSCs) is able to combat the symptoms of AD. Treating a subject suffering from AD symptoms with a composition comprising allogeneic MSCs has been discovered to improve the subject's brain morphology and ameliorate brain atrophy, and promote the expression of novel quantitative biomarkers, including but not limited to the previously-noted MRI biomarkers and blood serum biomarkers, for diagnosing and evaluating the progression of AD and the effectiveness of the treatment methods. The above discoveries are surprising due to the ambiguity surrounding the pathogenesis of AD and the general reservation of those skilled in the art to use allogeneic MSCs in treatments for AD since they were expected to perform poorly due to their inability to directly target beta-amyloids and their low residence time in the human body. They were also expected to perform poorly in AD treatments due to their large size, which led those skilled in the art to believe that they could not pass the blood-brain barrier and reach the site of inflammation and damage.

[000117] Another advantage of using allogeneic MSCs in treatments for AD is that they do not involve targeting a single pathway or biomarker, such as A β P accumulation. Instead, the use of allogeneic MSCs in AD treatments can allow multiple pathways to be targeted at once and thereby halt or significantly slow the progression of AD.

[000118] Following the surprising discoveries discussed above, one aspect of the present application relates to methods of determining the efficacy of alleviating the symptoms of Alzheimer's disease (AD) or treating Alzheimer's disease (AD) or inhibiting AD disease progression, in a subject suffering from symptoms of AD, by providing novel biomarkers, wherein one or more biomarkers are measured in the subject before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs).

[000119] In some embodiments, the method comprises determining the efficacy of alleviating the symptoms of Alzheimer's disease (AD) in a subject suffering from symptoms of AD, wherein one or more biomarkers are measured in the subject before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs).

[000120] In some embodiments, the method comprises determining the efficacy of treating Alzheimer's disease (AD) in a subject suffering from symptoms of AD, wherein one

or more biomarkers are measured in the subject before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs).

[000121] In some embodiments, the method comprises determining the efficacy of inhibiting AD disease progression in a subject suffering from symptoms of AD, wherein one or more biomarkers are measured in the subject before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs).

[000122] In some embodiments, the biomarker is an MRI biomarker.

[000123] In some embodiments, the method further comprises measuring the cognitive function of the subject suffering from symptoms of AD before and after administration of the composition comprising allogeneic MSCs.

[000124] In some embodiments, the method further comprises assessing the quality of life of the subject suffering from symptoms of AD before and after administration of the composition comprising allogeneic MSCs.

[000125] In some embodiments, the MRI biomarker comprises a volume measurement of whole brain, lateral ventricles, gray matter, hippocampus, temporal cortex, medial temporal cortex, hippocampus, thalamus, white matter, cingulate cortex, frontal cortex, or combinations thereof.

[000126] In some embodiments, the method comprises measuring the volume of the whole brain in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the whole brain. In preferred embodiments, a reduction in the decrease in the measured volume of the whole brain after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in whole brain volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000127] In some embodiments, the method comprises measuring the volume of gray matter in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of gray matter. In preferred embodiments, a reduction in the decrease in the measured volume of gray matter after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in gray matter volume decrease can range from 0% to

10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000128] In some embodiments, the method comprises measuring the volume of the temporal cortex in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the temporal cortex. In preferred embodiments, a reduction in the decrease in the measured volume of the temporal cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in temporal cortex volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000129] In some embodiments, the method comprises measuring the volume of the medial temporal cortex in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the medial temporal cortex. In preferred embodiments, a reduction in the decrease in the measured volume of the medial temporal cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in medial temporal cortex volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000130] In some embodiments, the method comprises measuring the volume of the hippocampus in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the hippocampus. In preferred embodiments, a reduction in the decrease in the measured volume of the hippocampus after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in hippocampus volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000131] In some embodiments, the method comprises measuring the volume of the frontal cortex in a subject suffering from symptoms of AD, before and after the administration

of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the frontal cortex. In preferred embodiments, a reduction in the decrease in the measured volume of the frontal cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in frontal cortex volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000132] In some embodiments, the method comprises measuring the volume of the thalamus in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the thalamus. In preferred embodiments, a reduction in the decrease in the measured volume of the thalamus after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in thalamus volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000133] In some embodiments, the method comprises measuring the volume of the lateral ventricle in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is an increase in the measured volume of the lateral ventricle. In preferred embodiments, a reduction in the increase in the measured volume of the lateral ventricle after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in lateral ventricle volume increase can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000134] In some embodiments, the method comprises measuring the volume of the white matter in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is an increase in the measured volume of the white matter. In preferred embodiments, a reduction in the increase in the measured volume of the white matter after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in white matter volume increase can range from

0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000135] In some embodiments, the method comprises measuring the volume of the cingulate cortex in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is an increase in the measured volume of the cingulate cortex. In preferred embodiments, a reduction in the increase in the measured volume of the cingulate cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in cingulate cortex volume increase can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000136] In some embodiments, the method comprises measuring the mean diffusivity or free water measurement via diffusion tensor imaging (DTI) or similar technology in the cingulate cortex in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In preferred embodiments, a reduction in mean diffusivity or free water measurement via diffusion tensor imaging (DTI) or similar technology in the cingulate cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy.

[000137] In other embodiments, the method comprises measuring a one or more of a whole blood, blood plasma, or blood serum biomarker or biomarkers in the subject suffering from symptoms of AD, before and after the administration of the composition comprising allogeneic MSCs.

[000138] In some embodiments, the blood serum or plasma biomarker comprises soluble TIE2 (sTIE2). In some embodiments, the biomarker is an increase in the level of sTIE2 in the blood serum or plasma. In preferred embodiments, a reduction in the level of sTIE2 in the blood serum or plasma after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy.

[000139] In some embodiments, the composition comprising allogeneic mesenchymal stem cells (MSCs) administered for alleviating the symptoms of AD, treating AD, and/or inhibiting AD disease progression, may comprise 20×10^6 allogeneic MSCs, 25×10^6 allogeneic MSCs, 100×10^6 allogeneic MSCs, or between 20×10^6 and 100×10^6 allogeneic MSCs, preferably 25×10^6 MSCs, or more preferably 100×10^6 MSCs.

[000140] In some embodiments, the composition comprising allogeneic mesenchymal stem cells (MSCs), administered for alleviating the symptoms of AD, treating AD, and/or inhibiting AD disease progression, is administered to a subject by intravenous or intra-arterial infusion.

[000141] In some embodiments, the composition comprising allogeneic mesenchymal stem cells (MSCs), administered for alleviating the symptoms of AD, treating AD, and/or inhibiting AD disease progression, is administered monthly.

[000142] In some embodiments, the method comprises the use of one or more biomarkers to determine the efficacy of alleviating the symptoms of Alzheimer's disease (AD) in a subject suffering from symptoms of AD, comprising the measurement of said one or more biomarkers in said subject before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs).

[000143] In some embodiments the method comprises the use of one or more biomarkers to determine the efficacy of treating Alzheimer's disease (AD) or inhibiting AD disease progression in a subject suffering from symptoms of AD, comprising the measurement of said one or more biomarkers in said subject before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs).

[000144] In some embodiments the method comprises the use of one or more biomarkers, wherein the biomarker is an MRI biomarker.

[000145] In some embodiments the method comprises the use of one or more biomarkers to determine the efficacy of alleviating the symptoms of AD, treating AD or inhibiting AD disease progression in a subject suffering from symptoms of AD, and further wherein the cognitive function of the subject suffering from symptoms of AD is measured before and after administration of the composition comprising allogeneic MSCs.

[000146] In some embodiments the method comprises the use of one or more biomarkers to determine the efficacy of alleviating the symptoms of AD, treating AD or inhibiting AD disease progression in a subject suffering from symptoms of AD, and further wherein the quality of life of the subject suffering from symptoms of AD is assessed before and after administration of the composition comprising allogeneic MSCs.

[000147] In some embodiments the method comprises the use of an MRI biomarker, wherein the MRI biomarker comprises a volume measurement of whole brain, lateral ventricles, gray matter, hippocampus, temporal cortex, medial temporal cortex, thalamus, white matter, cingulate cortex, frontal cortex, or combinations thereof.

[000148] In some embodiments, the method comprises the use of an MRI biomarker, wherein the biomarker comprises a volume measurement of the whole brain in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the whole brain. In preferred embodiments, a reduction in the decrease in the measured volume of the whole brain after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in whole brain volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000149] In some embodiments, the method comprises the use of an MRI biomarker, wherein the biomarker comprises a volume measurement of gray matter in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the gray matter. In preferred embodiments, a reduction in the decrease in the measured volume of the gray matter after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in gray matter volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000150] In some embodiments, the method comprises the use of an MRI biomarker, wherein the biomarker comprises a volume measurement of the temporal cortex in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the temporal cortex. In preferred embodiments, a reduction in the decrease in the measured volume of the temporal cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in temporal cortex volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000151] In some embodiments, the method comprises the use of an MRI biomarker, wherein the biomarker comprises a volume measurement of the medial temporal cortex in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments,

the biomarker is a decrease in the measured volume of the medial temporal cortex. In preferred embodiments, a reduction in the decrease in the measured volume of the medial temporal cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in medial temporal cortex volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000152] In some embodiments, the method comprises the use of an MRI biomarker, wherein the biomarker comprises a volume measurement of the hippocampus in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the hippocampus. In preferred embodiments, a reduction in the decrease in the measured volume of the hippocampus after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in hippocampus volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000153] In some embodiments, the method comprises the use of an MRI biomarker, wherein the biomarker comprises a volume measurement of the frontal cortex in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the frontal cortex. In preferred embodiments, a reduction in the decrease in the measured volume of the frontal cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in frontal cortex volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000154] In some embodiments, the method comprises the use of an MRI biomarker, wherein the biomarker comprises a volume measurement of the thalamus in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is a decrease in the measured volume of the thalamus. In preferred embodiments, a reduction in the decrease in the measured volume of the thalamus after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy.

The reduction in thalamus volume decrease can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000155] In some embodiments, the method comprises the use of an MRI biomarker, wherein the biomarker comprises a volume measurement of the lateral ventricles in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is an increase in the measured volume of the lateral ventricles. In preferred embodiments, a reduction in the increase in the measured volume of the lateral ventricles after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in lateral ventricles volume increase can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000156] In some embodiments, the method comprises the use of an MRI biomarker, wherein the biomarker comprises a volume measurement of the white matter in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is an increase in the measured volume of the white matter. In preferred embodiments, a reduction in the increase in the measured volume of the white matter after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in white matter volume increase can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000157] In some embodiments, the method comprises the use of an MRI biomarker, wherein the biomarker comprises a volume measurement of the cingulate cortex in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In some embodiments, the biomarker is an increase in the measured volume of the cingulate cortex. In preferred embodiments, a reduction in the increase in the measured volume of the cingulate cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy. The reduction in cingulate cortex volume increase can range from 0% to 10%, 1% to 5%, 1.0% to 10%, 3% to 10%, 5% to 10%, 7% to 10%, greater than 0% to less than or equal to 10%, 10% to 50%, 20% to 50%, 30% to 50% or greater than 50%.

[000158] In some embodiments, the method comprises the use of an MRI biomarker, wherein the MRI biomarker comprises a mean diffusivity or free water measurement via diffusion tensor imaging (DTI) or similar technology. In some embodiments, the mean diffusivity or free water measurement is a measurement of mean diffusivity in the cingulate cortex in a subject suffering from symptoms of AD, before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) to said subject. In preferred embodiments, a reduction in mean diffusivity or free water measurement in the cingulate cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy.

[000159] In other embodiments, the method comprises the use of one or more biomarkers, wherein the biomarker is one or more of a whole blood, blood plasma, or a blood serum biomarker or biomarkers. In some embodiments the blood serum or plasma biomarker comprises soluble TIE2 (sTIE2). In some embodiments, the biomarker is an increase in the level of sTIE2 in the blood serum or plasma. In preferred embodiments, a reduction in the level of sTIE2 in the blood serum or plasma after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy.

[000160] In some embodiments, the method comprises the use of one or more biomarkers, wherein a composition comprising allogeneic mesenchymal stem cells (MSCs) is administered for alleviating the symptoms of AD, treating AD, and/or inhibiting AD disease progression, and wherein the composition comprises 20×10^6 allogeneic MSCs, 25×10^6 allogeneic MSCs, 100×10^6 allogeneic MSCs, or between 20×10^6 and 100×10^6 allogeneic MSCs, preferably 25×10^6 MSCs, and or more preferably 100×10^6 MSCs.

[000161] In some embodiments, the method comprises the use of one or more biomarkers, wherein the composition comprising allogeneic mesenchymal stem cells (MSCs) is administered by intravenous or intra-arterial infusion to a subject for alleviating the symptoms of AD, treating AD, and/or inhibiting AD disease progression.

[000162] In some embodiments, the method comprises the use of one or more biomarkers, wherein the composition comprising allogeneic mesenchymal stem cells (MSCs) is administered as one or more of a single dose, a monthly dose, and a repeated interval dose, for alleviating the symptoms of AD, treating AD, and/or inhibiting AD disease progression.

Examples

Example 1

Lomecel-B Effects on Alzheimer's Disease: A Randomized, Double-Blinded, Placebo-Controlled Phase 2a Trial

Trial Design:

[000163] This double-blind, randomized, placebo-controlled 45-week trial (ClinicalTrials.gov: NCT05233774) enrolled patients (60-85 yrs.) with mild AD dementia (MMSE score 18-24); with evidence of amyloid on positron-emission tomography (PET) and brain MRI consistent with AD.

[000164] There were 4 study arms of Lomecel-B intravenous infusion: Group 1: placebo infusions once per month for four months (N=12); Group 2: 25 million cells at Day 0 followed by three monthly infusions of placebo (25Mx1; N=12); Group 3: 25 million cells infused monthly for four months (25Mx4; N=13); and Group 4: 100 million cells infused monthly for four months (100Mx4; N=11) (Fig. 1).

[000165] Demographics and baseline characteristics are summarized in Table 1.

Table 1: Demographics and baseline characteristics.

Parameter (%)	Group 1 (Placebo) {N=12}	Group 2 (25Mx1) {N=13}	Group 3 (25Mx4) {N=13}	Group 4 (100Mx4) {N=11}	All Patients {N=49}	P-value
Age in years	76.7 (4.40)	74.9 (7.51)	70.5 (7.14)	74.5 (6.07)	74.1 (6.65)	0.116
Sex						0.875
Male	5 (41.7)	5 (38.5)	7 (53.8)	5 (45.5)	22 (44.9)	
Female	7 (58.3)	8 (61.5)	6 (46.2)	6 (54.5)	27 (55.1)	
Race						0.066
Black/African American	0	0	0	2 (18.2)	2 (4.1)	
White	12 (100.0)	13 (100.0)	13 (100.0)	9 (81.8)	47 (95.9)	
Ethnicity						0.697
Hispanic/Latino	9 (75.0)	11 (84.6)	10 (76.9)	7 (63.6)	37 (75.5)	
Not Hispanic/Latino	3 (25.0)	2 (15.4)	3 (23.1)	4 (36.4)	12 (24.5)	
ApoE4 status						0.697
Non-carrier	10 (83.3)	8 (61.5)	7 (53.8)	10 (90.9)	35 (71.4)	
Heterozygous	2 (16.7)	5 (41.7)	5 (38.5)	1 (8.3)	13 (26.5)	
Homozygous	0	0	1 (7.7)	0	1 (2.0)	
AD Treatment	7 (58.3)	9 (69.2)	8 (61.5)	7 (63.6)	31 (63.3)	0.952
Donepezil	5 (41.7)	6 (46.2)	7 (53.8)	5 (45.5)	23 (46.9)	
Memantine	3 (25.0)	4 (30.8)	5 (38.5)	3 (27.3)	15 (30.6)	
Donepezil/Memantine	0	1 (7.7)	0	0	1 (2.0)	
Galantamine	0	0	1 (7.7)	0	1 (2.0)	
Height (cm)	165.0 (14.2)	170.2 (8.6)	166.5 (10.1)	167.1 (9.1)	167.3 (10.6)	0.665
Weight (kg)	79.5 (15.5)	78.6 (16.6)	84.1 (14.9)	77.8 (14.8)	80.1 (15.2)	0.781
BMI (kg/m ²)	30.4 (6.6)	27.1 (5.3)	30.4 (5.4)	27.8 (4.1)	28.9 (5.4)	0.757
MoCA Total Score	19.0 (5.63)	18.6 (14.51)	19.2 (4.18)	18.2 (4.58)	18.1 (4.83)	0.772
MMSE-2 Total Score	20.9 (2.27)	20.8 (2.23)	21.9 (2.06)	21.2 (1.60)	21.2 (2.05)	0.533
ADAS-Cog-13 Total Score	31.304 (11.821)	32.922 (8.871)	27.872 (8.968)	23.575 (4.131)	29.088 (9.363)	0.071
CDR-SB Total Score	9.75 (1.373)	9.19 (1.521)	9.79 (1.563)	4.23 (1.723)	4.23 (1.617)	0.071
ADCS-ADL Total Score	59.1 (9.10)	55.8 (9.24)	61.9 (13.43)	63.1 (8.95)	59.9 (10.49)	0.322

AD=Alzheimer's disease; BMI=Body Mass Index; SD=Standard Deviation. Group 1: Placebo (Dose x 4). Group 2: Lomecel-B (25M x 1). Group 3: Lomecel-B (25M x 4). Group 4: Lomecel-B (100M x 4). Baseline cognitive and functional tests represent the scores of the last test before the first dose. The intent to treat (ITT) population was N=49; after one withdrawal from Group 2, the modified intention to treat population (MITT) was N=48. Values represent means (SD).

Assessments:

[000166] To ensure safety, patients were monitored after each infusion of Lomecel-B (e.g., on Day 0 and Weeks 4, 8, and 12).

[000167] To analyze the efficacy of treatment, a Composite AD score (CADS) assessment was calculated at Baseline and Weeks 16, 26, and 39.

[000168] Neurocognitive, neuropsychiatric, QOL, ADL, and CSHA-CFS assessments were performed at screening, baseline, and Weeks 16, 26, and 39 to assess the change from baseline (CFB).

Biomarkers:

[000169] Serum-based biomarkers, including sTIE2, related to potential pro-vascular and anti-inflammatory activities of Lomecel-B, as well as Alzheimer's disease progression, were measured at Screening, Baseline, Day 0, and Weeks 4, 8, 12, 16, 26, and 39. The biomarker sTIE2 was analyzed using a Meso Scale Discovery (MSD) electrochemiluminescence (ECL) immunoassay machine (1300 MESO QuickPlex SQ120) and angiogenesis panel kit (Meso Scale Discovery; cat # K15190D-1). Quantification was completed using a MSD V-Plex Assay with duplicate samples per patient visit.

[000170] Brain volumetry was performed via MRI at Screening and Weeks 16, 26, and 39, to assess for volumetric changes in the hippocampus, overall brain size, ventricular volume, and other brain structures, each normalized for intracranial volume. Brain MRIs were performed using 3T (Tesla) scanners, 2 imaging centers were used by the 10 clinical centers, 1 MRI scanner at each location was used for the entire trial.

[000171] Diffusion Tensor Imaging (DTI) via MRI was conducted at Screening and Weeks 16, 26, and 39, to assess for changes in neuroinflammation.

Objectives and Endpoints:

[000172] Primary Objective: To demonstrate the safety of intravenous delivery of single or multiple dose Lomecel-B administered to patients with mild AD.

[000173] Primary Endpoint: Measuring the proportion of patients who experienced at least one treatment emergent serious adverse event (TE-SAE) within four weeks of each infusion.

[000174] Secondary Objective: To identify efficacy signals in single or multiple dose Lomecel-B versus placebo in the patient population.

[000175] Secondary Endpoint: Change from baseline to Week 39 in Composite AD Score (CADS) that equally incorporated z-scores of CDR-SB, ADAS-Cog-13, ADCS-ADL, and left hippocampal volume.

Statistical Analysis:

[000176] All statistical analyses were conducted using SAS software (v.9.4). Safety analyses were performed on the ITT population, based on the actual treatment received. For

the primary endpoint, Clopper-Pearson exact confidence intervals were calculated for percentage of patients with any SAEs within 4 weeks after an infusion for all treatment groups (Group 1 [Placebo], Group 2 [25M×1], Group 3 [25M×4] and Group 4 [100M×4]) as well as the Lomecel-B pooled 2,3,4 group (25M×1, 25M×4, 100M×4) and the Lomecel-B pooled 3 and 4 group (25M×4, 100M×4). Additional safety parameters (laboratory, vital signs, ECGs) were summarized descriptively by treatment group and assessment time.

[000177] The secondary endpoint, CADS, was calculated by combining z-scores for change from baseline (to study endpoint) values for multiple assessments: ADCS-ADL, CDR-SB, ADAS-Cog-13, and left hippocampal volume (normalized for intracranial volume). Each component comprised 25% of the final score (i.e., each equally weighted). Inverted measures of the ADAS-Cog-13 and CDR-SB were used in order to match directionality of the other measures with respect to improvement/decline changes. Change from baseline in CADS was statistically analyzed with a mixed model for repeated measures (MMRM) analysis. The model included fixed effects for visit, treatment group (4 level variable), visit by treatment interaction, sex, and baseline value of the outcome parameter. Patient was included as a random effect. Least-squares (LS) means, standard errors (SE), and 95% CIs for mean change from baseline were obtained from the model for each treatment group at each visit, including the pooled Lomecel-B treatment group. The LS-mean differences, SE, 95% CIs, and two-sided p-values for the differences between treatment groups were obtained for each active treatment group (Group 2, 3 and 4) relative to placebo (Group 1) at each visit. The pooled Lomecel-B treatment group effect relative to placebo was obtained from the same model. Because this study was a hypothesis generating study, for CADS, if the two-sided p-value for the difference was < 0.1, then the difference was considered statistically significant and further studies would be warranted. No adjustments for multiple comparisons were performed, and no primary or secondary outcome data were excluded.

[000178] Powering was performed based on the results of a completed Phase 1 study of Lomecel-B (Brody, M., et al. *Alzheimers Dement* 19, 261-273 (2023)). The study was not powered to detect a statistically significant difference in CADS. This study was originally designed to detect a difference in MMSE-2 of 3.87 points at 39 weeks between each Lomecel-B treatment group and placebo at 85% power. During the course of the study, the key secondary endpoint was changed to CADS score, following industry practice of adopting composite endpoints for AD. The study was not powered to detect a statistically significant difference in CADS. However, assuming a two-sided alpha of 0.1, the power to detect a 50% slowing of disease based on CADS comparing 3 active arms combined (N = 36) vs. Placebo

(N = 12) was 36% at 6 months. Effect sizes as small as 0.2-0.3 in CADS may be considered clinically meaningful. No interim analyses were conducted.

[000179] The Intention-to-Treat (ITT) Population included all randomized patients who received at least 1 full or partial dose of IP (any infusion of either Lomecel-B or Placebo). The Modified Intention-to-Treat (mITT) Population included all patients who were randomized and received at least 1 full or partial dose of the IP (any infusion of either Lomecel-B or Placebo) and completed at least 1 post-baseline efficacy assessment (biomarker data or cognitive test).

[000180] Similar MMRM analyses were conducted for the exploratory efficacy endpoints of ADAS-cog-13, MMSE-2, ADCS-ADL, CDR-SB, MoCA, NPI, QOL-AD (both caregiver and study subject), ADCS-ADL, ADRQL, brain volumetry (via MRI), diffusion tensor imaging.

Results:

Primary outcome

[000181] The study met its primary endpoint (Table 2). The primary endpoint was assessed by measuring the proportion of patients who experienced at least one treatment emergent serious adverse event (TE-SAE) within four weeks of each infusion. Additionally, safety assessments included the incidence of all adverse events (AEs) and SAEs throughout the study, which included changes from baseline in clinical laboratory measures and physical exams. Sequential brain MRI was performed and used to assess for the occurrence of ARIA. The lower confidence limit of each Lomecel-B group overlapped with the upper confidence limit for Group 1 (Placebo), indicating a lack of a statistically significant difference in the TE-SAE rate between Group 1 (Placebo) and any of the Lomecel-B groups within 4 weeks after infusion (95% CI for Group 1: 0 – 26.5; Group 2 and Group 3: 0.2 – 36.0; and Group 4: 0.2 – 41.3). Overall, the safety evaluations supported that Lomecel-B is safe and well tolerated in the study population, in both single and multiple dosing regimens.

Table 2: Safety Summary.

Adverse Events	Statistic	Group 1 {Placebo; N=12}	Group 2 {25Mx1; N=13}	Group 3 {25Mx4; N=13}	Group 4 {100Mx4; N=11}	All Patients (N=49)
Total Number of TE-AEs	n	18	21	20	24	83
Total Number of TE-SAEs	n	0	1	2	3	6
Total Number of Grade 3 or Grade 4 TEAEs	n	1	1	2	3	7
Total Number of Related TE-AEs	n	0	1	2	0	3
Total Number of Related TE-SAEs	n	0	0	0	0	0
Total Number of TE-AEs Leading to Study Discontinuation	n	0	0	0	0	0
Total Number of TE-AEs Leading to Treatment Discontinuation	n	0	0	0	0	0
Total Number of TE-AEs Leading to Treatment Interruption	n	3	0	1	0	4
Total Number of TE-SAEs with Outcome of Death	n	0	0	0	0	0
Total Number of TE-AEs within 4 Weeks of Any Infusion	n	15	15	9	19	58
Total Number of TE-SAEs within 4 Weeks of Any Infusion*	n	0	1	1	1	3
Number of Patients with:						
At Least One TE-AE	n (%)	3 (25.0)	7 (53.8)	7 (53.8)	6 (54.5)	23 (46.9)
At Least One TE-SAE	n (%)	0	1 (7.7)	2 (15.4)	2 (18.2)	5 (10.2)
At Least One Grade 3 or Grade 4 TE-AE	n (%)	1 (8.3)	1 (7.7)	2 (15.4)	2 (18.2)	6 (12.2)
At Least One Related TE-AE	n (%)	0	1 (7.7)	1 (7.7)	0	2 (4.1)
At Least One Related TE-SAE	n (%)	0	0	0	0	0
At Least One TE-AE Leading to Study Discontinuation	n (%)	0	0	0	0	0
At Least One TE-AE Leading to Treatment Discontinuation	n (%)	0	0	0	0	0
At Least One TE-AE Leading to Treatment Interruption	n (%)	1 (8.3)	0	1 (7.7)	0	2 (4.1)
At Least One TE-SAE with Outcome of Death	n (%)	0	0	0	0	0
At Least One TE-AE within 4 Weeks of Any Infusion	n (%)	3 (25.0)	7 (53.8)	6 (46.2)	4 (36.4)	20 (40.8)
At Least One TE-SAE within 4 Weeks of Any Infusion	n (%)	0	1 (7.7)	1 (7.7)	1 (9.1)	3 (6.1)

Secondary outcome

[000182] The pre-specified secondary endpoint of this study was change from baseline (CFB) to Week 39 in a Composite Alzheimer's Disease Score (CADS) in Lomecel-B treated groups vs. placebo. CADS was calculated using z-scores for the Alzheimer's Disease Cooperative Study Activities of Daily Living (ADCS-ADL), Clinical Dementia Rating scale

sum of boxes (CDR-SB), Alzheimer's Disease Assessment Scale-cognitive subscale 13 (ADAS-Cog-13) and left hippocampal volume via MRI. This is similar to other composite score approaches used to measuring AD progression, including ADCOMS (Wang, J., et al. J Neurol Neurosurg Psychiatry 87, 993-999 (2016)), Integrated AD Rating Scale (iADRS) (Wessels, A.M., et al. J Prev Alzheimers Dis 2, 227-241 (2015)), and Global Statistical Tests (GST) (Huang, P., et al. Mov Disord 24, 1732-1739 (2009)).

[000183] As depicted in Fig. 2a, progressive decline of the CADS score evident in the placebo group was attenuated in both Group 2 (25M×1) and a combined group consisting of all of the active treatment-group patients (Group 2: 25M×1, Group 3: 25M×4, and Group 4: 100M×4; hereafter referred to as "combined treatment groups 2-4") compared with placebo, providing provisional support for a slowing of disease progression (Group 2 vs. placebo change: 0.38; 95%CI: -0.06-0.82; $p = 0.091$; pre-specified $p < 0.1$ as a positive outcome due to a small sample size). When evaluated using the per-protocol groups, the CADS was positive for both Group 2 (change: 0.39, 95%CI: -0.06-0.85, $p = 0.086$) and Group 4 (change: 0.44, 95%CI: -0.07-0.95, $p = 0.09$) compared to placebo.

Exploratory outcomes

[000184] All patients underwent serial cognitive and function clinical assessments using the MoCA, MMSE-2, Clinical Dementia Rating scale sum of boxes (CDR-SB), and Alzheimer's Disease Assessment Scale-cognitive subscale 13 (ADAS-Cog-13) (Nasreddine, Z.S., et al. J Am Geriatr Soc 53, 695-699 (2005); Cummings, J.L., et al. Neurology 44, 2308-2314 (1994)). All Lomecel-B groups showed a trend toward improvement in the MoCA (Fig. 2b) and MMSE-2 (Fig. 2c) scores compared with placebo, reaching statistical significance for the MoCA in Group 2 (25M×1; $p = 0.009$; $N = 12$) and for the combined treatment effect group ($p = 0.015$; $N = 37$), trended towards improvement for MMSE-2 in Group 4 (100M×4) at 39 weeks ($p = 0.067$; $N = 11$) but did not impact the CDR-SB or ADAS-Cog13 (Fig. 2d and Fig. 2e). Nasreddine, Z.S., et al. J Am Geriatr Soc 53, 695-699 (2005); Cummings, J.L., et al. Neurology 44, 2308-2314 (1994).

[000185] The ADCS-ADL scores for the high dose (Group 4) and combined treatment Groups 2-4 showed improvement at week 39 compared with placebo, reaching statistical significance for Group 4 (100M×4, $p=0.040$; $N = 11$), with trending improvement for the other Lomecel-B doses (Fig. 2f). Quality of life as assessed by the caregiver for QOL-AD numerically improved for patients in Group 4 relative to Group 1 (Placebo) (Fig. 2g) but failed

to improve as assessed by the study patient (Fig. 2h). No statistically significant differences compared with placebo were observed at Week 39 for Lomecel-B treatment groups in the ADRQL (Fig. 2i).

[000186] MRI imaging revealed progressive atrophy at 39 weeks by volumetric MRI in the placebo group, affecting multiple brain regions and whole brain volume, accompanied by increased ventricular size (Fig. 3). Lomecel-B ameliorated this decline in all treatment groups relative to placebo (Fig. 3). Representative vMRI images depict changes in brain volume in Group 1 vs Group 4 at week 39 (increase: yellow; decrease: blue) (Fig. 3a). While the 25Mx1 treatment group (Group 2) showed a non-statistically significant reduction in whole brain atrophy, both 25Mx4 and 100Mx4 groups (Groups 3 and 4) showed statistically significant reduction in whole brain atrophy by week 39 (Group 3: $p=0.006$; Group 4: $p=0.009$) (Fig. 3b). Patients receiving Lomecel-B from the combined treatment groups 2-4 exhibited 48% slower progression of whole brain atrophy compared with placebo ($p=0.005$). Gray matter showed numerical improvement relative to placebo by week 39 (Fig. 3c). Ventricular enlargement, a characteristic feature of progressive AD decline, was observed in placebo (Group 1). Ventricular enlargement was numerically reduced in the Lomecel-B combined treatment groups by 34% for the left ventricle; ($p=0.097$), and bilaterally for combined treatment groups; 37%, ($p=0.066$) compared to placebo (Fig. 3d), and reached statistical significance in Group 3 at week 39 for the right ventricle ($N=12$; $p=0.042$) as well as for the combined treatment groups 2-4 compared to placebo; 40%, $N=33$; $p=0.044$).

[000187] Significantly reduced bilateral hippocampal atrophy was observed for Groups 2 and 3 ($p=0.029$ and $p=0.028$, respectively) at week 39, with numerical improvement for group 4 ($p=0.073$) (Fig. 3e). Combined Lomecel-B treatment Groups 2-4 also exhibited reduced left, right, and bilateral hippocampal atrophy by 62%, $p=0.021$, 53%, $p=0.073$ and 59%, $p=0.013$, respectively. The temporal cortex showed a clear decline in volume in Group 1 (placebo), while treatment groups 2 and 4 demonstrated statistically significant slowing of decline compared with placebo ($p=0.028$ and $p=0.042$, respectively), while Group 3 trended towards improvement ($p=0.076$) (Fig. 3f). The left medial temporal cortex showed progressive improvement towards week 39; while reduced atrophy in group 2 did not reach statistical significance, the higher dosage groups 3 and 4 showed significantly reduced atrophy (Group 3: $p=0.001$; Group 4: $p=0.032$) (Fig. 3g). Significantly reduced atrophy was also observed for the right medial temporal cortex for Group 3 and for the combined treatment groups by week 39 ($p=0.019$).

[000188] Rates of parietal, and occipital cortical atrophy were not significantly reduced. Cingulate cortex, striatum, and left thalamus similarly showed no significant change compared with placebo, but right thalamus showed statistically significant reduction in atrophy (Group 4: $p = 0.026$) (Fig. 5a, Fig. 5b). Frontal cortex atrophy was also significantly ameliorated by week 39 (left hemisphere: $p = 0.028$; right hemisphere: $p = 0.002$) in treatment Group 4 (100M×4), but not in Groups 2 and 3 (25M×1 and 25M×4; Fig. 5c, Fig. 5d).

[000189] Diffusion tensor imaging (DTI) is used to index tissue structure and neuroinflammation and has been a valuable tool in assessing AD brain pathology (Carlson, M.L., et al. *Alzheimers Dement (Amst)* 13, e12218 (2021)). In this study, DTI (mean diffusivity; MD) indicated a potential for increased inflammation in AD placebo over 39 weeks compared with baseline ($p=0.011$). In contrast, MD was reduced in all Lomecel-B treatment groups in the cingulate cortex, compared with placebo, reaching statistical significance for Group 2 ($p = 0.048$; Fig. 3h). Sato, T.N., et al. *Nature* 376, 70-74 (1995), Idowu, T.O., et al. *Elife* 9(2020)

[000190] Tyrosine kinase with immunoglobulin and epidermal growth factor homology domains (TIE2), the cognate receptor for angiopoietins 1 and 2, is expressed by endothelial cells, activates pro-angiogenic and anti-inflammatory downstream signaling pathways (Sato, T.N., et al. *Nature* 376, 70-74 (1995)), and can be degraded into a soluble form (sTIE2) that is released into the bloodstream (Idowu, T.O., et al. *Elife* 9(2020)). Results from this study demonstrated that sTIE2 levels rose progressively in the placebo group compared to baseline ($p=0.047$ at week 26), indicating cell-surface receptor shedding and therefore inactivation. Lomecel-B produced a statistically significant reduction in serum levels of sTIE2 relative to placebo for Group 3 (25M×4) at week 4 ($p=0.01$), week 8 ($p=0.02$), and week 16 ($p=0.045$) (Fig. 4d). sTIE2 levels also trended lower in groups 2 and 4 during the treatment period. sTIE2 levels increased back towards placebo values by weeks 26-39 in all treatment groups.

Post-hoc analysis

[000191] A Pearson correlation analysis was conducted to determine whether reduced brain atrophy correlated with improved clinical scores. Improvements in bilateral hippocampal volume ($R = 0.41$; $p = 0.008$), global brain volume ($R = 0.35$; $p = 0.023$), and bilateral lateral ventricle volume ($R = -0.35$; $p = 0.0213$), all correlated significantly with improvements in MMSE-2 scores (Figs. 4a-c).

Discussion

[000192] Statistically significant improvements in brain atrophy were not usually visible at week 16, four weeks after the final Lomecel-B treatment, but statistically significant changes became evident with subsequent follow-up visits, particularly at week 39. The reduction in brain atrophy was significant on a whole brain level and was enhanced in several brain regions. Areas that showed the greatest improvements by week 39 included the temporal lobes including the hippocampus—areas that show early atrophy progression and disease pathology in AD—as well as the frontal lobes.

[000193] Lomecel-B was not expected to directly target β -Amyloid or p-Tau, but instead is aimed at the neurovascular and inflammatory components of AD and related disorders. Because this study reported no evidence for ARIA, infusion with Lomecel-B could indicate a potentially new class of treatment that is safer than anti-amyloid antibody treatments, potentially complimentary, and addresses a unique mechanism of disease. Moreover, it is speculated that Lomecel-B expresses protective neurovascular and anti-inflammatory effectors that have the potential to help offset ARIA in a combination treatment. In addition, a strong correlation was observed between performance on the cognitive scales with hippocampal volume preservation. Reduced neuroinflammation was suggested via DTI imaging (mean diffusivity) in the cingulate cortex, an area that shows volume loss in early AD (Scheff, S.W., et al. *J Alzheimers Dis* 43, 1073-1090 (2015); Planche, V., et al. *Brain Commun* 4, fcacl09 (2022)), consistent with one of the principal mechanisms of action of Lomecel-B. Prior evidence indicates that MSCs and their potential therapeutic effectors (e.g. exosomes) are active in animals and humans for at least 7-10 days (Assis, A.C., et al. *Cell Transplant* 19, 219-230 (2010); Preda, M.B., et al. *Cell Death Dis* 12, 566 (2021); Kaushal, S., et al. *Eur Heart J Open* 3, oead002 (2023); Gholamrezanezhad, A., et al. *Nucl Med Biol* 38, 961-967 (2011)), but in the present study the data suggest a potential for a more durable therapeutic benefit. Similar findings of sustained physiologic/clinical improvements have also been observed in studies of MSC administration to patients with congestive heart failure (Hare, J.M., et al. *JAMA* 308, 2369-2379 (2012)) and aging frailty (Tompkins, B.A., et al. *J Gerontol A Biol Sci Med Sci* 72, 1513-1522 (2017)).

[000194] Results of this study indicated significant increases in levels of circulating TIE2 in the placebo group that were offset by Lomecel-B. Consistent with previous studies, the reduced TIE2 levels observed in blood serum could reflect a decrease in the degradation and “shedding” of the extracellular portion of the TIE2 receptor after cleavage by metalloproteases,

which are reported to be upregulated in AD (Idowu, T.O., et al. *Elife* 9(2020); Liao, M.C. & Van Nostrand, W.E. *Biochemistry* 49, 1127-1136 (2010); Yamada, T., et al. *Acta Neuropathol* 90, 421-424 (1995)). The increased TIE2 levels observed at week 26 are supportive of a role for TIE2 degradation in the pathology of AD. In principle, these findings are consistent with a provascular/anti-inflammatory effect of Lomecel-B, mediated by circulating secreted factors, including but not limited to, protein mediators and exosomes (Kaushal, S., et al. *Eur Heart J Open* 3, oead002 (2023); Behnke, J., et al. *J Clin Med* 9(2020)), in preserving TIE2 signaling in brain vascular endothelial cells (Joussen, A.M., et al. *Eye (Lond)* 35, 1305-1316 (2021)). This has the potential to preserve BBB function and improve the brain's ability to cope with amyloid- and tau-related disease mechanisms. The temporal delay in observing rescue of regional and global atrophy is consistent with an acute but long-lasting effect during the treatment period. The effects of Lomecel-B thus might improve neurovascular unit functioning and slow neuroinflammation enough to temper the declining trajectory of neuronal death and atrophy over time during AD progression. It remains unknown whether anti-inflammatory effects in AD could be due to direct anti-inflammatory effectors produced by Lomecel-B, or indirectly due to improved clearance of amyloid (Kinney, J.W., et al. *Alzheimers Dement (N Y)* 4, 575-590 (2018)) through preserved BBB function.

[000195] Not all clinical and imaging biomarker results showed clear dose responses, but repeated dosing (Groups 3 and 4) produced better responses than single dosing (Group 1) in 27 of 35 total clinical and vMRI measures, and the highest dosage group (Group 4) produced the best response in 15 of these, indicating a bias towards increasing effect sizes with higher dose regimens. On a clinical level, caregiver variability could explain some noise in the data, but on the other hand, volumetric MRI data could be considered more precise. Other contributing factors to data variability, apart from the small sample size, could include variances in the potency of different lots of Lomecel-B, as potency may depend partly on the bone marrow donor.

[000196] Areas that showed the greatest improvements by week 39 included the temporal lobes including the hippocampus—areas that show early atrophy progression and disease pathology in AD (Whitwell, J.L. *Neurotox Res* 18, 339-346 (2010))—as well as the frontal lobes.

[000197] This study had limitations that warrant mention. First, there was a relatively small sample size with most patients of Hispanic ethnicity, and some variables such as patient education level were not ascertained. Second, the 39-week study duration was relatively short for trials of disease modifying agents in the early AD patient population, and longer studies are

warranted. Additionally, while global statistical testing principles support using a composite endpoint in small studies for AD, the CADS used here is not a validated composite endpoint and it should be assessed with future studies.

Study procedures and timelines.

[000198] Screening activity was performed within 6 weeks prior to the first infusion treatment and included informed consent, medical history and physical examination collection, ECG, clinical assessments including MMSE-2 (Kueper, J.K., et al. J Alzheimers Dis 63, 423-444 (2018)), ADAS-Cog-13 (Tzeng, R.C., et al. Front Aging Neurosci 14, 1021792 (2022)), CDR-SB (Kahle-Wroblewski, K., et al. Alzheimers Dement (Amst) 6, 82-90 (2017)), QOL-AD (Potashman, M., et al. BMC Geriatr 23, 124 (2023)), ADCS-ADL (Kasper, J.D., et al. Alzheimer Dis Assoc Disord 23, 275-284 (2009)), ADRQL (Kasper, J.D., et al. Alzheimer Dis Assoc Disord 23, 275-284 (2009)), NPI (Tompkins, B.A., et al. J Gerontol A Biol Sci Med Sci 72, 1513-1522 (2017)), specimen collection for safety labs, biomarker samples and urine sample analysis. Imaging via MRI and Amyloid PET scan completed the screening procedure (McKay, N.S., et al. Nat Neurosci 26, 1449-1460 (2023)). The Baseline visit activity was performed within 4 weeks prior to the first infusion and included a review of the medical history for any changes, MoCA testing, re-administration of the cognitive testing except for MMSE-2 as well as specimen collection. Screening and Baseline were separated by at least 2 weeks. All visit dates were planned with respect to the first infusion, defined as time zero (0). Infusions were conducted on Day 0, Weeks 4, 8 and 12. During the infusion visits, 80mL of Lomecel-B or placebo (80mL of PlasmaLyte containing 1% HSA) were administered via peripheral Intravenous infusion over 40 minutes. Prior to infusion a review of concomitant medications and adverse events was conducted as well as administration of the CDR-SB and specimen collection. Follow-up visits were completed at Weeks 16, 26 and 39, which included clinical assessment and review, ECG, patient and caregiver assessments, specimen collection and MRI brain scans.

CLAIMS

1. A method of determining the efficacy of alleviating the symptoms of Alzheimer's disease (AD) in a subject suffering from symptoms of AD, wherein one or more biomarkers are measured in the subject before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs).
2. A method of determining the efficacy of treating Alzheimer's disease (AD) or inhibiting AD disease progression in a subject suffering from symptoms of AD, wherein one or more biomarkers are measured in the subject before and after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs).
3. The method according to claims 1 or 2, wherein the biomarker is an MRI biomarker.
4. The method according to any one of claims 1-3, wherein the method further comprises measuring the cognitive function or quality of life of the subject suffering from symptoms of AD before and after administration of the composition comprising allogeneic MSCs.
5. The method according to claim 3 wherein the MRI biomarker comprises a volume measurement of whole brain, lateral ventricles, gray matter, hippocampus, temporal cortex, medial temporal cortex, thalamus, white matter, cingulate cortex, frontal cortex, or combinations thereof.
6. The method according to claim 5, wherein the volume measurement is a measurement of the volume of whole brain, gray matter, temporal cortex, medial temporal cortex, hippocampus, frontal cortex, or thalamus, and wherein a reduction in the decrease in the measured volume after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy.

7. The method according to claim 6, wherein the reduction in the decrease in the measured volume after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is from 1% to 5%, 5% to 10%, 10% to 50%, or greater than 50%.
8. The method according to claim 5, wherein the volume measurement is a measurement of the volume of lateral ventricles, white matter, or cingulate cortex, and wherein a reduction in the increase in the measured volume after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy.
9. The method according to claim 8, wherein the reduction in the increase in the measured volume after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is from 1% to 5%, 5% to 10%, 10% to 50%, or greater than 50%.
10. The method according to any one of claims 3-9, wherein the MRI biomarker further comprises a mean diffusivity or free water measurement via diffusion tensor imaging (DTI) or similar technology.
11. The method according to claim 10 wherein the mean diffusivity measurement is a measurement of mean diffusivity in the cingulate cortex, and wherein a reduction in mean diffusivity in the cingulate cortex after the administration of a composition comprising allogeneic mesenchymal stem cells (MSCs) is indicative of efficacy.
12. The method according to claims 1 or 2, wherein the method comprises measuring one or more of a whole blood, blood plasma, or blood serum biomarker or biomarkers, in the subject suffering from symptoms of AD, before and after the administration of the composition comprising allogeneic MSCs.

13. The method according to claim 12 wherein the blood serum or plasma biomarker comprises soluble TIE2 (sTIE2), eotaxin 1, eotaxin 2, or eotaxin 3.
14. The method according to claim 13 wherein a measured reduction in the level of sTIE2 in the blood serum or plasma in the subject in need thereof, after the administration of the composition comprising allogeneic MSCs, is indicative of efficacy.
15. The method according to any one of claims 1-14, wherein the composition comprises 25×10^6 MSCs.
16. The method according to any one of claims 1-14, wherein the composition comprises 100×10^6 MSCs.
17. The method according to any one of claims 1-16 wherein the composition is administered by intravenous or intra-arterial infusion to a subject in need thereof.
18. The method according to any one of claims 1-17 wherein the composition is administered as one or more of a single dose, a monthly dose, and a repeated interval dose, to a subject in need thereof.