

Allogeneic mesenchymal stem cell therapy with laromestrocel in mild Alzheimer's disease: a randomized controlled phase 2a trial

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Alzheimer's disease (AD) is characterized by progressive cognitive decline, severe brain atrophy and neuroinflammation. We conducted a randomized, double-blind, placebo-controlled, parallel-group phase 2a clinical trial that tested the safety and efficacy of laromestrocel, a bone-marrow-derived, allogeneic mesenchymal stem-cell therapy, in slowing AD clinical progression, atrophy and neuroinflammation. Participants across ten centers in the United States were randomly assigned 1:1:1:1 to four infusion groups: group 1 (placebo; four monthly infusions, $n = 12$); group 2 (25 million cells, one infusion followed by three monthly infusions of placebo, $n = 13$); group 3 (25 million cells; four monthly doses, $n = 13$); and group 4 (100 million cells; four monthly doses, $n = 11$). The study met its primary end point of safety; the rate of treatment-emergent serious adverse events within 4 weeks of any infusion was similar in all four groups: group 1, 0% (95% CI 0–26.5%); group 2, 7.7% (95% CI 0.2–36%); group 3, 7.7% (95% CI 0.2–36%) and group 4, 9.1% (95% CI 0.2–41.3%). Additionally, there were no reported infusion-related reactions, hypersensitivities or amyloid-related imaging abnormalities. Laromestrocel improved clinical assessments at 39 weeks compared to placebo, as measured by a composite AD score (secondary end point was met: group 2 versus placebo change: 0.38; 95% CI –0.06–0.82), Montreal cognitive assessment and the Alzheimer's Disease Cooperative Study Activities of Daily Living. At 39 weeks, Laromestrocel slowed the decline of whole brain volume compared to placebo ($n = 10$) by 48.4% for all treatment groups combined (groups 2–4: $P = 0.005$; $n = 32$) and left hippocampal volume by 61.9% (groups 2–4, $P = 0.021$; $n = 32$), and reduced neuroinflammation as measured by diffusion tensor imaging. The change in bilateral hippocampal atrophy correlated with the change in mini-mental state exam scores ($R = 0.41$, $P = 0.0075$) in all study patients ($N = 42$). Collectively these results support safety of single and multiple doses of laromestrocel treatment for mild AD and provide indications of efficacy in combating decline of brain volume and potentially cognitive function. Larger-scale clinical trials of laromestrocel in AD are warranted. ClinicalTrials.gov registration: [NCT05233774](https://clinicaltrials.gov/ct2/show/study/NCT05233774).

AD is a devastating and progressive neurocognitive disorder representing the primary cause of dementia^{1–5}. Until recently, AD treatments lacked disease-modifying effects and an AD diagnosis led to inevitable and disabling cognitive decline in all affected individuals⁶. Attempts to modify the disease have focused largely on the amyloid and neurofibrillary tangle hypotheses of disease pathogenesis⁷. After many failures of monoclonal antibodies attempting to remove amyloid proteins from the brain, three drugs were approved by the US Food and Drug Administration (FDA) between 2021 and 2024⁸.

In a phase 3 trial of lecanemab, randomization to active treatment resulted in a 27% improvement in Clinical Dementia Rating scale sum of boxes (CDR-SB) at 18 months^{9–12}. Despite this therapeutic advance, lecanemab is associated with a 21.5% risk of amyloid-related imaging abnormalities (ARIAEs)^{13,14} and the possibility of continued progression of brain atrophy^{15,16}. Additionally, lecanemab resulted in a 26.4% rate of infusion-related reactions that can be devastating for a patient. Aducanumab carried a 35% risk of ARIA-edema (ARIA-E) and 19% risk of ARIA-hemorrhage (ARIA-H)¹⁷. Further, a third anti-amyloid drug, donanemab, carries a 36.8% risk of ARIAs (ARIA-E or ARIA-H), with drug-induced inflammation and brain bleeding reported¹⁸. Accordingly, it is imperative to continue to develop novel therapeutic strategies.

Nonamyloid and non-tau contributions to AD include cerebrovascular degradation and a neuroinflammatory component contributing to neuronal death and brain atrophy^{19,20}. The neurovascular unit, composed of endothelial cells lining cerebral vessels in tight association with pericytes and astrocytes, regulates cerebral blood flow and the exchange of nutrients and waste products generated by the substantial energetic demands of active neural networks, and is the central component of the blood–brain barrier (BBB)^{20,21}. Dysregulation of this system is often observed along with hallmark neuroinflammation in AD, but the precise relationship of these features with amyloid and tau pathology remains a matter of debate^{22–24}.

Cell-based therapy with mesenchymal stem cells (MSCs) is a particularly attractive treatment candidate as it encompasses provascular, anti-inflammatory, immunomodulatory and tissue repair mechanisms of action, with findings validated in a murine AD model^{25,26}. For AD, these effects could represent a neuroprotective dimension to treatment that mitigates some of the more destructive aspects of the disease and augments anti-amyloid/tau regimens. We have previously shown in a phase I study that laromestrol (Lomecel-B) is safe in patients with mild AD and does not cause ARIAs, including in ApoE4 homozygous carriers²⁷. Laromestrol is immunoprivileged and is administered as

an allogeneic cell therapy, due in part to undetectable expression of major histocompatibility complex (MHC)-II molecules, low-level MHC-I expression, and immunomodulatory properties^{25,26,28,29}.

To advance our understanding of the potential therapeutic use of laromestrol in AD, we conducted a 49-patient randomized, double-blind, placebo-controlled phase 2a study testing three different dosing regimens of laromestrol. Our objectives were to test the hypothesis that intravenous administration of laromestrol was safe, could ameliorate both the clinical disease progression of AD and its associated brain atrophy, and to gather mechanistic insight on potential roles in influencing disease trajectory.

Results

Patient disposition

A total of 120 patients with a clinical diagnosis of mild AD were screened and 50 were enrolled. The first patient was enrolled on 29 December 2021 and the last on 21 November 2022, with the last patient study completion on 21 August 2023. All eligible patients were randomized 1:1:1:1 in block sizes of four, with each site receiving separate blocks. Randomization was additionally stratified by sex. Forty-nine randomized participants received double-blind treatment and were analyzed for the primary end point, safety, as part of the intention-to-treat (ITT) population (Fig. 1). One patient randomized to group 4 was not treated based on the investigator's decision. The remaining 48 patients completed treatment with at least one post-baseline efficacy assessment and were analyzed for the secondary and exploratory end points as part of the modified ITT (mITT) population. Laromestrol was administered through intravenous infusions of approximately 40 min duration with no pre-medication required. The treatment groups in the mITT population included: group 1, placebo infusions once per month for 4 months ($n = 12$); group 2, 25 million cells at day 0 followed by three monthly infusions of placebo ($25 \text{ M} \times 1$; $n = 12$); group 3, 25 million cells infused monthly for 4 months ($25 \text{ M} \times 4$; $n = 13$); and group 4: 100 million cells infused monthly for 4 months ($100 \text{ M} \times 4$; $n = 11$) (Fig. 1).

Baseline demographics and disease characteristics for the ITT population are presented in Table 1. Overall, the mean $\pm$ s.d. age was 74.1 ± 6.65 years ($n = 49$), with a lower proportion of male (44.9%) versus female (55.1%) patients. The majority of patients were white (95.9%) with a higher proportion of Hispanic/Latino ethnicity (75.5%). Medication for symptomatic AD at baseline was taken by 63.3% of patients. Baseline volumetric magnetic resonance imaging (MRI) values are shown in Supplementary Table 1 and inclusion/exclusion criteria are shown in Supplementary Table 2. All study patients, caregivers and

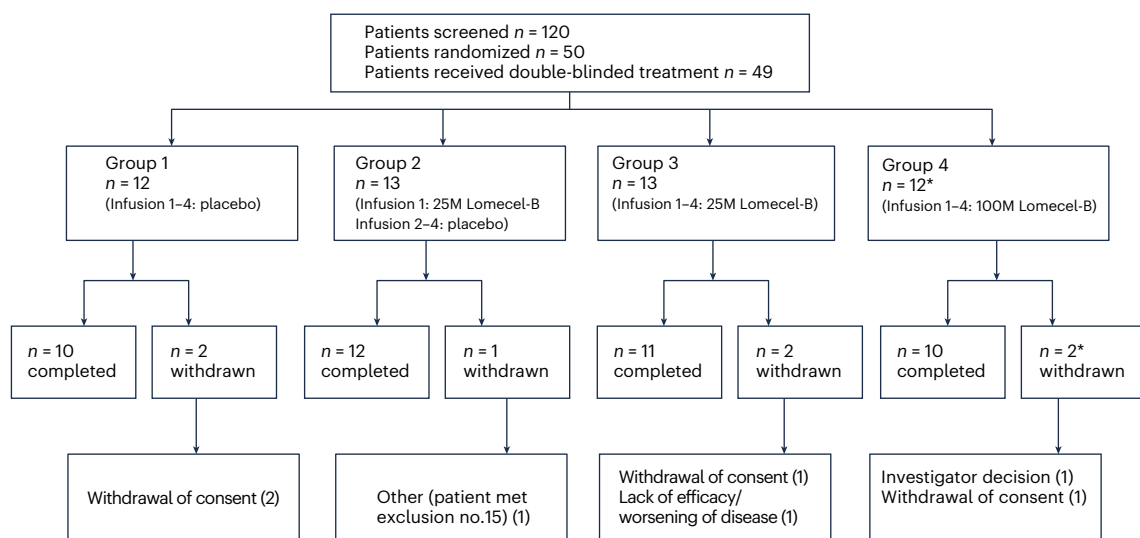


Fig. 1 | Consort diagram depicting study design. Full analysis set (FAS): $N = 120$; per-protocol population (PP): $N = 41$; ITT population: $N = 49$; mITT population: $N = 48$.

Table 1 | Demographics and baseline characteristics (ITT population)

Parameter	Group 1 (placebo) (N=12)	Group 2 (25M×1) (N=13)	Group 3 (25M×4) (N=13)	Group 4 (100M×4) (N=11)	All patients (N=49)	P value
Age, years, mean (s.d.)	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, n (%)	5 (41.7)	5 (38.5)	7 (53.8)	5 (45.5)	22 (44.9)	
Female, n (%)	7 (58.3)	8 (61.5)	6 (46.2)	6 (54.5)	27 (55.1)	
Race						0.066
Black/African American, n (%)	0	0	0	2 (18.2)	2 (4.1)	
White, n (%)	12 (100.0)	13 (100.0)	13 (100.0)	9 (81.8)	47 (95.9)	
Ethnicity						0.697
Hispanic/Latino, n (%)	9 (75.0)	11 (84.6)	10 (76.9)	7 (63.6)	37 (75.5)	
Not Hispanic/Latino, n (%)	3 (25.0)	2 (15.4)	3 (23.1)	4 (36.4)	12 (24.5)	
ApoE4 status						0.697
Non-carrier, n (%)	10 (83.3)	8 (61.5)	7 (53.8)	10 (90.9)	35 (71.4)	
Heterozygous, n (%)	2 (16.7)	5 (41.7)	5 (38.5)	1 (8.3)	13 (26.5)	
Homozygous, n (%)	0	0	1 (7.7)	0	1 (2.0)	
AD treatment, n (%)	7 (58.3)	9 (69.2)	8 (61.5)	7 (63.6)	31 (63.3)	0.952
Donepezil, n (%)	5 (41.7)	6 (46.2)	7 (53.8)	5 (45.5)	23 (46.9)	
Memantine, n (%)	3 (25.0)	4 (30.8)	5 (38.5)	3 (27.3)	15 (30.6)	
Donepezil/memantine, n (%)	0	1 (7.7)	0	0	1 (2.0)	
Galantamine, n (%)	0	0	1 (7.7)	0	1 (2.0)	
Height (cm), mean (s.d.)	165.0 (14.2)	170.2 (8.6)	166.5 (10.1)	167.1 (9.1)	167.3 (10.6)	0.665
Weight (kg), mean (s.d.)	79.5 (15.5)	78.6 (16.6)	84.1 (14.9)	77.8 (14.8)	80.1 (15.2)	0.741
BMI (kg m ⁻²), mean (s.d.)	30.4 (6.63)	27.1 (5.35)	30.4 (5.39)	27.8 (4.06)	28.9 (5.49)	0.707
MoCA total score, mean (s.d.)	19.0 (5.83)	16.0 (4.51)	19.2 (4.18)	18.2 (4.58)	18.1 (4.83)	0.772
MMSE-2 total score, mean (s.d.)	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, mean (s.d.)	31.30 (11.82)	32.92 (8.87)	27.87 (8.91)	23.58 (4.13)	29.09 (9.36)	0.071
CDR-SB total score, mean (s.d.)	3.75 (1.37)	5.19 (1.52)	3.73 (1.56)	4.23 (1.72)	4.23 (1.62)	0.071
ADCS-ADL total score, mean (s.d.)	59.1 (9.10)	55.8 (9.24)	61.9 (13.43)	63.1 (8.95)	59.9 (10.49)	0.322

BMI, body mass index. Group 1, placebo (dose × 4). Group 2, laromestrol (25 M × 1). Group 3, laromestrol (25 M × 4). Group 4, laromestrol (100 M × 4). Baseline cognitive and functional tests represent the scores of the last test before the first dose. The ITT population was *n* = 49; after one withdrawal from group 2, the mITT was *n* = 48.

all personnel involved in the conduct and interpretation of the study, including investigators, site personnel and sponsor staff, were blinded to treatment group.

Primary outcome

The study met its primary end point (Table 2). The primary end point was assessed by measuring the proportion of patients who experienced at least one treatment-emergent (TE) serious adverse event (SAE) within 4 weeks of any infusion. Additionally, safety was evaluated by the incidence of all adverse events (AEs) and SAEs throughout the study, clinical laboratory assessments, physical examinations and ARIAs (ARIA-H or ARIA-E) or clinically asymptomatic microhemorrhages as assessed by MRI.

With regard to the primary end point, three patients experienced a TE-SAE within 4 weeks of administering an infusion, corresponding to one patient in each of the laromestrol treatment groups. These TE-SAEs were: (1) grade 3 anemia, 5 days after the third infusion (group 3, 25 M × 4); (2) grade 4 lumbar radiculopathy, 19 days after the fourth infusion (group 2, 25 M × 1, after placebo infusion); and (3) grade 4 acute respiratory failure, 28 days after the fourth infusion (group 4, 100 M × 4). All SAEs were related to pre-existing conditions, none of these events was assessed as related to study product, and all resolved. Thus, based on a medical evaluation, the primary end point was met.

Based on the pre-specified statistical safety analysis, the primary end point was met; the lower confidence limit of each laromestrol 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 laromestrol 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%).

With regard to other safety events, the most frequent AEs were COVID-19 and urinary tract infections (Table 2), there were no deaths on the study and no TE-AEs leading to study or treatment discontinuation. No clinically meaningful changes were observed in hematology, chemistry, coagulation or urinalysis parameters. Overall, 23 patients presented with 83 TE-AEs (18 in group 1 (placebo), 21 in group 2 (25 M × 1), 20 in group 3 (25 M × 4) and 24 in group 4 (100 M × 4)). Five patients out of 49 presented with six TE-SAEs (one in group 2 (25 M × 1), two in group 3 (25 M × 4) and three in group 4 (100 M × 4)). Two patients had three TE-AEs (one in group 2 (25 M × 1) and two in group 3 (25 M × 4)) that were assessed as related to the investigational product (IP) (chills and headache). Four patients experienced six infusion interruptions during the course of the study, none of which was associated with AEs. There were no hypersensitivities or infusion-related reactions observed during the study. Additionally, there were no events of ARIAs (ARIA-H or ARIA-E), including clinically asymptomatic microhemorrhages detected in

Table 2 | Safety summary (ITT)

Adverse events	Statistic	Group 1 (placebo; <i>N</i> =12)	Group 2 (25 M×1; <i>N</i> =13)	Group 3 (25 M×4; <i>N</i> =13)	Group 4 (100 M×4; <i>N</i> =11)	All patients (<i>N</i> =49)
Total number of TE-AEs	<i>n</i>	18	21	20	24	83
Total number of TE-SAEs	<i>n</i>	0	1	2	3	6
Total number of grade 3 or grade 4 TE-AEs	<i>n</i>	1	1	2	3	7
Total number of related TE-AEs	<i>n</i>	0	1	2	0	3
Total number of related TE-SAEs	<i>n</i>	0	0	0	0	0
Total number of TE-AEs Leading to study discontinuation	<i>n</i>	0	0	0	0	0
Total number of TE-AEs leading to treatment discontinuation	<i>n</i>	0	0	0	0	0
Total number of TE-AEs leading to treatment interruption	<i>n</i>	3	0	1	0	4
Total number of TE-SAEs with outcome of death	<i>n</i>	0	0	0	0	0
Total number of TE-AEs within 4 weeks of any infusion	<i>n</i>	15	15	9	19	58
Total number of TE-SAEs within 4 weeks of any infusion*	<i>n</i>	0	1	1	1	3
Number of patients with:						
At least one TE-AE	<i>n</i> (%)	3 (25.0)	7 (53.8)	7 (53.8)	6 (54.5)	23 (46.9)
At least one TE-SAE	<i>n</i> (%)	0	1 (7.7)	2 (15.4)	2 (18.2)	5 (10.2)
At least one grade 3 or grade 4 TE-AE	<i>n</i> (%)	1 (8.3)	1 (7.7)	2 (15.4)	2 (18.2)	6 (12.2)
At least one related TE-AE	<i>n</i> (%)	0	1 (7.7)	1 (7.7)	0	2 (4.1)
At least one related TE-SAE	<i>n</i> (%)	0	0	0	0	0
At least one TE-AE leading to study discontinuation	<i>n</i> (%)	0	0	0	0	0
At least one TE-AE leading to treatment discontinuation	<i>n</i> (%)	0	0	0	0	0
At least one TE-AE leading to treatment interruption	<i>n</i> (%)	1 (8.3)	0	1 (7.7)	0	2 (4.1)
At least one TE-SAE with outcome of death	<i>n</i> (%)	0	0	0	0	0
At least one TE-AE within 4 weeks of any infusion	<i>n</i> (%)	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	<i>n</i> (%)	0	1 (7.7)	1 (7.7)	1 (9.1)	3 (6.1)

*Study primary end point.

any study patient. Overall, laromestrol was safe and well tolerated in the study population, in both single and multiple dosing regimens.

Secondary outcomes

The pre-specified secondary end point of this study was change from baseline (CFB) to week 39 in a novel Composite Alzheimer's Disease Score (CADS) in laromestrol treated groups versus placebo. The 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, and is similar to other composite score approaches used to measure AD progression, such as the ADCOMS^{9,30}, Integrated AD Rating Scale (iADRS)³¹ and Global Statistical Tests (GST)³². As depicted in Fig. 2a, the progressive decline of the CADS score (disease worsening) evident in the placebo group was attenuated in both group 2 (25 M × 1) and a combined group consisting of the patients in the active-treatment groups (group 2, 25 M × 1; group 3, 25 M × 4; and group 4, 100 M × 4; hereafter referred to as 'combined treatment groups 2–4') compared to placebo, providing provisional support for a slowing of disease progression (group 2 (*n* = 11) versus placebo (*n* = 10) difference 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) (Supplementary Table 3). When evaluated using the per-protocol groups, the CADS was positive for both group 2 (difference 0.39, 95% CI −0.06–0.85, *P* = 0.086; *n* = 11) and group 4 (change 0.44, 95% CI −0.07–0.95, *P* = 0.09; *n* = 7) compared to placebo (*n* = 9).

Exploratory outcomes

All patients underwent serial cognitive and function clinical assessments using the Montreal Cognitive Assessment (MoCA), mini-mental state evaluation (MMSE-2), CDR-SB and ADAS-Cog-13 (refs. 33,34). All laromestrol groups showed a trend toward improvement in the MoCA (Fig. 2b and Supplementary Table 4) and MMSE-2 (Fig. 2c and Supplementary Table 5) scores compared to placebo (*n* = 10) at week 39, reaching statistical significance for the MoCA in group 2 (25 M × 1; *P* = 0.009; *n* = 11) and for the combined treatment effect group (groups 2–4, *P* = 0.015; *n* = 32). There was a trend toward improvement for MMSE-2 in group 4 (100 M × 4) at 39 weeks (*P* = 0.067; *n* = 10) but treatment did not significantly impact the CDR-SB (Fig. 2d) or ADAS-Cog13 (Fig. 2e).

The ADCS-ADL scores for the high-dose (group 4) and combined treatment groups 2–4 showed improvement at week 39 compared to placebo (*n* = 10), reaching statistical significance for group 4 (100 M × 4, *P* = 0.040; *n* = 10), with trending improvement for the other laromestrol doses (Fig. 2f and Supplementary Table 6). Quality of life (QoL) 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 treatment effect compared to placebo was observed at week 39 for laromestrol treatment groups in the Alzheimer's disease related quality of life (ADRQL) score (Fig. 2i).

Brain atrophy in AD involves multiple brain regions and begins relatively early in disease progression, affecting broad areas of the temporal, occipital, parietal and frontal lobes, and notably the hippocampus, up to 8 years before AD diagnosis^{35–38}. In our study, volumetric

MRI revealed progressive atrophy at 39 weeks in the placebo group, affecting multiple brain regions and whole brain volume, accompanied by increased ventricular size (Fig. 3). Laromestrocel ameliorated this decline in all treatment groups relative to placebo (Fig. 3). Representative volumetric MRI (vMRI) images depict changes in brain volume in group 1 versus group 4 at week 39 (increase, yellow; decrease, blue) (Fig. 3a). While the 25 M × 1 treatment group (group 2) showed a non-statistically significant reduction in whole brain atrophy versus the placebo group, both 25 M × 4 and 100 M × 4 groups (groups 3 and 4) showed statistically significant reduction in whole brain atrophy compared to placebo ($n = 10$) by week 39 (by up to 57% for group 3, $P = 0.006$, $n = 11$; group 4, 0.009 , $n = 10$) (Fig. 3b). Patients receiving laromestrocel from the combined treatment groups 2–4 ($n = 32$) exhibited 48.4% slower progression of whole brain atrophy at 39 weeks compared to placebo ($n = 10$; $P = 0.005$). 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) at week 39. Ventricular enlargement was numerically reduced in the laromestrocel combined treatment groups 2–4 ($n = 32$) by 34% for the left ventricle; ($P = 0.097$) and bilaterally for combined treatment groups 2–4 ($n = 32$) compared to placebo ($n = 10$; 37%, $P = 0.066$) (Fig. 3d) and reached statistical significance for the right ventricle in group 3 ($n = 11$) at week 39 (49%, $P = 0.042$) as well as for the combined treatment groups 2–4 ($n = 32$) compared to placebo ($n = 10$; 40%, $P = 0.044$).

Significantly reduced bilateral hippocampal atrophy was observed for groups 2 and 3 ($P = 0.029$, $n = 11$ and $P = 0.028$, $n = 11$ respectively) at week 39 compared to placebo ($n = 10$), with numerical improvement for group 4 ($P = 0.073$; $n = 10$) (Fig. 3e). Combined laromestrocel 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, versus placebo. The left temporal cortex showed a clear decline in volume at week 39 in group 1 (placebo; $n = 10$), whereas treatment groups 2 and 4 demonstrated statistically significant slowing of decline compared to placebo ($P = 0.042$, $n = 11$ and $P = 0.028$, $n = 10$, respectively) and group 3 trended toward improvement ($P = 0.076$, $n = 11$) (Fig. 3f). The left medial temporal cortex also showed progressive improvement at week 39 versus placebo; while reduced atrophy in group 2 did not reach statistical significance, the higher-dosage groups 3 and 4 demonstrated significant reduction in atrophy relative to placebo ($n = 10$) (group 3, $P = 0.001$, $n = 11$; group 4, $P = 0.032$, $n = 10$) (Fig. 3g). Significantly reduced atrophy was also observed for the right medial temporal cortex for group 3 ($P = 0.019$; $n = 11$) and trending for the combined treatment groups 2–4 compared to placebo ($n = 10$) by week 39 ($P = 0.064$; $n = 32$).

Notably, the rates of parietal and occipital cortical atrophy were not significantly reduced. Cingulate cortex, striatum and left thalamus similarly showed no significant change compared to placebo, but right thalamus showed statistically significant reduction in atrophy in group 4 ($P = 0.026$, $n = 10$) (Extended Data Fig. 1a,b). Frontal cortex atrophy was also significantly ameliorated by week 39 (left hemisphere:

$P = 0.028$; right hemisphere: $P = 0.002$) in group 4 (100 M × 4; $n = 10$), but not in groups 2 and 3 (25 M × 1 and 25 M × 4; Extended Data Fig. 1c,d) compared to placebo ($n = 10$).

Diffusion tensor imaging (DTI) is used to index tissue structure and neuroinflammation and has been a valuable tool in assessing AD brain pathology³⁹. In our study, DTI (mean diffusivity; MD) indicated a potential for increased inflammation in AD placebo at 39 weeks compared to baseline ($P = 0.011$; $n = 10$). In contrast, MD was reduced in all laromestrocel treatment groups in the cingulate cortex, compared to placebo ($n = 10$), reaching statistical significance for group 2 ($p = 0.048$; $n = 11$; Fig. 3h).

We examined blood serum biomarkers to gain insight into the mechanisms of action of laromestrocel in AD. 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 pathways⁴⁰, and can be degraded into a soluble form (sTIE2) that is released into the bloodstream⁴¹. We found that sTIE2 levels rose progressively in the placebo group compared to baseline ($P = 0.047$ at week 26; $n = 11$), indicating cell-surface receptor shedding and therefore likely inactivation. Laromestrocel produced a statistically significant reduction in serum levels of sTIE2 relative to placebo for group 3 (25 M × 4) at week 4 ($P = 0.01$; $n = 12$), week 8 ($P = 0.02$; $n = 12$) and week 16 ($P = 0.045$; $n = 12$) (Fig. 4d). sTIE2 levels also trended lower in groups 2 and 4 during the treatment period. sTIE2 levels increased back toward placebo values by weeks 26–39 in all treatment groups.

Post hoc analysis

We hypothesized that patients with the greatest improvement in vMRI measures would also show the most improvement in cognitive scores. To determine whether reduced brain atrophy correlated with improved clinical scores, we performed a Pearson correlation analysis of week 39 data in all study patients. The change in bilateral hippocampal volume ($R = 0.41$; $P = 0.008$; $n = 42$), whole brain volume ($R = 0.35$; $P = 0.023$; $n = 42$) and bilateral lateral ventricle volume ($R = -0.35$; $P = 0.0213$; $n = 42$), all correlated significantly with change in MMSE-2 scores (Fig. 4a–c).

Discussion

AD is a disorder of neurocognition with a major unmet medical need. The recent introduction of the disease-modifying class of anti-amyloid monoclonal antibody treatments has ushered in new hope that the natural history of AD could be favorably impacted¹³. Although this class of medicine is now approved, other classes of treatment are still needed. Here we present a phase 2a study of a novel therapeutic class of AD therapy – cell-based therapy that possesses provascular and anti-inflammatory properties¹¹. This study provides additional support for the safety profile of this treatment previously seen in a phase 1 study, indicates safety of both single and multiple dosing, and provides preliminary indications of efficacy²⁷. The study findings support the potential for laromestrocel to improve both cognitive function and

Fig. 2 | Clinical assessments. Groups 1–4 are color coded: group 1 (placebo; black), group 2 (25 M × 1; blue), group 3 (25 M × 4; salmon) and group 4 (100 M × 4; magenta). Two-sided P values are associated with testing for statistically significant differences in least-squares (LS) means between laromestrocel and placebo obtained from a mixed model for repeated measures (MMRM) (F-statistic). No adjustment for multiple comparisons was made. Statistical significance was pre-set at $P < 0.10$ for the CADS scores. For CADS, the placebo group showed a trending decline in CADS scores at 39 weeks (a), while treatment groups showed numerical improvement relative to placebo, reaching statistical significance at 39 weeks (group 2, $P = 0.091$; $n = 11$) compared to placebo ($n = 10$). Statistical significance was met for MoCA at 39 weeks versus placebo ($n = 10$) (group 2, $P = 0.009$; $n = 11$) (b) as well as for combined groups 2–4, $P = 0.015$; $n = 32$). MMSE-2 (group 4, $P = 0.067$, $n = 10$) (c) and CDR-SB (group 4, $P = 0.270$;

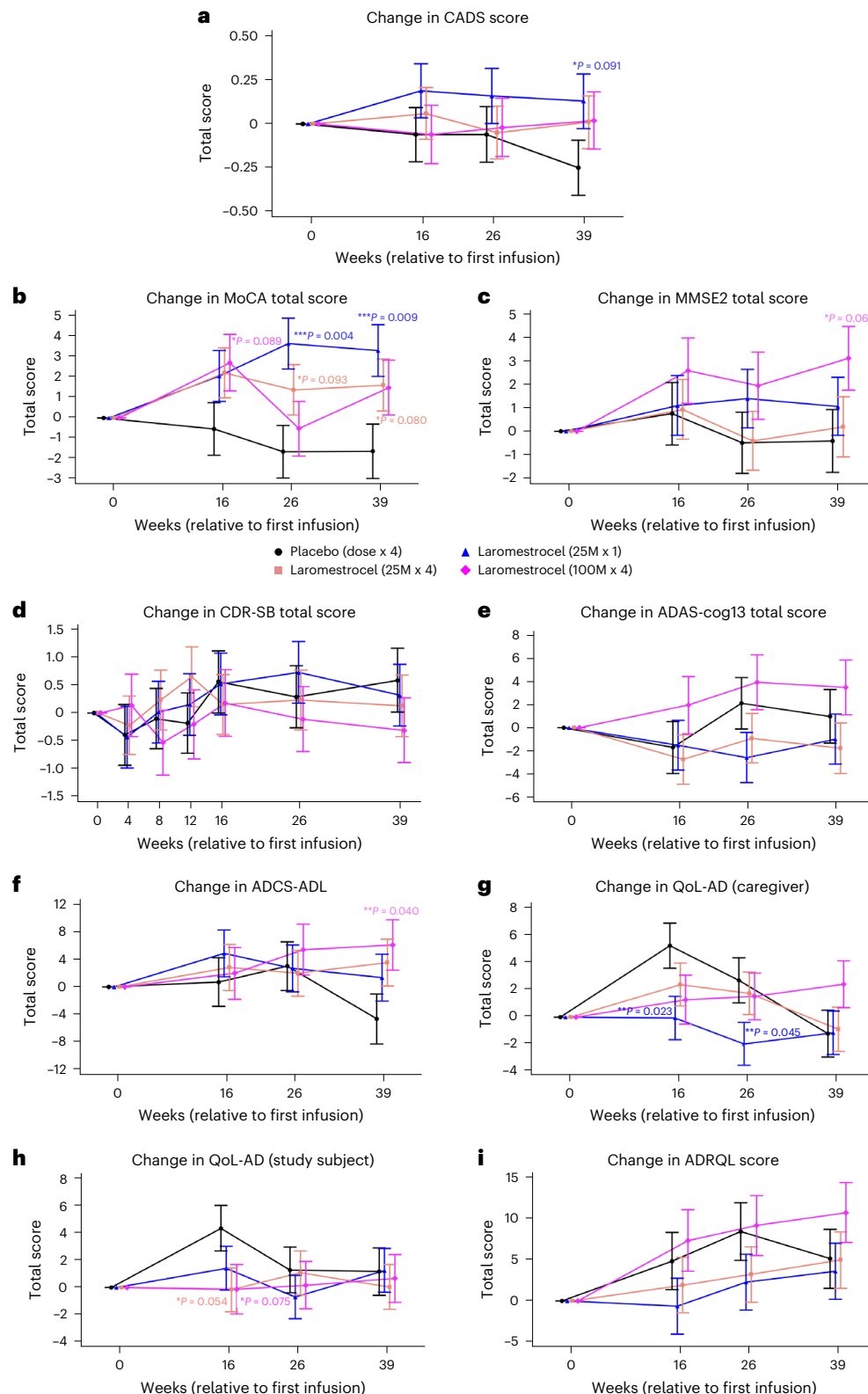
$n = 10$) (d) scores showed numerical improvement for all treatment groups at week 39 compared to placebo ($n = 10$), while ADAS-cog13 measures (combined groups 2–4, $P = 0.780$; $n = 33$) were not statistically different from placebo ($n = 10$) (e). f–i. For QoL measures, statistical significance was met with group 4 for ADCS-ADL at 39 weeks (group 4, $P = 0.04$; $n = 10$) compared to placebo ($n = 10$) (f), while group 2 ($P = 0.228$, $n = 12$) and group 3 ($P = 0.103$, $n = 11$) showed numerical improvement relative to placebo ($n = 10$). Numerical improvement was observed in group 4 at 39 weeks for QoL-AD (caregiver) ($P = 0.142$, $n = 10$) compared to placebo ($n = 10$) (g), but no improvement was found in QoL-AD (study participant) scores (combined groups 2–4, $P = 0.797$, $n = 33$) compared to placebo ($n = 10$) (h) while a numeric trend towards improvement was found in group 4 ($P = 0.275$, $n = 10$) at 39 weeks for ADRQL scores compared to placebo ($n = 10$) (i). Error bars represent $\pm$ s.e.m.

QoL in mild AD. Furthermore, detailed brain-imaging studies reveal potential improvements compared to placebo in brain architecture with laromestrocel administration.

A single previous trial of human umbilical cord derived MSCs explored intracerebroventricular injection in patients with AD. The efficacy of this approach remains to be established and this route of administration was associated with AEs such as fever and headache⁴². The present study further confirms the absence of ARIAs even in

individuals who are ApoE carriers, the group previously shown to be at highest risk for ARIAs¹⁴. Moreover, while both the previous and current studies—limited to 1 year or less—show improvements in some but not all indices of cognitive function and QoL, we hypothesize that longer treatment and longer follow-up times could reveal greater impact.

Statistically significant improvements in brain atrophy were not usually visible at week 16, 4 weeks after the final laromestrocel treatment, but statistically significant changes became evident with



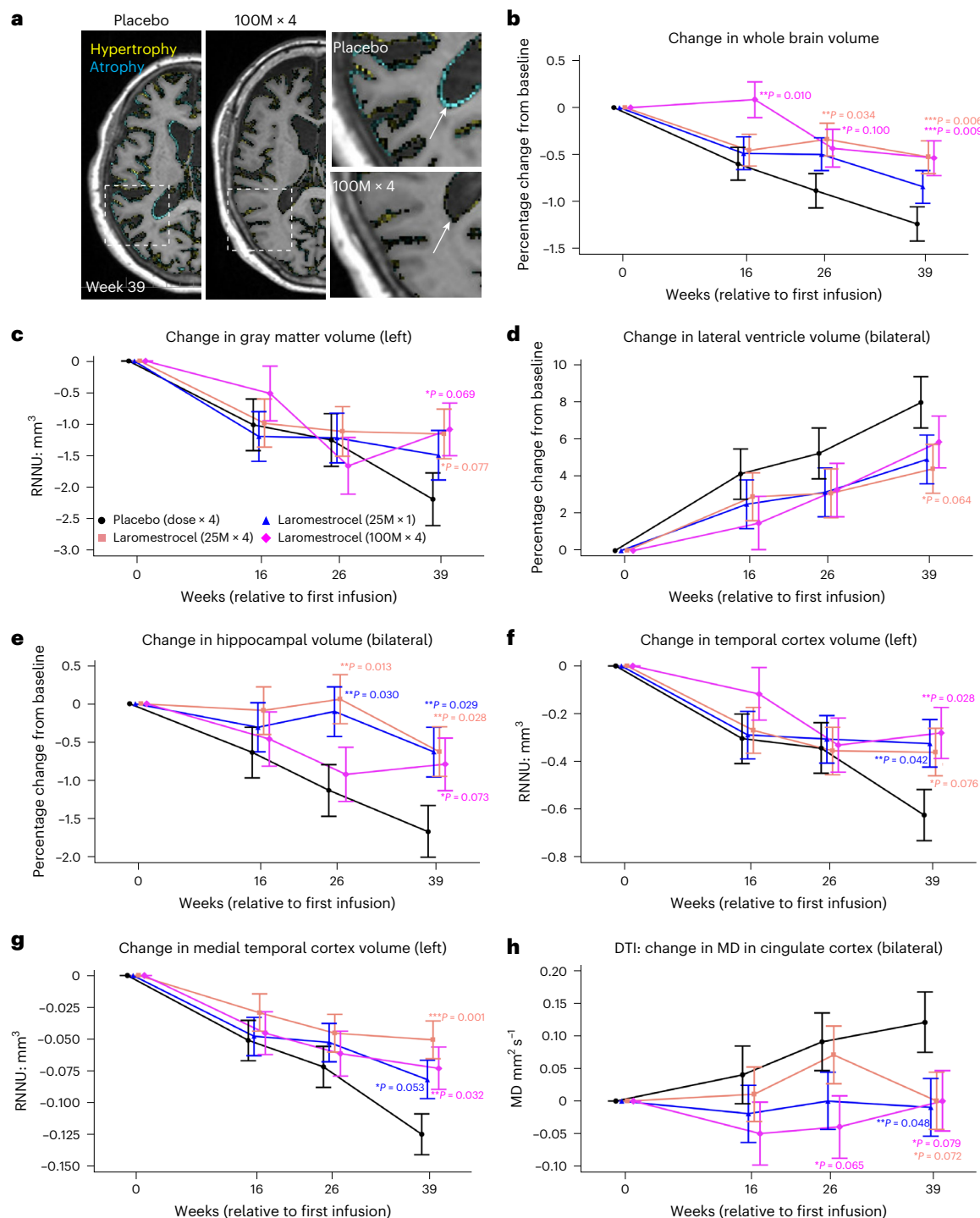


Fig. 3 | Volumetric MRI assessments of AD patients treated with laromestrocel versus placebo. Two-sided *P* values are associated with testing for statistically significant differences in LS means between laromestrocel and placebo obtained from a MMRM (F-statistic). No adjustment for multiple comparisons was made. **a**, vMRI images depicting changes in brain volume in group 1 (placebo) versus group 4 (high repeated dose) at week 39 (increase, yellow; decrease, blue). Insets in **a** are higher magnification regions of the boxed regions for groups 1 and group 4. **b**, Whole brain volume steadily decreased by ~1.2% in the placebo group during the 39-week trial period, whereas treatment groups showed statistically significant slowing of whole brain atrophy (group 3, *P* = 0.006, *n* = 11; group 4, *P* = 0.009, *n* = 10) compared to placebo (*n* = 10). **c**, Gray matter volume (left) declined in placebo by about 2.2% at week 39, whereas treatment group 4 (*P* = 0.069, *n* = 10) showed numerical improvement compared to placebo (*n* = 10). **d**, Lateral ventricles enlarged in placebo over time, but all treatment groups trended toward improvement at all time points, compared to placebo

(*n* = 10), reaching statistical significance for group 3 (right ventricle) at week 39, (*P* = 0.042; *n* = 11). **e**, Bilateral hippocampal volume similarly decreased by approximately 1.6% in placebo (*n* = 10) while treatment groups showed less decline (group 2, *P* = 0.029, *n* = 11; group 3, *P* = 0.028, *n* = 11). **f**, Left temporal cortex showed progressive decline in volume in the placebo group (*n* = 10), but partial rescue with group 2 (*P* = 0.042; *n* = 11) and group 4 (*P* = 0.028; *n* = 10). **g**, Left medial temporal cortex also showed progressive decline in volume in the placebo group (*n* = 10) while treated groups showed slower decline (group 3, *P* = 0.001; *n* = 11; group 4, *P* = 0.032; *n* = 10). Units are indicated as raw non-normalized units ratio to intracranial volume ($\times 10^{-3}$). **h**, Patients with AD receiving placebo showed increasing cingulate cortex diffusion tensor imaging (DTI) values for mean diffusivity (MD) indicating increasing inflammation and disease progression, whereas all groups receiving laromestrocel showed numerically improved MD at 39 weeks, reaching statistical significance for group 2; *P* = 0.048; *n* = 11. Error bars represent $\pm$ s.e.m.

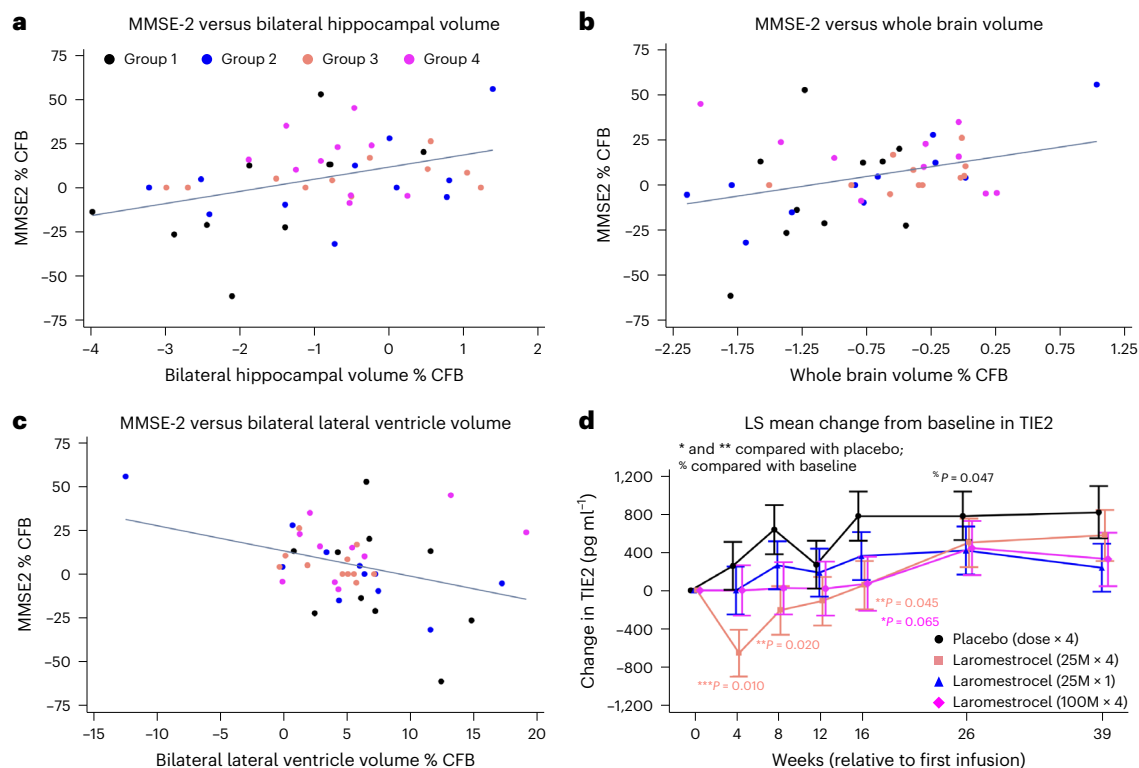


Fig. 4 | Reduction of bilateral hippocampal, whole brain atrophy correlates with improved MMSE scores. **a, b**, Pearson correlation analysis showing correlation between bilateral hippocampal (**a**) and whole brain (**b**) atrophy reduction and improved MMSE-2 scores at week 39 ($R = 0.41$; $P = 0.0075$; $n = 42$ and $R = 0.35$; $P = 0.0227$; $n = 42$, respectively). Correlation of reduced bilateral ventricular enlargement with improved MMSE-2 scores was also evident ($R = -0.35$; $P = 0.0213$; $n = 42$) (**c**). Blood serum collected at the indicated time points was analyzed by Meso Scale Discovery (MSD) for TIE2 (**d**). Two-sided P values are associated with testing for statistically significant differences

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 AD⁴³—as well as the frontal lobes. It is unclear why these areas showed the greatest benefit; it could indicate a regional bias in the vascular or anti-inflammatory effects of laromestrol.

Laromestrol is 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 we found no evidence for ARIAs, infusion with laromestrol could indicate a potentially new class of treatment with a favorable safety profile compared to existing anti-amyloid antibody treatments, potentially complementary to other therapeutic strategies. Moreover, we speculate that laromestrol expresses protective neurovascular and anti-inflammatory effectors that have the potential to help offset ARIAs in a combination treatment. In addition, we found a correlation of performance on the cognitive scales with hippocampal volume preservation in our study population. Reduced neuroinflammation was suggested via DTI imaging (MD) in the cingulate cortex, an area that shows volume loss in early AD^{44,45}, consistent with one of the principal mechanisms of action of laromestrol. Previous evidence indicates that MSCs and their potential therapeutic effectors (for example exosomes) are active in animals and humans for at least 7–10 days^{46–49}, 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 LS means between laromestrol and placebo obtained from a MMRM (F-statistic). No adjustment for multiple comparisons was made. Soluble TIE2 levels are represented as raw CFB and were reduced in treatment groups compared to placebo, particularly during the treatment period (to week 16) and reached statistical significance in group 3 at week 4 ($P = 0.010$; $n = 13$), week 8 ($P = 0.020$; $n = 12$) and week 16 ($P = 0.045$; $n = 12$). TIE2 levels were significantly increased in the placebo group ($n = 11$) at week 26 compared to baseline ($P = 0.047$; $n = 11$). Error bars represent $\pm$ s.e.m.

in studies of MSC administration to patients with congestive heart failure^{50,51} and aging frailty²⁹.

We sought evidence of vascular target engagement, and found a statistically significant increase in levels of circulating sTIE2 in the placebo group that were offset by laromestrol. Consistent with previous studies, the reduced TIE2 levels we 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^{41,52–54}. Indeed, the increased TIE2 levels we observed at week 26 are supportive of a role for TIE2 degradation in the pathology of AD. In principle, our findings are consistent with a provascular/anti-inflammatory effect of laromestrol, mediated by circulating secreted factors including but not limited to protein mediators and exosomes^{48,55} in preserving TIE2 signaling in brain vascular endothelial cells⁵⁶. 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 laromestrol 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 laromestrol or indirect due to improved clearance of amyloid⁵⁷ through preserved BBB function. New studies are planned to distinguish between these possibilities.

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 toward 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, vMRI 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 laromestrocel, as potency may depend partly on the bone-marrow donor. Further research on defining potency of laromestrocel is underway^{11,12,25}.

This study had limitations that warrant mentioning. First, there was a relatively small sample size with a high proportion of 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 end point in small studies for AD, the CADS used here is not a validated composite end point and it should be assessed with future studies.

Taken together, the findings of this study suggest that treatment of patients with mild AD with laromestrocel is safe, administered in both single and multiple dosing regimens. New studies using laromestrocel in the treatment of mild AD will examine the safety and efficacy profile of extended treatment durations and follow-up periods with greater statistical power, and we hypothesize longer treatment duration would slow AD progression further. The safety results presented here, coupled with signals of potential efficacy, support ongoing clinical development of this novel class of therapeutics for AD.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-025-03559-0>.

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Methods

Study design and enrollment

The CLEAR-MIND study entitled ‘Lomestrel-B Effects on Alzheimer’s disease: A Randomized, Double-Blinded, Placebo-Controlled Phase 2a Trial’ (ClinicalTrials.gov registration: [NCT05233774](https://clinicaltrials.gov/ct2/show/study?term=NCT05233774)) was conducted using our trial protocol under US FDA IND no. 16524, which is available in the trial master file. Institutional Review Board (IRB) approval was granted by Western-Copernicus Group Institutional Review Board (WCG IRB) on 24 November 2021. The following items were approved at that time: Lomestrel-B Drug Brochure (31 August 2023) Protocol v.2.0 (9 November 2021) and Template Consent Form. The primary goal of the study was to demonstrate the safety of intravenous delivery of single- or multiple-dose lomestrel (Lomestrel-B) administered to patients with mild AD. The secondary objective of the study was to identify efficacy signals in single- or multiple-dose lomestrel versus placebo in the patient population. Efficacy was measured in three domains: cognitive function, QoL and brain architecture measured with vMRI. Patient enrollment occurred at ten clinical centers in South Florida from Martin County to Miami-Dade County between 29 December 2021 and 21 November 2022, with final follow-up on 21 August 2023. All patients provided written consent on the Western IRB-approved protocol. An independent Data and Safety Monitoring Board (DSMB) was responsible for safety and oversight, and recommended study continuation after each planned meeting. Randomization was performed electronically using block sizes of four, equally allocated, with each site receiving separate blocks. Randomization was additionally stratified by sex. Patients were randomized using a 1:1:1:1 ratio into each of four parallel treatment groups. The clinical research organization (CRO), Biorasi, generated the random allocation sequence and assigned patients to intervention groups, while patients were enrolled by the clinical sites. Group 1 patients ($n = 12$) received four infusions of placebo on day 0, weeks 4, 8 and 12. Group 2 patients ($n = 13$) received an infusion of lomestrel at a dose of 25×10^6 cells (25 million) on day 0, followed by placebo infusions at weeks 4, 8 and 12. Group 3 patients ($n = 13$) received four infusions of lomestrel 25 M on day 0, weeks 4, 8 and 12. Group 4 patients ($n = 11$) received four infusions of lomestrel at a dose of 100×10^6 cells (100 million) on day 0, weeks 4, 8 and 12. Stopping rules were used to indicate boundaries requiring DSMB discussion and to assist the DSMB in its duties. The stopping rules were based on continuous monitoring for repeated testing for SAEs using a Pocock-type boundary. We chose to consider the treatment group safe if the proportion of patients with unexpected and possibly related SAEs within 4 weeks of any infusion was 10% or less. Three SAEs occurred during the study and were unrelated to the treatment. The stopping rules were not triggered during the study and the trial was completed.

Lomestrel and placebo sourcing and manufacturing

Lomestrel is an investigational allogeneic adult human MSC therapy, consisting of a suspension of human bone-marrow-derived MSCs^{27,29,48,58}. Lomestrel is obtained from bone-marrow aspirates from the iliac crest of unrelated and human leukocyte antigen (HLA)-unmatched healthy adult donors (ages 18–45 years). Marrow donor characteristics and cell count are provided in Supplementary Table 13. All bone-marrow collections are regulated under 21 CFR 1271, Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/PS). All donors are screened in accordance with 21 CFR 1271 Subpart C Donor Eligibility. The material is shipped to Longeveron for processing under aseptic conditions for further processing in a GMP facility. Lomestrel MSCs were isolated from bone marrow using density gradient separation.

Lomestrel is culture expanded in complete medium (α -minimum essential medium (Gibco), containing FBS (Corning)) and grown to approximately 85–90% confluency in multilayer vessels. At each passage, lomestrel is trypsinized, pooled, subjected to cell

count and viability assessments and multilayer vessels are then seeded. All passages utilize the same complete medium. All passages must have cell viability $\geq 70\%$. At collection, conditioned medium (supernatant) samples are collected and frozen at -80°C , then lomestrel is trypsinized, pooled, subjected to cell count/viability assessment and filled at specific concentrations into CS50 cryobags in a suspension containing a cryoprotectant composed of Hesperan, HSA and dimethylsulfoxide (DMSO). Lomestrel is frozen utilizing controlled rate freezers and stored at $\leq -135^\circ\text{C}$ in vapor-phase LN_2 freezers. Each lot of lomestrel is tested to confirm MSC identity and to ensure all critical quality attributes meet product specifications (Supplementary Table 13). If all specifications are met, the cryopreserved product is released. Identity confirmation is performed via immunophenotyping via flow cytometry. Lomestrel lots must be positive for cell-surface markers, CD105, CD90 and CD73 ($\geq 95\%$). Lomestrel must be negative ($\leq 2\%$) for CD45, CD11b, CD19, HLA-DR isotype HLA-DR (MHC II cell-surface receptor) and ($\leq 5\%$) for CD34 (Extended Data Fig. 2). Lomestrel must be negative for *Mycoplasma*, adventitious viruses via culture on three cell types and negative for Parvo B19, HIV 1, HIV 2, hepatitis B, hepatitis C, HTLV 1, HTLV 2, CMV and EBV by PCR, pass USP 71 sterility with no growth and have post-thaw viability $\geq 70\%$.

At the time of infusion, the IP is prepared for administration by Longeveron or a qualified third-party facility. As such, the cryobag containing lomestrel cryopreserved drug product is thawed in a $37 \pm 1^\circ\text{C}$ water bath. The thawed cell suspension is diluted with medium containing Plasmalyte-A supplemented with human serum albumin and transferred to an appropriate container. A sample of the cell suspension is counted to determine cell count and viability. The viability must be greater than or equal to 70%. The calculated number of viable cells required to deliver the dose are resuspended and the cell suspension is added to the infusion bag for administration to the patient. The prepared lomestrel final formulation ready for infusion is maintained at $2-8^\circ\text{C}$ in a container validated to hold refrigerated temperatures for up to 48 h. Stability testing is performed on all lots of lomestrel to ensure that infusion product meets viability criteria for a minimum of 8 h. The formulated product for administration is released for patient use following gram stain and endotoxin testing in addition to the cell count and viability.

Flow cytometry

The identity of MSCs was confirmed by performing immunophenotyping through flow cytometry according to standard methods. In brief, cells were thawed using thaw medium; cell counts and viability were analyzed using a cell counter (NucleoCounter; NC-200; ChemoMetec). Cells were washed with flow staining buffer (cat. no. 00422226, Thermo Fisher) and centrifuged. After centrifugation, cell pellets were resuspended in flow buffer. For the blocking step, cells were added to each tube with appropriate antibodies and incubated in the dark at $2-4^\circ\text{C}$. Cells were labeled with live/dead dye; 7-AAD (cat. no. 559925 or cat. no. 00-6993-50; Invitrogen lots 3115592, 1095376 and 2238457); negative controls included: FITC IgG1 (cat. no. 556649, clone MOPC-21, lots 7072907, 7179618, 7165880 and 8274649), PE IgG1 (cat. no. 556650, clone MOPC-21, lots 7165880, 9024919 and 1060595), PCP5.5 IgG1 (cat. no. 552834, clone MOPC-21, lots 9282777 and 1193612 or cat. no. 400149, lot B272763 or cat. no. 400251, lot B236966), APC IgG1 (cat. no. 550854, clone MOPC-21, lots 7215834, 7215839, 9038519 and 2005498). The live/dead and all isotype control IgG1 antibodies were used undiluted at $20 \mu\text{l}$. All antibodies were used undiluted and were purchased from BD Biosciences or BioLegend. The cells were labeled with cell-surface marker antibodies for CD105 PE (cat. no. 560839, clone 266, lots 9155633 and 8137932, $5 \mu\text{l}$), CD90 FITC (cat. no. 555595 or cat. no. 328108, clone SE10, lots B2053317, B241990, B205312 and B291322, $5 \mu\text{l}$), CD73 APC (cat. no. 560847 or cat. no. 344006, lot B252653 or cat. no. 344005, lot B218129, clone AD2, $5 \mu\text{l}$), CD45 FITC (cat. no. 555482 or cat. no. 560976, clone HI30, lots 9077614, 1285678,

7058663 and 8232661, 20 µl), CD34 PE (cat. no. 560941, clone 581, lots 7033536, 203612 and 8221866, 20 µl), CD19 PE (cat. no. 555413 or cat. no. 561741 clone HIB19, lots 8081579, 6124709 and 6124705, 20 µl), CD11b PE (cat. no. 561001, clone ICRF44, lots 6251566 and 0066711, 20 µl) and HLA-PC5.5 (cat. no. 560652, clone G46-6, lots 7152686, 8159976, 30984 and 1193612, 5 µl). Unstained cells served as an additional experimental control. After the incubation step, cells were washed with flow cytometry staining buffer solution and centrifuged. Cell pellets were resuspended in flow cytometry staining buffer. All samples were run in triplicate. All data were collected on the CytoFLEX V5-B5-R3 flow cytometer using CytExpert software (Beckman Coulter) and analyzed by FlowJo (Tree Star) (Extended Data Fig. 2).

Exploratory testing of laromestrol conditioned medium for bioactive effectors

Conditioned medium samples acquired at the time of collection were tested for the presence of potential bioactive secreted proteins, including TIMP2 (by ELISA; Abcam cat. no. 100653, using lots 1012154 and 1065326 following the manufacturer's instructions) and VEGF-A, VEGF-D, PIGF, TIE2, interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10, IL-12/p70, IL-13, IL-1 β , TNF and IFN γ (V-PLEX and V-PLEX Plus Angiogenesis Panel 1 (Human) kits; cat. no. 15190G, 15190D and V-PLEX Plus Proinflammatory Panel 1 Human kit cat. no. K15049G-1 from MSD), according to the manufacturer's instructions. TIMP2 concentrations in the samples (in ng ml⁻¹) were further normalized to the cell density at the time of laromestrol collection (cells per ml) and represented as ng per cell. MSD analyses were performed using an MSD instrument (MESO QuickPlex SQ 120MM) and DISCOVERY WORKBENCH v.4.0 software. (Supplementary Table 14).

Patient population

Eligible patients had a clinical diagnosis of mild AD in accordance with the National Institute of Aging and the Alzheimer's Association criteria at the time of enrollment. Additionally, a positron emission tomography (PET) scan using a US FDA-approved tracer (for example, florbetapir-fluorine-18 (AMYVID), Vizamyl or Neuraceq) was required, consistent with the diagnosis of AD. Further criteria included APOe4 positivity, MMSE-2 score of 18–24, age 60–85 years, presence of an adult caregiver willing and able to participate in the study and accompany the patient to all study visits and Brain MRI consistent with AD, excluding any other brain abnormalities that can cause dementia (such as stroke, mass lesions or hydrocephalus). Patients were excluded with any other neurodegenerative disease, history of seizure disorder or evidence of previous macro hemorrhages.

Study procedures and timelines

Screening activity was performed within 6 weeks before the first infusion treatment and included informed consent, medical history and physical examination collection, ECG, clinical assessments including MMSE-2 (ref. 59), ADAS-Cog-13 (ref. 60), CDR-SB⁶¹, QoL-AD⁶², ADCS-ADL⁶³, ADRQL⁶³, Neuropsychiatric Inventory (NPI)²⁹, specimen collection for safety labs, biomarker samples and urine sample analysis. Informed consent was obtained for all study participants. Participants were not compensated for their participation in the study. Only self-reported sex categories were collected during the study and included the categories 'male' and 'female'. This was used as a stratification factor as AD affects women disproportionately compared to men. No other sex analysis was performed at screening. Imaging via MRI and amyloid PET scan completed the screening procedure⁶⁴.

The baseline visit activity was performed within 4 weeks before 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, laromestrol or placebo (PlasmaLyte containing HSA) were administered via peripheral intravenous infusion over 40 min. Before infusion a review of concomitant medications and AEs 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.

Study end points

Primary end point. To ensure safety, the patients were monitored after each infusion of laromestrol (for example, on day 0 and weeks 4, 8 and 12). The safety assessments were measured by evaluating the incidence of any AEs and SAEs within 4 weeks of infusion; any changes in blood and urine sample analysis; and any ARIAs or clinically asymptomatic microhemorrhages as revealed by MRI in the study. The primary end point was the difference from placebo in the rate of TE-SAEs occurring within 4 weeks of any infusion. The TE-SAE criteria employed in this trial captured all SAEs between the first infusion and end of study.

Secondary end point. To analyze the efficacy of treatment, a CADS assessment was calculated at baseline and weeks 16, 26, and 39. The CADS was evaluated at each visit by averaging equally weighted z-scores for the following components: ADCS-ADL total score, CDR-SB total score (inverted), overall ADAS-Cog-13 total score (inverted) and left hippocampal volume (normalized for intracranial volume). The estimated marginal mean CADS at week 39 was used as our secondary end point in comparing each laromestrol group versus placebo as well as the combined laromestrol groups versus placebo.

Exploratory end points. The neurocognitive, neuropsychiatric, QoL, activities of daily living and Canadian Study of Health and Aging Clinical Frailty Score (CSHA-CFS) assessments described above were performed at screening, baseline and weeks 16, 26 and 39 to assess the CFB. In addition, biomarkers were assessed as exploratory efficacy measurement: serum-based biomarkers related to potential provascular and anti-inflammatory activities of laromestrol, as well as AD progression, were measured at screening, baseline, day 0 and weeks 4, 8, 12, 16, 26 and 39. A vascular-related biomarker, sTIE2, was evaluated based on previous studies that implicate laromestrol as having the potential to inhibit the cleavage of TIE2 from the cell membrane, and as such, offsetting its inactivation^{41,52–54}. sTIE2 was analyzed using an MSD electrochemiluminescence immunoassay machine (1300 MESO QuickPlex SQ120) and angiogenesis panel kit (MSD; cat. no. K15190D-1). Quantification was completed using a MSD V-Plex Assay with duplicate samples per patient visit.

Brain volumetry was performed via MRI at screening and weeks 16, 26 and 39, to assess volumetric changes in the hippocampus, overall brain size, ventricular volume and other brain structures, each normalized for intracranial volume (refs). DTI via MRI was conducted at screening and weeks 16, 26 and 39, to assess for changes in neuroinflammation. DTI scalars include fractional anisotropy (FA)⁶⁵, mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD)⁶⁶. Furthermore, the neurocognitive, neuropsychiatric, QoL, activities of daily living and Canadian Study of Health and Aging Clinical Frailty Score assessments were performed at screening (excluding MoCA), baseline (excluding MMSE-2) and weeks 16, 26 and 39 to assess the CFB.

vMRI and PET imaging

Independent central imaging services were supported by Bioclinica, a Clario company. Bioclinica developed imaging protocols, trained the imaging centers, performed quality assurance on all images and completed the blinded data analysis. Brain MRIs were performed using 3T (Tesla) scanners, two imaging centers were used by the ten clinical centers, one MRI scanner at each location was used for the entire trial.

Whole and regional brain volumes were calculated via MRI, including hippocampal and ventricular volume, each normalized for intracranial volume, and collected at screening and weeks 16, 26 and 39. The DTI indices (FA, AD, RD and MD) were calculated from the native DTI acquisition. Whole-brain vMRI scans were used in this study, with atlas-based subregion identification. For vMRI and atrophy analysis, all 3DT1 scans were N4 bias-corrected, registered to a 256-mm³ template space and isotropically resampled to achieve a voxel size of 1 mm³. Preprocessed 3DT1 scans were then analyzed in FreeSurfer 6 to generate baseline regional volume measures of all available cortical/subcortical, white matter and ventricular regions. For atrophy measures, follow-up imaging time points were registered with baseline scans to a log-symmetric midway space for tensor-based morphometry analysis. All volume and atrophy measures are reported as either % CFB or as regional/intracranial volume ratio using raw non-normalized units (mm³) as applicable. For 3DT1 MPAGE, parameters included sagittal acquisition with 176 slices, a field of view of 240 × 240 mm; slice thickness of 1.2 mm; TR/TE of 1,800ms/2.49 ms; TI = 900 ms; flip angle of 10 degrees; and a matrix size of 192 × 192.

PET⁶⁴ imaging was collected at screening using a USFDA-approved tracer (for example, florbetapir-fluorine-18 (AMYVID), Vizamyl or Neuraceq) consistent with the diagnosis of AD. A previous positive PET scan was allowed with sponsor approval.

DTI imaging

DTI parameters were axial spin echo; echo planar imaging acquisition with 80 slices; field of view = 232 × 232 mm; slice thickness 2 mm; phase encode direction, AP, TR/TE, min/min (but still T2-weighted, dependent on scanner performance); matrix size, 116 × 116; bandwidth, 1,486 Hz/Px; directions, 30; b-value = 1,000 s mm⁻²; and 2 b0 averages. In addition, a DTI b0 reverse sequence with identical parameters (including TR/TE/BW) but with phase encode direction, PA.

A standardized DTI sequence (2 mm³ voxel size, 30 directions, b-value = 1,000 s mm⁻²) was deployed to each site in conjunction with a reverse-phase encode DTI sequence (blip down) with identical contrast parameters (TR/TE/bandwidth) for distortion correction. Scans were received and quality controlled to ensure adherence to approved acquisition settings. Pre-processing included eddy and motion correction that was performed using a linear affine transformation on all gradient images with the baseline image of b = 0 s mm⁻². The reverse encoding DTI image (blip down acquisition) was employed for correcting the distortions in the main DTI image (blip up acquisition) using DRBUDDI, which is based on symmetric diffeomorphic registration to estimate the distortion field. Diffusion tensors were then computed using a standard LS approximation and the scalar maps of FA, MD, axial diffusivity and RD were derived from the tensor image. The FA maps were then registered to the FreeSurfer processed 3DT1 image in standard 256 × 256 × 256 space and the deformation was applied to remaining maps (MD, AD and RD). Finally, the FA image was registered to The Johns Hopkins University (JHU) atlas's FA image using advanced normalization tools (ANTs). Symmetric normalization (SyN) deformable registration and deformation were applied to all the maps. This allowed for a seamless overlay of cortical, subcortical and white matter labels for regional value extraction. For each subject, the registrations and overlays were checked manually and reprocessed or rejected in case of failures^{65,66}.

Statistical analysis

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 end point, 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 (25 M × 1), group 3 (25 M × 4) and group 4 (100 M × 4)) as well as the laromestrol pooled 2, 3 and 4 group

(25 M × 1, 25 M × 4, 100 M × 4) and the laromestrol pooled 3 and 4 group (25 M × 4, 100 M × 4). Additional safety parameters (laboratory, vital signs and ECGs) were summarized descriptively by treatment group and assessment time.

The secondary end point, CADS, was calculated by combining z-scores for CFB (to study end point) 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 (each equally weighted). Inverted measures of the ADAS-Cog-13 and CDR-SB were used to match directionality of the other measures with respect to improvement/decline changes. Change from baseline in CADS was statistically analyzed with an 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. LS means, s.e. and 95% CIs for mean CFB were obtained from the model for each treatment group at each visit, including the pooled laromestrol treatment group. The LS-mean differences, s.e., 95% CIs and two-sided *P* values for the differences between treatment groups were obtained for each active-treatment group (groups 2, 3 and 4) relative to placebo (group 1) at each visit. The pooled laromestrol 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.

Powering was performed based on the results of a completed phase 1 study of laromestrol²⁷. 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 laromestrol treatment group and placebo at 85% power. During the course of the study, the key secondary end point was amended in the trial protocol to the CADS score. A protocol amendment was approved by the US FDA before unblinding, guided by the practice of adopting composite end points for AD; however, assuming a two-sided α of 0.1, the power to detect a 50% slowing of disease progression based on CADS comparing three active arms combined (*n* = 36) versus 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.

The ITT population included all randomized patients who received at least one full or partial dose of IP (any infusion of either laromestrol or placebo). The mITT population included all patients who were randomized and received at least one full or partial dose of the IP (any infusion of either laromestrol or placebo) and completed at least one post-baseline efficacy assessment (biomarker data or cognitive test).

Similar MMRM analyses were conducted for the exploratory efficacy end points of ADAS-cog-13, MMSE-2, ADCS-ADL, CDR-SB, MoCA, Neuropsychiatric Inventory (NPI), QoL-AD (both caregiver and study participant), ADCS-ADL, ADRQL, brain volumetry (via MRI) and DTI.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All requests for data access should be sent to the corresponding authors, B.G.R. or J.M.H. The minimum dataset, without individual patient data, used for the primary, secondary and exploratory conclusions, may be shared under a data-use agreement for IRB-approved research. Requests will be considered and responded to within 1 month of receipt. The trial protocol and statistical analysis plan under US FDA IND no. 16524 can be shared upon academic or research request.

Code availability

SAS v.9.4 was used for all analyses included in the manuscript. SAS code for the minimum dataset is on file and can be made available under a code use agreement for IRB-approved research. Requests for this code should be sent to the corresponding authors, B.G.R. or J.M.H. Code for volumetric MRI and DTI image analysis was generated by Clario and is not publicly available; special requests for this code can be directed to Clario.

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Author contributions

B.G.R., K.N.R., N.A., J.B. and J.M.H. wrote the paper. A.A.O., K.N.R. and J.M.H. designed the trial. B.G.R., K.N.R., N.A., E.N., Z.Z., A.O.O., J.B. and J.M.H. analyzed data and prepared figures. K.N.R. and A.A.O. interacted with the CRO, Biorasi, Imaging CRO, Clario and managed the trial. A.A.O. and E.N. interacted with the statistical consulting group, Pentara Corporation, to develop the secondary end point. L.M.M. supervised the manufacturing of laromestrol and provision to clinical sites. B.G.R., Z.Z., S.K. and B.V. performed and analyzed the TIMP2 ELISA or MSD experiments. K.P., M.B. and T.L. performed laboratory and manufacturing work. R.C., P.P., M.B., B.H., A.F., S.R., A.F.J., R.L., M.P. and J.B.S. enrolled the patients at clinical sites. J.M.H. supervised the project and provided the intellectual property.

Competing interests

J.M.H. reports having a patent for cardiac cell-based therapy and holds equity in Vestion and maintains a professional relationship with Vestion as a consultant and member of the Board of Directors and Scientific Advisory Board. Vestion did not play a role in the design, conduct, or funding of the study. J.M.H. is the Chief Scientific Officer, a compensated consultant and board member for Longeveron and holds equity in Longeveron. J.M.H. is also the co-inventor of intellectual property licensed to Longeveron. The University of Miami is an equity owner in Longeveron, which has licensed intellectual property from the University of Miami. B.G.R., N.A., L.M.M., Z.Z., B.V., K.P., M.B. and T.L. are active employees of Longeveron. The other authors declare no competing interests.

Additional information

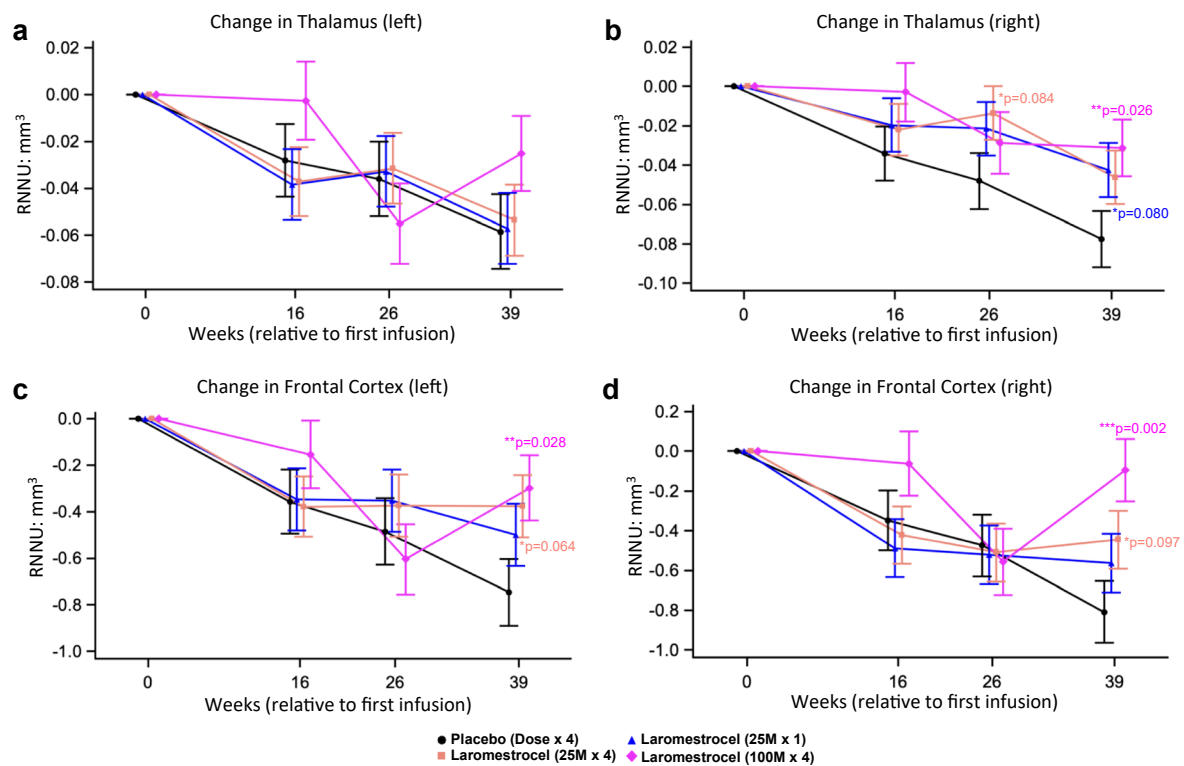
Extended data is available for this paper at <https://doi.org/10.1038/s41591-025-03559-0>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41591-025-03559-0>.

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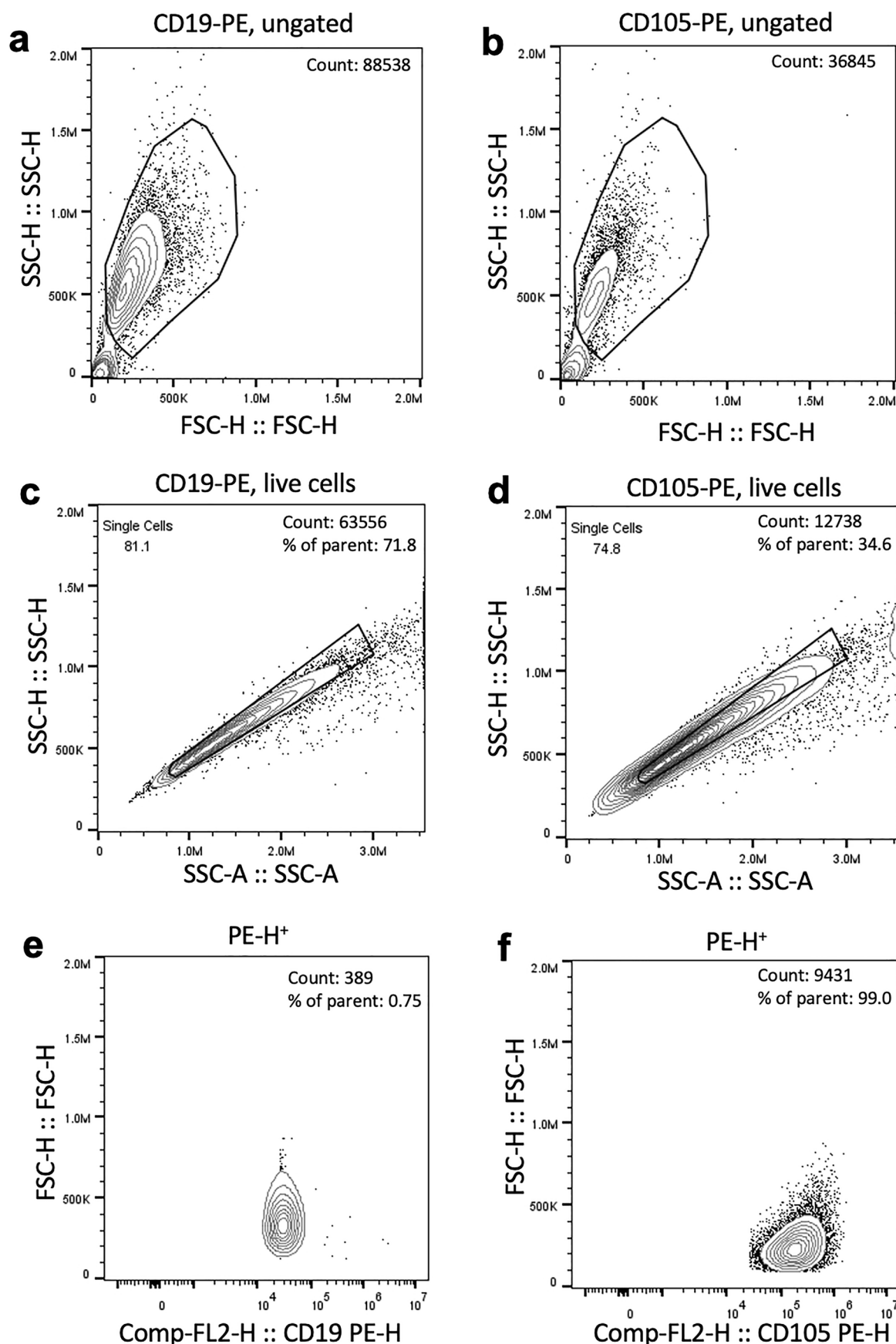
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Extended Data Fig. 1 | Volumetric MRI assessments of thalamus and frontal cortex of AD patients treated with laromestrol vs placebo. Two-sided p values are associated with testing for statistically significant differences in LS means between laromestrol and placebo obtained from a MMRM (F-statistic). No adjustment for multiple comparisons was made. Statistically significant slowing of right thalamus atrophy compared with placebo (N = 10) was observed

in Group 4 at week 39 ($p = 0.026$; N = 10), with numeric slowing in Groups 2 and 3, while no statistically significant change was observed in left thalamic volume (**a, b**). Group 4 showed statistically significant slowing of frontal cortical atrophy on both the left ($p = 0.028$) and right sides ($p = 0.002$; N = 10) compared with placebo (N = 10) (**c, d**). Error bars represent $\pm$ SEM.



Extended Data Fig. 2 | Example flow cytometry workflow for CD19 (negative marker) and CD105 (positive marker) for identity assessment of laromestrocel. Ungated cells (**a**, **b**) were subcategorized through detection of live cells using the live/dead marker 7-AAD (**c**, **d**). Single stained cells were distinguished by

gating 0.01% of non-stained cells (**e**, **f**). Laromestrocel lots must be positive for cell surface markers, CD105, CD90, and CD73 ($\geq 95\%$). Laromestrocel must be negative ($\leq 2\%$) for CD45, CD11b, CD19, and $\leq 5\%$ for CD34. Abbreviations: SSC-H, side scatter height; FSC-H, forward scatter height; SSC-A, side scatter area.

Reporting Summary

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| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
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| ☐ | ☒ A description of all covariates tested |
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| ☐ | ☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

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Software and code

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Data collection	FreeSurfer 6
Data analysis	SAS 9.4 was used for all analyses included in the manuscript. SAS code is on file and can be made available according to our code availability statement (below): Code availability SAS version 9.4 was used for all analyses included in the manuscript. SAS code for the minimum dataset is on file and can be made available under a code use agreement for IRB approved research. Requests for this code should be sent to the corresponding authors, BGR or JMH. Code for volumetric MRI and DTI image analysis was generated by Clario and is not publicly available; special requests for this code can be directed to Clario.

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- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Data availability

Please send all requests for data access to the corresponding authors, BGR or JMH. The minimum dataset, without individual patient data, used for the primary, secondary, and exploratory conclusions, may be shared under a data use agreement for IRB approved research. Requests will be considered and responded to within 1 month of receipt. The trial protocol and statistical analysis plan under US FDA IND # 16524 can be shared upon academic or research request.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Sex and gender information was self-reported by study participants and recorded within the EDC. Informed consent was obtained for all study participants. Participants were not compensated for their participation in the study.

Only the self-reported sex categories were collected during the study and included the categories "male" and "female." This was used as a stratification factor as AD affects women disproportionately compared to men. No other sex analysis was performed.

Reporting on race, ethnicity, or other socially relevant groupings

Race and ethnicity information was self-reported by study participants and recorded within the EDC.

Population characteristics

A total of 120 patients with a clinical diagnosis of mild AD were screened and 50 were enrolled. Eligible patients had a clinical diagnosis of mild Alzheimer's Disease in accordance with the National Institute of Aging and the Alzheimer's Association (NIA-AA) criteria at the time of enrollment. Additionally, a Positron Emission Tomography (PET) scan using a Food and Drug Administration (FDA) -approved tracer (e.g., Florbetapir-fluorine-18 (AMyViD), Vizamyl, or Neuraceq) was required, consistent with the diagnosis of AD. Further criteria included APOE4 positivity, Mini-Mental State Evaluation (MMSE-2) score of 18-24, age 60-85 years, presence of an adult caregiver willing and able to participate in the study and accompany the patient to all study visits and Brain Magnetic Resonance Imaging (MRI) consistent with AD, excluding any other brain abnormalities which can cause dementia (such as stroke, mass lesions or hydrocephalus). Patients were excluded with any other neurodegenerative disease, history of seizure disorder or evidence of prior macro hemorrhages. Sex was used as a covariate in all mixed effects repeated measures models.

Recruitment

Patient enrollment occurred at 10 clinical centers in South Florida from Martin County to Miami-Dade County between December 29, 2021, and November 21, 2022, with final follow-up on August 21, 2023. All patients provided written consent on the Western Institutional Review board- approved protocol. An independent Data and Safety Monitoring Board (DSMB) was responsible for safety and oversight, and recommended study continuation after each planned meeting. There are no known self-selection or other recruitment biases that could impact study results; the study employed a double blind, placebo-controlled, randomized design. 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.

Ethics oversight

The CLEAR-MIND study entitled "Lomecel-B Effects on Alzheimer's disease: A Randomized, Double-Blinded, Placebo-Controlled Phase 2a Trial" (ClinicalTrials.gov registration: NCT05233774) was conducted using our trial protocol under US FDA IND # 16524, which is available in the trial master file. Protocol granted by U.S. Food and Drug Administration, under IND 16524. IRB approval was granted by WCG IRB on 11/24/2021. The following items were approved at that time: Lomecel-B Drug Brochure (08-31-2023) Protocol Version 2.0 (11-09 2021) and Template Consent Form.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Assuming a two-sided alpha of 0.1, the power for the CADS comparing 3 active arms combined (N=36) vs placebo (N=12) at 50% slowing is 0.14 at 3 months or 0.36 at 6 months (insufficient 9-month data). This study was not powered for statistical significance of the CADS endpoint, however effect sizes as small as 0.2-0.3 may be considered clinically meaningful.
Data exclusions	The mITT population was used for all analyses described within the manuscript. The Modified Intention-to-Treat Population (mITT) includes all patients who are randomized and received at least one full or partial dose of the investigational product (any infusion, either Lomecel-B or placebo) and completed at least 1 (one) post-baseline efficacy assessment (biomarker data or cognitive test).patient
Replication	To ensure reproducibility of the findings, the trial firstly implemented a randomized, double-blind, placebo-controlled design. Secondly, for the core prespecified analysis (primary, secondary, key secondary, exploratory analyses, laboratory, and imaging data), Longeveron used a validated data set of tables, figures, and listings (TFL), which was reproduced by two independent biostatisticians at our contracted CRO, Biorasi. We are not aware of any failures in reproducing the results. Additionally, we have implemented several rounds of QC of the manuscript to ensure accuracy of all statistics, figures, and tables, in correspondence with the tables, figures, and listings associated with and contained within the clinical study report. These numbers have been independently verified by multiple individuals at Longeveron, including our clinical biostatisticians. A small number of typeos were found and corrected.
Randomization	This was a placebo-controlled, double-blinded trial. Patients were randomized using a 1:1:1:1 ratio of each active treatment group (Group 2, 3 or 4) and placebo (Group 1). Randomization was performed in block sizes of 4 for each gender, and separate blocks per site.
Blinding	The patient and clinical teams were blinded regarding the treatment arms. Only the Investigational Product (IP) depot was unblinded. As applicable and when possible, biological samples were analyzed and evaluated by blinded staff or an independent third party.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☐	☒ MRI-based neuroimaging

Antibodies

Antibodies used	Cells were labeled with live/dead dye; 7-AAD (cat# 559925 or cat#00-6993-50; Invitrogen lots#3115592, 1095376, 2238457); negative controls included: FITC IgG1 (cat#556649, clone MOPC-21, lots#7072907, 7179618, 7165880, 8274649), PE IgG1 (cat#556650, clone MOPC-21, lots#7165880, 9024919, 1060595), PCP5.5 IgG1 (cat#552834, clone MOPC-21, lots# 9282777, 1193612 or cat#400149, lot#B272763 or cat#400251, lot#B236966), APC IgG1 (cat#550854, clone MOPC-21, lots#7215834, 7215839, 9038519, 2005498). The live/dead and all isotype control IgG1 antibodies were used undiluted at 20uL. All antibodies were used undiluted and were purchased from BD Biosciences or BioLegend. The cells were labeled with cell surface marker antibodies for CD105 PE (cat#560839, clone 266, lots#9155633, 8137932, 5uL), CD90 FITC (cat#555595 or cat#328108, clone 5E10, lots#B2053317, B241990, B205312, B291322, 5uL), CD73 APC (cat# 560847, or cat#344006, lot# B252653 or cat#344005, lot# B218129, clone AD2, 5uL), CD45 FITC (cat#555482, or cat#560976, clone HI30, lots# 9077614, 1285678, 7058663, 8232661, 20uL), CD34 PE (cat# 560941, clone 581, lots#7033536, 203612, 8221866, 20uL), CD19 PE (cat# 555413 or cat#561741clone H1B19, lots#8081579, 6124709, 6124705, 20uL), CD11b PE (cat# 561001, clone ICRF44, lots#6251566, 0066711, 20uL), HLA-PC5.5 (cat#560652, clone G46-6, lots# 7152686, 8159976, 30984, 1193612, 5uL). TIMP2 ELISA kit Abcam cat# 100653 VEGF-A, VEGF-D, PIGF, TIE-2, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12/p70, IL-13, IL-1β, TNF-α, and IF-γ (V-PLEX and V-PLEX Plus Angiogenesis Panel 1 (Human) kits cat#15190G, 15190D, and V-PLEX Plus Proinflammatory Panel 1 Human Kit cat# K15049G-1 from Meso Scale Discovery (MSD), Maryland, USA),
Validation	All antibodies used in this study were commercially available, and have been stated to recognize the human analyte target for each intended assay application.¹ CD105 PE, cat#560839, clone 266 Description: The 266 monoclonal antibody specifically binds to CD105. CD105 is a type I transmembrane glycoprotein that is encoded by END (Endoglin) and belongs to the transforming growth factor-β (TGF-β) type III receptor family. CD105 is expressed on cells as a homodimer comprised of ~95 kDa subunits. CD105 is expressed on vascular endothelial cells and placental syncytiotrophoblasts and

at lower levels on stromal fibroblasts. It is also expressed on mesenchymal stem cells, erythroid precursors, activated macrophages, pre-B cells, and some tumor cells and cell lines including U937 cells. CD105 serves as a regulatory component of the TGF- β receptor system. In association with TGF- β RI or TGF- β RII, CD105 binds TGF- β 1 and TGF- β 3 with high affinity but does not bind to TGF- β 2. Expression of CD105 is increased on activated endothelium in tissues undergoing angiogenesis, such as in tumors, or in cases of wound healing or dermal inflammation.

Flow cytometric analysis of CD105 expression on human U937 cells. U937 cells (ATCC, Cat No.

CRL-1593.2) were stained with PE Mouse anti-Human CD105 antibody (Cat. No. 5060839; solid line histogram) or a PE mIgG1, κ isotype control (Cat. No. 554680; dashed line histogram). Flow cytometric fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable cells. Flow cytometry was performed using a BD LSRTM II flow cytometry system.

2. CD90 FITC, cat#555595, clone 5E10

Description: The 5E10 monoclonal antibody specifically binds to human CD90 which is also known as Thy-1. CD90 is a 25-35 kDa glycosphosphatidylinositol-anchored membrane glycoprotein of the Ig superfamily that is expressed on 1-4% of human fetal liver cells, cord blood cells, and bone marrow cells. The anti-CD90 antibody binds to a subset of immature CD34+ cells and a distinct subset of mature CD34- cells that are CD3+CD4+. The CD90+CD34+ population is highly enriched for cells capable of long-term culture. The anti-CD90 antibody is useful for enriching high proliferative potential colony-forming cells (HIPP-CFC) that are primitive progenitor cells.

Flow cytometric analysis of CD90 expression on HEL

cell line. HEL cells were stained with either FITC Mouse IgG1, κ Isotype Control (Cat. No. 555748; dashed line histogram) or FITC Mouse Anti-Human CD90 (Cat. No. 555595/561969; solid line histogram). Fluorescent histograms were derived from gated events with the side and forward light-scatter characteristics of viable HEL cells.

or cat#328108, clone 5E10

Human Erythroleukemic cell line (HEL) stained with 5E10 FITC

3. CD73 APC, cat# 560847 Clone AD2

Description: The AD2 monoclonal antibody specifically binds to ecto-5'-nucleotidase, a 70 kDa, glycosyl phosphatidylinositol (GPI)-anchored glycoprotein. CD73 is expressed on subsets of T and B lymphocytes, follicular dendritic cells, epithelial cells, endothelial cells and mesenchymal stem cells. Its expression on lymphocytes increases during T and B cell development. CD73 has enzymatic activity and catalyzes the dephosphorylation of adenosine monophosphate (AMP) converting it to adenosine. It has been suggested that CD73 can mediate costimulatory signals in T cell activation and adhesion of lymphocytes to endothelium.

Flow cytometric analysis of CD73 on human lymphocytes. Whole blood was stained with APC Mouse anti-Human CD73 (Cat. No. 560847; solid line fluorescence histogram) and compared with whole blood stained with APC Mouse IgG1, κ Isotype Control (Cat. No. 554681; used at a matching concentration; dashed line histogram). The erythrocytes were lysed with BD PharmLyse™ Lysing Buffer (Cat. No. 555899). Flow cytometric fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD LSRTM II flow cytometry system.

OR CD73 cat# 344002 Clone AD2

Human peripheral blood lymphocytes stained with AD2 PE and CD3 APC

Human peripheral blood lymphocytes stained with AD2 PE and CD19 APC

4. CD45 FITC, cat#555482, 560976 clone HI30

Description: The HI30 monoclonal antibody specifically binds to the 180, 190, 205, 220 kDa protein isoforms of CD45. CD45 is encoded by the PTPRC (Protein tyrosine phosphatase receptor type C) gene. CD45, also known as the leukocyte common antigen (LCA), is a member of the protein tyrosine phosphatase (PTP) family. It is present on all human leukocytes including lymphocytes, monocytes, granulocytes, eosinophils, and thymocytes. CD45 is absent from circulating erythrocytes, platelets, or mature erythroid cells of bone marrow and non-hemopoietic tissues.

Flow cytometric analysis of CD45 expression on human peripheral blood lymphocytes. Human whole blood was stained with either FITC Mouse IgG1, κ Isotype Control (Cat. No. 555748; dashed line histogram) or FITC Mouse Anti-Human CD45 antibody (Cat. No. 555482/560976/561865; solid line histogram). The erythrocytes were lysed with BD FACSTM Lysing Solution (Cat. No. 349202). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of intact lymphocytes.

5. CD34 PE, cat# 560941, clone 581

Description: The 581 monoclonal antibody specifically binds to CD34, a sialomucin-like type I transmembrane glycoprotein. This single-chain, 105-120 kDa, heavily O-glycosylated protein is expressed on hematopoietic progenitor cells, vascular endothelium, bone marrow stromal cells and embryonic fibroblasts. The cytoplasmic region of the CD34 antigen is a target for phosphorylation by activated protein kinase C suggesting CD34 may play a role in signal transduction. CD34 may also play a role as an adhesion molecule

since it binds to CD62E and CD62L. Clone 581 binds to the class III CD34 epitope. It is resistant to neuraminidase, chymopapain and glycoprotease. The 581 antibody blocks reactivity of another anti-CD34 monoclonal antibody, 8G12.

Flow cytometric analysis of CD34 expression by human peripheral blood mononuclear cells. Human PBMCs were stained PE Mouse Anti-Human CD34 antibody (Cat. No. 555822/560941). Flow cytometric dot plot showing side-scattered light versus CD34 were derived from gated events based on the light scattering characteristics viable cells. Flow cytometry was performed on a BD FACScan™ system.

11. CD19 PE, cat# 555413 or cat#561741, clone HIB19

Description: The HIB19 monoclonal antibody specifically binds to the 95 kDa type I transmembrane CD19 glycoprotein. CD19 is expressed during all stages of B-cell maturation and differentiation, except on plasma cells. CD19 is also present on follicular dendritic cells. It is not found on T cells or on normal granulocytes. CD19 is a signal transduction molecule that regulates B cell development, activation, proliferation and differentiation. It associates with the complement receptor 2 (CD21), TAPA-1 (CD81), Leu 13, and/or MHC class II to form a signal transduction complex on the surface of B cells. Anti-CD19 clone HIB19 partially blocks the binding of clone B43, another CD19-specific monoclonal antibody.

Profile of peripheral blood lymphocytes analyzed on a FACScan (BDIS, San Jose, CA)

12. CD11b PE, cat# 561001, clone ICRF44

Description: The ICRF44 monoclonal antibody specifically binds to CD11b, the 165-kDa adhesion glycoprotein that associates with the 95-kDa integrin $\beta 2$ (CD18) to form the CD11b/CD18 complex, also known as Mac-1 or CR3. CD11b is a type I transmembrane glycoprotein that is encoded by ITGAM (Integrin alpha M). It is expressed on activated lymphocytes, monocytes, granulocytes, and a subset of NK cells. CD11b functions in cell-cell and cell-substrate interactions and is a receptor for iC3b, CD54 (ICAM-1), CD102 (ICAM-2) and CD50 (ICAM-3). This antibody significantly inhibits polymorphonuclear leukocyte aggregation in response to fMLP. This clone also cross-reacts with granulocytes, a subset of peripheral blood lymphocytes and some monocytes of baboon, and both rhesus and cynomolgus macaque monkeys. The distribution on lymphocytes and granulocytes is similar to that observed with peripheral blood from normal human donors. There are fewer CD11b-positive monocytes present in the non-human primate blood than in normal human donor samples.

Flow cytometric analysis of CD11b expression on human lymphocytes (Left Plot) or granulocytes (Right Plot). Human whole blood was stained with either PE Mouse IgG1, κ Isotype Control (Cat. No. 555749; dashed line histogram) or PE Mouse Anti-Human CD11b (Cat. No. 555388/561001/557321; bold line histogram). Erythrocytes were lysed with Pharm Lyse™ Lysing Buffer (Cat. No. 555899). Fluorescence histograms depicting CD11b (or Ig isotype control) expression were derived from gated events with the side and forward light-scattering characteristics of viable lymphocytes or granulocytes. Flow cytometry was performed on a BD FACScan™ system.

13. HLA-PC5.5 (cat#560652, clone G46-6

The G46-6 monoclonal antibody specifically binds to HLA-DR, a major histocompatibility complex (MHC) class II antigen. HLA-DR antigens are encoded by genes within the Human Leukocyte Antigen (HLA) Complex located on chromosome 6. HLA-DR is a transmembrane heterodimeric glycoprotein composed of an α chain (36 kDa) and a β subunit (27 kDa) expressed primarily on antigen presenting cells: B cells, dendritic cells, monocytes, macrophages, and thymic epithelial cells. HLA-DR is also expressed on activated T cells. This molecule plays a major role in mediating cellular interactions during antigen presentation to CD4-positive T cells.

Flow cytometric analysis of HLA-DR on human lysed whole blood. Human whole blood was lysed with BD FACSTM Lysing Solution (Cat. No. 349202) and stained with the PerCP-CyTM5.5 Mouse Anti-Human HLA-DR antibody (Cat. No. 560652/552764; unshaded histogram) or with a PerCP-CyTM5.5 Mouse IgG2a, κ isotype control (Cat. No. 550927; shaded histogram). Fluorescent histograms showing expression of HLA-DR (or Ig isotype staining) were derived from gated events based on forward and side light scattering characteristics for intact lymphocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry system.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Lomecel-B is an investigational allogeneic adult human mesenchymal stem cell (hMSC) therapy, comprised of a suspension of human bone marrow-derived mesenchymal stem cells^{27,29,48,58}. Lomecel-B is obtained from bone marrow aspirates from the iliac crest of unrelated and human leukocyte antigen (HLA)-unmatched healthy adult donors (ages 18-45). Marrow donor

characteristics and cell count are provided in Table S13. All bone marrow collections are regulated under 21 CFR 1271, "Human Cells, Tissues, and Cellular and Tissue Based Products (HCT/PS). All donors are screened in accordance with 21 CFR 1271 Subpart C Donor Eligibility. The material is shipped to Longevion for processing under aseptic conditions for further processing in a GMP facility. Lomecel-B MSCs were isolated from bone marrow using density gradient separation. The sex of each donor cell line was: LMSC024 (F) LMSC037 (M) LMSC040 (F) LMSC042 (M) LMSC045 (M) LMSC053 (M) LMSC055 (M) LMSC064 (F). See Table S13 and Materials and methods.

Authentication

Lomecel-B is culture expanded in complete medium (alpha-minimum essential medium (MEM; Gibco), containing FBS (fetal bovine serum; Corning)) and grown to approximately 85-90% confluency in multilayer vessels. At each passage, Lomecel-B is trypsinized, pooled, subjected to cell count and viability assessments, and multilayer vessels are then seeded. All passages utilize the same complete medium. All passages must have cell viability $\geq 70\%$. At harvest, conditioned media (supernatant) samples are collected and frozen at -80°C , then Lomecel-B is trypsinized, pooled, subjected to cell count/viability assessment and filled at specific concentrations into CS50 cryobags in a 20mL suspension containing a cryoprotectant composed of Hespan, HSA, and DMSO. Lomecel-B is frozen utilizing controlled rate freezers and stored at $\leq -135^{\circ}\text{C}$ in vapor phase LN2 freezers. Each lot of Lomecel-B is tested to confirm MSC (mesenchymal stem cell; also known as medicinal stem cell) identity and ensure all critical quality attributes meet product specifications (Table S13). If all specifications are met the cryopreserved product is released. Identity is performed via immunophenotyping via flow cytometry. Lomecel-B lots must be positive for surface cell markers, CD105, CD90, and CD73 ($\geq 95\%$). Lomecel-B must be negative ($\leq 2\%$) for CD45, CD11b, CD19, Human Leukocyte Antigen DR Isotype HLA-DR (MHC II cell surface receptor) and ($\leq 5\%$) for CD34. Lomecel-B must be negative for mycoplasma, adventitious viruses via culture on three cell types and negative for Parvo B19, HIV 1, HIV 2, Hepatitis B, Hepatitis C, HTLV 1, HTLV 2, CMV and EBV by PCR, pass USP 71 sterility with no growth and have post thaw viability $\geq 70\%$.

Mycoplasma contamination

All cell lines tested negative for mycoplasma (see Table S13).

Commonly misidentified lines (See [ICLAC](#) register)

n/a

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration NCT05233774

Study protocol The study protocol can be found in the trial master file at ClinicalTrials.gov

Data collection Patient enrollment occurred at 10 clinical centers in South Florida from Martin County to Miami-Dade County between December 29, 2021, and November 21, 2022, with final follow-up on August 21, 2023.

Outcomes The primary endpoint entails tabulation of the percentage of patients having SAEs within 4 weeks after any infusion with investigational product (Lomecel-B or placebo) by treatment group. Rate is calculated on subject-level (number and percentage of patients experiencing an SAE within 4 weeks of any infusion). Rates and Clopper Pearson exact confidence intervals for each treatment group will be analyzed.

The secondary endpoint is the composite AD score (CADS) for dementia progression at 39 weeks, and 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. Each component comprised 25% of the final score (i.e., each equally weighted). Inverted measures of the ADAS-Cog-13 and CDR-SB will be used in order to match directionality of the other measures with respect to improvement/decline changes.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Cells were thawed using thaw media; cell counts and viability were analyzed using a cell counter (NucleoCounter; NC-200; ChemoMetec, Allerod, Denmark). Cells were washed with flow staining buffer (cat # 00422226, Thermo Fisher) not to exceed 50 mL and centrifuged at $500 \times g$ for 10 min at 20°C . After centrifugation, cell pellets were resuspended in flow buffer at a concentration of 1×10^6 to 1×10^7 cells per mL. 100 μL of cells were added to each tube with appropriate antibodies and incubated for 60 min. in the dark at $2-4^{\circ}\text{C}$. Cells were labeled with live/dead dye; 7-AAD (cat# 559925); negative controls included: FITC IgG1 (cat#556649), PE IgG1 (cat#556650), PCP5.5 IgG1 (cat#552834), APC IgG1 (cat#550854). The live/dead and all isotype control IgG1 antibodies were used undiluted at 20 μL . The cells were labeled with cell surface marker

antibodies for CD105 PE (cat#560839, 5uL), CD90 FITC (cat#555595, 5uL), CD73 APC (cat# 560847, 5uL), CD45 FITC (cat#555482, 20uL), CD34 PE (cat# 560941, 20uL), CD19 PE (cat# 555413, 20uL), CD11b PE (cat# 561001, 20uL), HLA-PC5.5 (cat#560652, 5uL). Unstained cells served as an additional experimental control. All cell surface marker antibodies were used undiluted and were purchased from BD Biosciences. After the incubation step, cells were washed with 1 mL of flow cytometry staining buffer solution and centrifuged for 10 min at 500 x g, at room temperature. Cell pellets were resuspended in 300uL flow cytometry staining buffer. All samples were run in triplicates.

Instrument	CytoFLEX V5-B5-R3
Software	CytExpert and FlowJo
Cell population abundance	Similar to the definition of bone marrow derived MSCs following the ISCT criteria, passing Lomemel-B lots must be positive for the surface cell markers CD105, CD90, and CD73 ($\geq 95\%$). Lomemel-B must be negative ($\leq 2\%$) for CD45, CD11b, CD19, and $\leq 5\%$ for CD34.
Gating strategy	A standard gating strategy was employed using side scatter (SSC)-H and -A and forward scatter (FSC)-H, where cells were first subcategorized as single cells vs doublets, further categorized as live cells using a live/dead dye, and assessed as positive using a gate permitting a maximum of 0.01% of unstained cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	Resting state
Design specifications	This was a longitudinal vMRI and DTI assessment at baseline, 16, 26, and 39 weeks after initiation of the treatment regimens, with vMRI and DTI data collected at each timepoint.
Behavioral performance measures	n/a: No behavioral tasks were provided to study participants during MRI imaging.

Acquisition

Imaging type(s)	Structural (volumetric), and diffusion tensor imaging
Field strength	3T
Sequence & imaging parameters	3DT1 MPRAGE : Sagittal acquisition with 176 slices, FOV = 240mmx240mm, Slice thickness = 1.2mm, TR/TE = 1800ms/2.49ms, TI = 900ms, flip angle = 10 degrees, matrix size = 192x192
Area of acquisition	Whole brain vMRI scans were used in this study, with atlas-based sub-region identification. Volumetric MRI and Atrophy analysis: All 3DT1 scans were N4 bias corrected, registered to a 256mm ³ template space and isotropically resampled to achieve a voxel size of 1mm ³ . Preprocessed 3DT1 scans were then analyzed in FreeSurfer 6 to generate baseline regional volume measures of all available cortical/sub-cortical, white matter, and ventricular regions. For atrophy measures, follow up imaging timepoints are registered with baseline scans to a log-symmetric midway space for tensor based morphometry analysis. All volume and atrophy measures are reported in mm ³ and converted to cm ³ as applicable.
Diffusion MRI	☒ Used ☐ Not used

Parameters	DTI Parameters: Axial spin echo, echo planar imaging acquisition with 80 slices, FOV = 232mmx232mm, Slice thickness = 2mm, Phase encode direction = AP, TR/TE = min/min (but still T2 weighted, dependent on scanner performance), matrix size = 116x116, bandwidth = 1486 Hz/Px, directions = 30, b-value = 1000 s/mm², 2 b0 averages. In addition, a DTI b0 reverse sequence with identical parameters (including TR/TE/BW)but with phase encode direction = PA. DTI analysis: A standardized DTI sequence (2mm³ voxel size, 30 directions, b-value = 1000 s/mm²) was deployed to each site in conjunction with a reverse phase encode DTI sequence (blip down) with identical contrast parameters (TR/TE/Bandwidth) for distortion correction. Scans were received and quality controlled to ensure adherence to approved acquisition settings. Pre-processing included eddy and motion correction that was performed using a linear affine transformation on all gradient images with the baseline image of b=0 s/mm² (1). The reverse encoding DTI image (blip down acquisition) was employed for correcting the distortions in the main DTI image (blip up acquisition) using DRBUDDI which is based on symmetric diffeomorphic registration (1-3) to estimate the distortion field. Diffusion tensors were then computed using a standard least square approximation and the scalar maps of fractional anisotropy (FA), mean diffusivity (MD) axial diffusivity (AD) and radial diffusivity (RD) were derived from the tensor image. The FA maps were then registered to the FreeSurfer (4) processed 3DT1 image in standard 256*256*256 space and the deformation was applied to remaining maps (MD, AD and RD). Finally, the FA image was registered to JHU atlas's (6) FA image using ANTS (5) SyN deformable registration and the deformation was applied to all the maps. This allowed for a seamless overlay of cortical, subcortical, and white matter labels for regional value extraction. For each subject, the registrations and overlays were checked manually, and reprocessed or rejected in case of failures. 1. C. Pierpaoli, L. Walker, M. O. Irfanoglu, A. Barnett, P. Bassar, L-C. Chang, C. Koay, S. Pajevic, G. Rohde, J. Sarlls, and M. Wu,
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2010, TORTOISE: an integrated software package for processing of diffusion MRI data, ISMRM 18th annual meeting, Stockholm, Sweden, abstract #1597, (pdf)

2. Mustafa Okan Irfanoglu^{1,2,3}, Amritha Nayak^{1,2,3}, Jeffrey Jenkins^{1,2}, and Carlo Pierpaoli^{1,2}, TORTOISEv3: Improvements and New Features of the NIH Diffusion MRI Processing Pipeline, ISMRM 25th annual meeting, Honolulu, HI, abstract #3540, (pdf)

3. Irfanoglu MO, Modi P, Nayak A, Hutchinson EB, Sarlls J, Pierpaoli C. DR-BUDDI (Diffeomorphic Registration for Blip-Up blip-Down Diffusion Imaging) method for correcting echo planar imaging distortions, Neuroimage. 2015 Feb 1;106:284-99. doi: 10.1016/j.neuroimage.2014.11.042. Epub 2014 Nov 26. (pdf)

4. Fischl, Bruce. "FreeSurfer." Neuroimage 62.2 (2012): 774-781.

5. Avants, Brian B., Nick Tustison, and Gang Song. "Advanced normalization tools (ANTs)." Insight j 2.365 (2009): 1-35.

6. Mori, Susumu, et al. "Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template." Neuroimage 40.2 (2008): 570-582.

Preprocessing

Preprocessing software	Detailed description provided above. For the 3DT1 scans, FreeSurfer 6 was used to generate the segmentation maps. Tensor based morphometry measures were calculated using in-house created software. DTI analysis leveraged DRBUDDI for distortion correction and ANTS for registration. DTI calculations were derived using in-house created software.
Normalization	3DT1 scans were normalized to MNI average_305 template per FreeSurfer's processing steps. The JHU atlas was used for registration within the DTI pipeline to allow for cortical/sub-cortical gray matter statistics in addition to the white matter tracts defined in JHU space.
Normalization template	Described above but the MNI 305_template and JHU templates were used.
Noise and artifact removal	Based on the definition here, no noise/artifact removal was performed. All calculated maps were visually QCed for accuracy of the derived result.
Volume censoring	Not used

Statistical modeling & inference

Model type and settings	MMRM. 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.
Effect(s) tested	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.
Specify type of analysis:	☐ Whole brain ☒ ROI-based ☐ Both

For volumetric MRI the following regions were calculated (left, right, and bilateral):

- Whole Brain
- Lateral Ventricles
- Hippocampus
- Whole Gray Matter
- Cingulate cortex: Caudal anterior cingulate, Rostral anterior cingulate, Isthmus cingulate, Posterior cingulate
- Frontal cortex: Superior frontal, Caudal middle frontal, Rostral middle frontal, Pars opercularis, Pars orbitalis, Pars triangularis, Lateral orbitofrontal, Medial orbitofrontal, Frontal pole, Precentral gyrus, Paracentral lobule
- Medial temporal cortex: Hippocampus, Amygdala, Entorhinal, Parahippocampal gyrus, Temporal pole, Fusiform
- Temporal cortex: Entorhinal, Parahippocampal gyrus, Temporal pole, Fusiform, Inferior temporal, Middle temporal, Superior temporal, Transverse temporal, Banks of the superior temporal sulcus
- Occipital cortex: Lateral occipital, Lingual gyrus, Cuneus, Pericalcarine sulcus
- Parietal cortex: Inferior parietal, Superior parietal, Postcentral gyrus, Supramarginal gyrus, Precuneus
- Striatum: Caudate, Putamen
- Thalamus

For DTI all available FreeSurfer regions were reported for FA, MD, AD, and RD in addition to free water fraction and free water corrected values. For JHU the following white matter tracts were calculated (left, right, bilateral where appropriate):

- Whole White Matter
- Cerebral Peduncle
- Cingulum
- Corona Radiata (Anterior, Posterior, Superior, Whole)
- Corpus Collosum (Body, Genu, Splenium, Whole)
- Corticospinal tract
- External Capsule
- Fornix
- Inferior Cerebellar Peduncle
- Internal Capsule (Anterior, Posterior, Retrolenticular, Whole)
- Medial Lemniscus
- Middle Cerebellar Peduncle
- Pontine Crossing Tract
- Sagittal Stratum
- Superior Cerebellar Peduncle
- Superior Fronto-Occipital Fasciculus
- Superior Longitudinal Fasciculus
- Tapetum
- Thalamic Radiation (Posterior)
- Uncinate Fasciculus

Anatomical location(s)

Statistic type for inference

Voxel-wise

(See [Eklund et al. 2016](#))

Correction

None used.

Models & analysis

n/a | Involved in the study

- ☐ ☒ Functional and/or effective connectivity
- ☒ ☐ Graph analysis
- ☐ ☒ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

To determine whether reduced brain atrophy correlated with improved clinical scores, we performed a Pearson correlation analysis.

Multivariate modeling and predictive analysis

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. 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.