

Laromestrocel Stabilizes Brain Inflammation In Key Alzheimer's Disease Gray And White Matter Regions As Assessed Using Free Water MRI

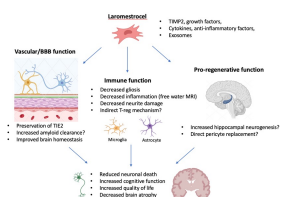
Brian G. RASH¹, Jeffrey BOTBYL², Sofya LEVITINA¹, Chris CONKLIN³, Madhura INGALHALIKAR⁴, Zarin ZAINUL¹, Joshua M. HARE¹

¹ Longeveron INC, Miami, United States, ² Provonix Inc, ³ Clario, Philadelphia, United States, ⁴ Clario, Miami, United States

- Laromestrocel, a stem cell therapy, was administered IV in subjects with mild AD to test pro-vascular and anti-inflammatory effects
- Free water, a measure of neuroinflammation, was assessed using diffusion tensor imaging data from the CLEAR-MIND trial (Rash et al., 2025 Nat. Med.)
- Free water increased in placebo over 39 weeks, consistent with disease progression, particularly in hippocampus, temporal cortex and white matter
- Laromestrocel substantially reduced or eliminated free water increase, correlating with improved brain volume and clinical scores
- Group level MMRM and patient level machine learning analysis indicated an immunomodulatory signature involving IL-13

INTRODUCTION

A key contributor to Alzheimer's disease (AD) brain atrophy and clinical pathology is progressive neuroinflammation, which can be indexed using free water MRI. Free water rises substantially during AD pathogenesis, particularly in the hippocampus, temporal lobe, and other key regions, including white matter tracts associated with memory. We previously showed that laromestrocel, a human bone marrow-derived, mesenchymal stem cell therapy with anti-inflammatory and pro-vascular effects, substantially rescued brain atrophy and lowered free water in a proof of concept, randomized Phase 2a trial (CLEAR-MIND), especially in the hippocampus, temporal lobe, and other areas, correlating with improved cognitive and quality of life scores. Here, we investigate whether brain neuroinflammatory status using free water measurements from the MRI data of participants in the CLEAR-MIND trial correlates with volume and clinical scores, changes in white matter free water, and immunomodulatory signatures of laromestrocel treatment.



METHODS

This double-blind, randomized, placebo-controlled 39-week proof-of-concept trial (ClinicalTrials.gov: NCT05233774)⁴ enrolled patients (60-85 yrs.) with mild AD (NIA-AA criteria, MMSE score 18-24, evidence of amyloid on positron-emission tomography (PET) and brain MRI consistent with AD). There were 4 study arms of laromestrocel intravenous infusion: Group 1: 4 IV infusions of Placebo (N=12); Group 2: 25 million (25M) cells once followed by 3 infusions of placebo (N=13); Group 3: 25M cells (N=13) monthly for 4 months; and Group 4: 100 million (100M) cells (N=11) monthly for 4 months. Laromestrocel treatment groups were evaluated for individual and pooled (25Mx1, 25Mx4, 100Mx4) groups vs placebo, n=32. Neuroinflammation was indexed using free water fraction components extracted from diffusion tensor imaging (DTI) by Clario. Briefly, longitudinal data were collected using 3T MRI at baseline and weeks 16, 26, and 39. Diffusion MRI images were modeled using a single shell bi-tensor model that separates isotropic (free water) and anisotropic (tissue) diffusion components^{5,6}. **Statistical processing.** All statistical analyses utilized the CLEAR-MIND modified intention-to-treat population. Brain volume and free water endpoints were evaluated using a mixed effects repeated measures model. Least squares mean differences between each active treatment group and placebo were estimated and formally tested against zero. The p-values shown in the figures correspond to these tests. No adjustments for multiple comparisons were applied. Spearman correlations were computed for each brain volume and free water region pair, and the resulting values are visualized on the heat maps using color intensity.

Disclosures: Dr. Joshua M. Hare reports having a patent for cardiac cell-based therapy and holds equity in Vestion Inc., maintaining a professional relationship with Vestion Inc. as a consultant and member of the Board of Directors and Scientific Advisory Board. Vestion Inc. did not play a role in the design, conduct, or funding of the study. Dr. Hare is the Chief Scientific Officer, a compensated consultant and board member for Longeveron Inc. and holds equity in Longeveron. Dr. Hare is also the co-inventor of intellectual property licensed to Longeveron. The University of Miami is an equity owner in Longeveron Inc., which has licensed intellectual property from the University of Miami.

RESULTS

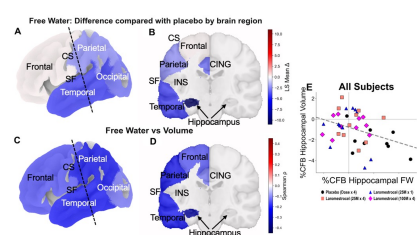


Figure 1. Decreased inflammation and atrophy in core AD brain regions after laromestrocel treatment. Pooled treatment group free water changes (LS mean differences) were mapped onto the brain using the Harvard-Oxford atlas for volumetric regions and the Desikan atlas for cortical surface parcels, with values displayed using a symmetric seismic (blue, white, and red) colormap. Blue shades represent a negative difference of pooled treatment groups compared with placebo. Lateral (A), and coronal section (B) views showing the hippocampus. The hippocampus shows the largest anti-inflammatory effect of laromestrocel treatment compared with placebo, followed by the temporal lobe. Spearman correlation analyses at Week 39 across all subjects (N = 40) showed that greater regional free water reduction was associated with greater brain volume preservation, with the strongest inverse correlation in the left hippocampus ($p = -0.33$, $p = 0.040$) and trends in the temporal ($p = -0.22$, $p = 0.177$) and parietal lobes ($p = -0.24$, $p = 0.128$), mapped onto brain surfaces (C, D). Per-subject scatter plot of left hippocampal free water vs hippocampal volume percent change from baseline at Week 39 for all subjects (E) and the dashed line indicates the linear regression fit. Dotted lines in A, C indicate the approximate plane of coronal section in B, D. FW, free water; SF, Sylvian Fissure; CING, cingulate cortex; INS, insular cortex; CS, central sulcus.

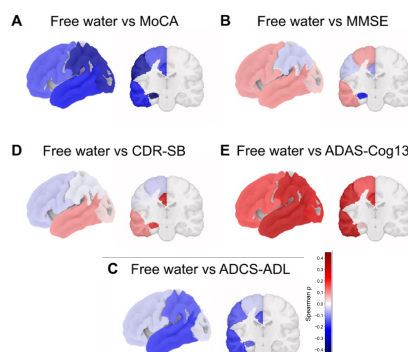


Figure 2. Reduced neuroinflammation correlates with improved clinical outcomes. Left-hemisphere brain surface maps of Spearman correlation coefficients (r) between placebo-adjusted percent change from baseline (CFB) in regional free water and placebo-adjusted percent CFB in clinical scores at Week 39: (A) MoCA, (B) MMSE, (C) ADAS-ADL, (D) CDR-SB, and (E) ADAS-Cog13. Cool colors indicate inverse associations; warm colors indicate positive associations (scale shown). For scores where clinical improvement is a positive change (MoCA, MMSE, ADAS-ADL), free water reduction showed mostly inverse correlations. The strongest regional inverse correlations were for free water vs MoCA (left hippocampus: $r = -0.40$, $p = 0.011$, $N = 39$), parietal cortex ($r = -0.35$, $p = 0.030$, $N = 39$) and occipital cortex ($r = -0.32$, $p = 0.045$, $N = 39$). For CDR-SB and ADAS-Cog13, where clinical improvement is a negative change, regional correlations were mostly positive, reaching statistical significance for temporal cortex in ADAS-Cog13 ($r = 0.322$, $p = 0.043$, $N = 40$).

RESULTS (continued)

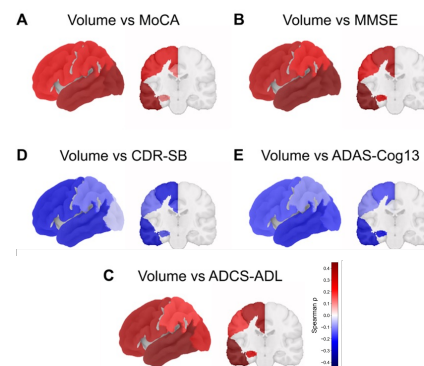


Figure 3. Brain volume preservation correlates with improved clinical outcomes. Left-hemisphere brain surface maps of Spearman correlation coefficients (r) between placebo-adjusted percent CFB in regional brain volume and placebo-adjusted percent CFB in clinical scores at Week 39 (N = 42): (A) MoCA, (B) MMSE, (C) ADAS-ADL, (D) CDR-SB, and (E) ADAS-Cog13. Warm colors indicate that volume preservation correlates with clinical improvement on positive-direction scales; cool colors indicate inverse associations (scale shown). The strongest regional correlations were observed for volume vs MMSE (Frontal Cortex: $r = 0.35$, $p = 0.025$; Temporal Cortex: $r = 0.47$, $p = 0.002$; Occipital Cortex: $r = 0.49$, $p = 0.001$; Cingulate Cortex: $r = 0.36$, $p = 0.018$, $N = 42$); MoCA (Temporal Cortex: $r = 0.40$, $p = 0.009$; Occipital Cortex: $r = 0.42$, $p = 0.007$, $N = 41$), and ADAS-ADL (Frontal Cortex: $r = 0.370$, $p = 0.016$; Temporal Cortex: $r = 0.46$, $p = 0.002$; Cingulate Cortex: $r = 0.390$, $p = 0.011$, $N = 42$). Placebo adjustment reflects change relative to the placebo arm mean prior to correlation analysis.

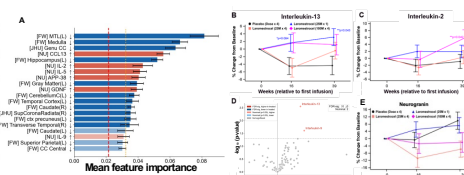


Figure 4. An unbiased machine learning analysis and plasma protein screen identifies Th2 cytokines as top treatment-responsive signals, with converging peripheral immune activation. (A) Top 20 features ranked by mean random forest importance across folds. Bar color encodes the univariate direction of each feature's Week 39 percent-change contrast (treatment minus placebo): red bars: increased in the treatment group relative to placebo; blue bars: decreased. Red dashed lines indicate mean shadow-feature importance; orange dotted lines mark the 1.5x shadow retention threshold. MTL: medial temporal lobe, CC, corpus callosum. (B) IL-13 increased in all treatment groups at Week 39, reaching statistical significance for 25Mx1 ($p = 0.043$, $N = 10$). (C) IL-2, a regulator of T-regulatory cell expansion, showed statistically significant elevation at Week 39 (100Mx4: $p = 0.042$, $N = 7$). (D) Volcano plot of OLS regression beta coefficients (treatment minus placebo, % change from baseline at Week 39) versus $-\log_{10}$ nominal p-value across 117 NULISA CNS120 proteins, adjusted for sex and APOE-epsilon4 status (N = 48). Two proteins reached significance (dashed line, $p = 0.05$), elevated in the treatment arm: IL-13 ($\beta = +7.52\%$, $p = 0.003$) and IL-9 ($\beta = +8.75\%$, $p = 0.025$); zero proteins reached FDR significance. (E) Neurogranin was reduced in plasma compared with placebo at Week 39, consistent with peripheral immune activation (25Mx1: $p = 0.020$, $N = 10$). Statistical comparisons in B, C, E utilized an MMRM model. Error bars: ± SEM.

RESULTS (continued)

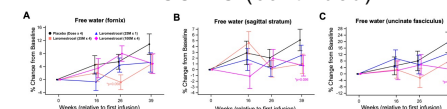


Figure 5. Free water in key AD white matter tracts at week 39. Free water in the fornix, a key white matter tract serving the hippocampus, steadily increased by more than 10% in the placebo group (black line), while treatment groups showed consistent trends for less increase at week 39 (A). Similar trends were found for sagittal striatum (B) and uncinate fasciculus (C). Statistical comparisons utilized an MMRM model. Error bars: ± SEM.

CONCLUSIONS

- After 39 weeks, treatment with laromestrocel was associated with a reduction in neuroinflammation as assessed by free water compared to placebo across multiple brain regions, including key AD-associated regions—most notably in the hippocampus and temporal lobe.
- Placebo showed increasing free water in multiple brain regions, representing continued inflammatory drive and disease progression.
- Free water changes were correlated with improved clinical scores and reduced brain atrophy
- Unbiased machine learning analysis confirms a primary anti-inflammatory effect of laromestrocel enriched in key AD-associated gray and white matter regions and implicate IL-13 and other plasma biomarkers in an immunomodulatory signature.
- These findings support the hypothesis that laromestrocel exerts a sustained anti-inflammatory effect in the treatment of mild Alzheimer's disease.

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