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FEATURED ARTICLE

Results and insights from a phase I clinical trial of Lomecel-B for Alzheimer's disease

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Abstract

Hypothesis: We hypothesized that Lomecel-B, an allogeneic medicinal signaling cell (MSC) therapeutic candidate for Alzheimer's disease (AD), is safe and potentially disease-modifying via pleiotropic mechanisms of action.

Key Predictions: We prospectively tested the predictions that Lomecel-B administration to mild AD patients is safe (primary endpoint) and would provide multiple exploratory indications of potential efficacy in clinical and biomarker domains (prespecified secondary/exploratory endpoints).

Strategy and Key Results: Mild AD patient received a single infusion of low- or high-dose Lomecel-B, or placebo, in a double-blind, randomized, phase I trial. The primary safety endpoint was met. Fluid-based and imaging biomarkers indicated significant

improvement in the Lomecel-B arms versus placebo. The low-dose Lomecel-B arm showed significant improvements versus placebo on neurocognitive and other assessments.

Interpretation: Our results support the safety of Lomecel-B for AD, suggest clinical potential, and provide mechanistic insights. This early-stage study provides important exploratory information for larger efficacy-powered clinical trials.

KEYWORDS
Alzheimer disease, anti-inflammatory agents, biological therapy, bone marrow mesenchymal stem cell, clinical trial, cytokines, hippocampus, human bone marrow, inflammation, inflammation mediators, interleukins, Lomecel-B, medicinal signaling cell, mesenchymal stem cell, mesenchymal stromal cell, multipotent stem cells, neuroimaging, neuroinflammatory diseases, randomized controlled trial, regenerative medicine, vascular, vascular endothelial cell growth factor

1 | NARRATIVE (EXECUTIVE SUMMARY)

Developing treatments for Alzheimer's disease (AD) has been characterized by substantial challenges in converting mechanistic strategies into decisively successful therapeutic outcomes.[1] Given that complex pathophysiology underlies AD, targeting a single pathological feature, such as amyloid beta (Aβ), may lack comprehensive disease-targeting properties required to substantially alter clinical progression. Lomecel-B, a medicinal signaling cell (MSC; also known as mesenchymal stromal cell or mesenchymal stem cell), holds promise as a novel therapeutic candidate, either alone or as part of combinatorial therapy, via pleiotropic mechanisms of action (MOAs) potentially targeting several pathological features of AD.

1.1 | Background

While substantial scientific evidence supports involvement of Aβ and cytotoxic tau isoforms in AD pathophysiology, it has become increasingly clear that other mechanisms contribute to disease pathogenesis.[1] A pro-inflammatory state is increasingly recognized as a major contributor to the manifestation of dementia.[2,3] Proinflammatory cytokines are abundant in the vicinity of amyloid deposits and neurofibrillary tangles, and there is an association between systemic inflammation and Aβ accumulation.[3] Additionally, impaired neurovasculature function appears to be a contributing factor.[4] This includes compromise of the blood-brain barrier (BBB)[5] and impaired exchange across the endothelium, leading to inefficient clearance and accumulation of Aβ peptides in the brain.[6]

MSCs are multipotent cells with pleiotropic mechanisms of action, possessing anti-inflammatory, pro-vascular, and pro-regenerative properties exerted via hetero-cellular coupling, the release of cytokines, growth factors, exosomes, and other biologically active molecules.[7,8] MSCs are chemoattracted to sites of inflammation and damage,[7,8] and likely target sites of neuroinflammation in AD even when systemically administered. MSCs are also immune-evasive/immunoprivileged, facilitating allogeneic use, and have an acceptable safety profile[9] in clinical trials for other conditions, such as aging-related frailty[10] and cardiac-related conditions.[11]

AD animal model studies support the therapeutic potential of MSCs. Systemically administered MSCs inhibit Aβ deposition and promote clearance, promote neurogenesis and reduce apoptosis, improve neuronal morphology, cross the BBB, and improve behavioral and spatial memory performance.[12–14] These beneficial effects are associated with decreased inflammation, increased Aβ-degrading factors and Aβ clearance, and decreased hyperphosphorylated tau. These appear, at least in part, due to Aβ-induced release of chemoattractants from MSCs that recruit alternatively activated microglia to reduce Aβ deposition.[15] Together, these preclinical studies support the hypothesis that MSCs possess therapeutic properties that are clinically effective for treating AD.

1.2 | Phase I AD trial

To address this hypothesis and obtain important safety data, we conducted a phase I clinical trial ($N = 33$) to evaluate safety of Lomecel-B in patients with mild AD (Table S1 in supporting information) as a first-in-human study. While not powered for efficacy, we conducted preliminary efficacy assessments to generate hypothesis-driving results to inform next-phase trials. Subjects in this trial were randomized to receive a single intravenous infusion of a low-dose (2×10^7 cells) or high-dose (1×10^8 cells) of Lomecel-B, or a placebo.

Safety was blindly assessed throughout the trial, and included evaluation of types and rates of adverse events (AEs) and serious AEs (SAEs). Additional safety measures included evaluation for changes in blood chemistries, complete blood count with differential, coagulation, echocardiography, and amyloid-related imaging abnormalities (ARIA).[16]

The primary endpoint was the treatment-emergent SAEs (TE-SAE) stopping rule, defined by rate of SAEs within 30 days post-infusion. The stopping rule was never triggered, thus meeting the primary study

endpoint. Only one TE-SAE occurred within 30 days of infusion (Table 1), which was at day 27 in the high-dose Lomecel-B arm for back pain resulting in 24-hour hospitalization, and deemed unrelated to study product (Table S2 in supporting information). The overall incidence of AEs and SAEs was lower in each Lomecel-B treatment arm versus placebo. No AEs or SAEs were deemed related to study product. There was one death on study, occurring at day 144 in the high-dose Lomecel-B arm. No infusions were interrupted, terminated prematurely, or had an associated AE/SAE. There were no reports of ARIA.[16] Hematology, coagulation, blood chemistry, vital signs, urinalysis, and echocardiogram data showed no trends or issues of concern.

1.2.1 | Exploratory findings regarding mechanism of action and bioactivity in humans

To gain a preliminary understanding of potential Lomecel-B MOAs involved in treating AD, we ran panels of serum-based vascular-, inflammatory-, and neuronal-related biomarkers. Several vascular-related serum biomarkers were significantly higher in the Lomecel-B arms versus placebo post-treatment, including vascular endothelial cell growth factor (VEGF), interleukin (IL) 4, and IL-6. VEGF significantly decreased in the placebo arm versus the change from baseline in both the low- ($P < 0.0128$) and high-dose Lomecel-B arms ($P < 0.0012$; Figure 1A). VEGF has neuroprotective and neurorestorative effects, and positively associates with increased hippocampal volume[17] (also observed in this study—*see below*).

Similarly, IL-4 significantly decreased in the placebo arm versus both the low- ($p < 0.0054$) and high-dose Lomecel-B arms ($p < 0.0180$) (Figure 1B). IL-4 is a pleiotropic cytokine that regulates vascular function, cell proliferation and apoptosis, and decreases pro-inflammatory profiles of a variety of cell types, including microglia, and can induce brain-derived neurotrophic factor (BDNF) production from astrocytes.[18] IL-4 can also improve Aβ-inhibited long-term potentiation by suppressing Aβ-induced upregulation of IL-1β from M1 microglial activation.[19] IL-4 also leads to clearance of oligomeric Aβ peptides by increasing expression of the Aβ-degrading enzyme CD10 in microglia.[20] Furthermore, IL-4 can activate a M2 microglia

RESEARCH IN CONTEXT

1. **Systematic review**: Medicinal signaling cells (MSCs) have pleiotropic mechanisms of action (MOAs), and thus have potential for treating multiple aspects of the complex pathophysiology associated with Alzheimer's disease (AD). These MOAs include pro-vascular, anti-inflammatory, and pro-regenerative activities. We conducted a double-blind, randomized, placebo-controlled trial to evaluate the safety and effects of Lomecel-B, an allogeneic MSC formulation, in patients with mild AD.
2. **Interpretation**: Our findings support the safety of Lomecel-B in mild AD patients, meeting the primary endpoint of the trial. Secondary endpoints indicated that with a single dose, Lomecel-B has potential to improve clinical outcomes relative to placebo, and that the biological basis of these effects may include pro-vascular and anti-inflammatory activities.
3. **Future directions**: These results indicate that Lomecel-B is a safe and potentially effective cell therapy approach to AD. A next-phase trial powered for effect is warranted.

TABLE 1 Incidence of AEs and SAEs

	Placebo	20 M Lomecel-B	100 M Lomecel-B
Primary endpoint: Number of TE-SAEs occurring within 30 days after treatment (*n*)	0	0	1
Number of subjects [*n* (%)]	0	0	1 (10.0%)
Number of SAEs occurring over entire trial (*n*)	4	2	3
Number of subjects [*n* (%)]	3 (37.5%)	2 (13.3%)	2 (20.0%)
Number of TE-AEs occurring within 30 days after treatment (*n*)	3	3	2
Number of subjects [*n* (%)]	2 (25.0%)	3 (20.0%)	1 (10.0%)
Number of AEs occurring over entire trial (*n*)	33	23	15
Number of subjects [*n* (%)]	7 (87.5%)	10 (66.7%)	5 (50.0%)
Number of deaths on study (*n*)	0	1[a]	0
Number of AEs related to study drug (*n*)	0	0	0
Number of SAEs related to study drug (*n*)	0	0	0
Number of infusions interrupted or stopped prematurely (*n*)	0	0	0
Number of patients with ARIA (*n*)	0	0	0

Abbreviation: AEs, adverse events; ARIA, Alzheimer's related imaging abnormalities; SAEs, serious adverse events; TE-AE, treatment-emergent AE occurring within 30 days post-infusion; TE-SAE, treatment-emergent SAE occurring within 30 days post-infusion.

[a]The patient withdrew from the trial first and subsequently died in an assisted-living facility at day 144 after the infusion.

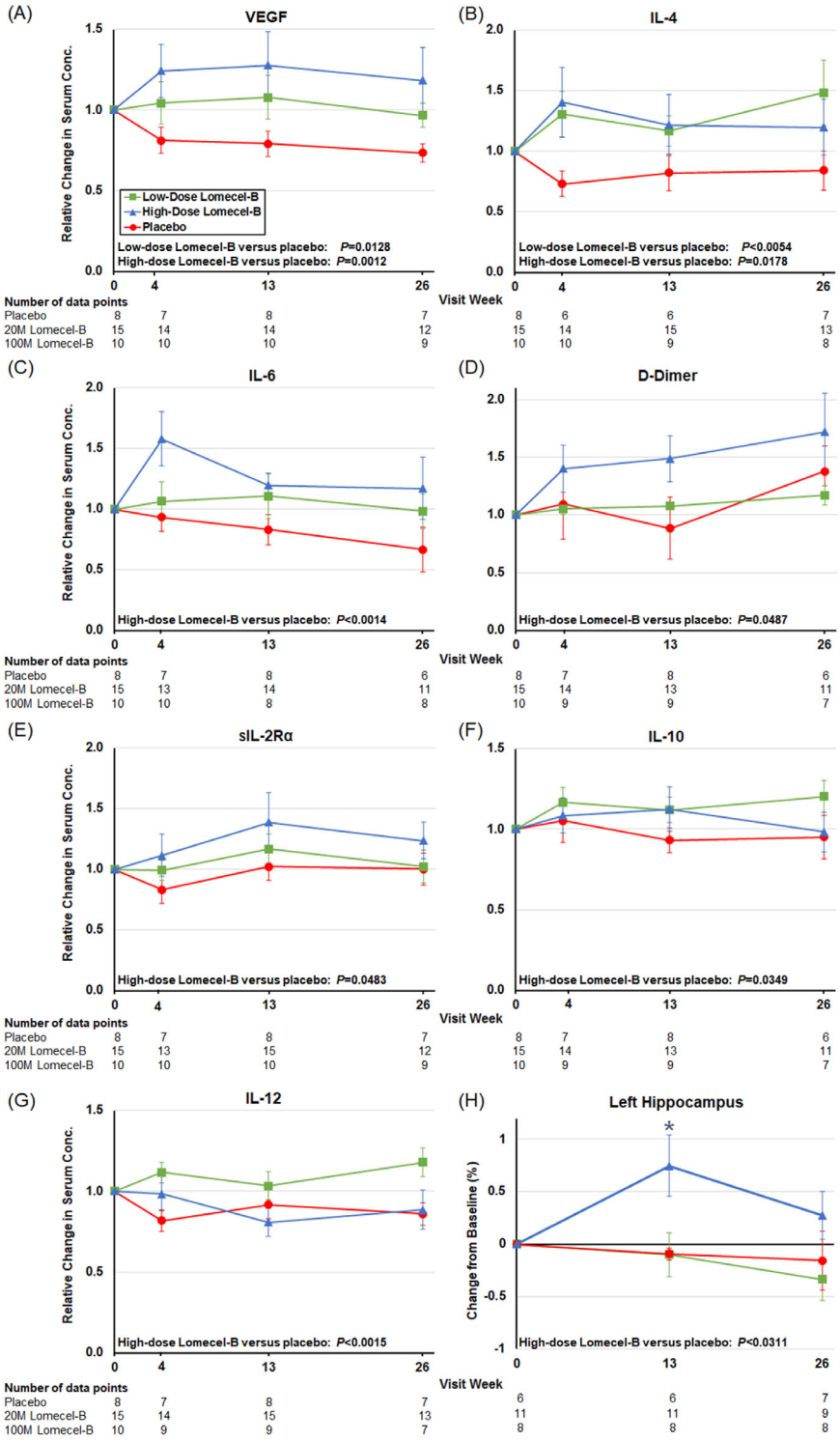

FIGURE 1 Secondary and exploratory biomarker endpoints: changes in serum biomarkers related to inflammation and vascular functioning, and hippocampus volume. A to C, Declines in vascular-related biomarkers occurred in the placebo arm, but not the Lomecel-B arms. A, Vascular endothelial growth factor (VEGF) significantly decreased in the placebo arm versus the changes in the low-dose Lomecel-B arm ($P < 0.0128$) and the high-dose Lomecel-B arm ($P < 0.0012$). B, Interleukin (IL)-4 was significantly higher in both the low-dose and high-dose Lomecel-B arms versus placebo ($P < 0.0054$ and $P < 0.0180$, respectively). C, IL-6 was significantly higher for the high-dose Lomecel-B arm versus placebo ($P < 0.0014$). D, D-Dimer significantly increased in the high-dose Lomecel-B arm versus placebo ($P < 0.0488$). E to G, Significant increases in anti-inflammatory biomarkers occurred in the Lomecel-B arms versus the changes in placebo. E, Soluble IL-2 receptor α (sIL-2Rα) significantly increased in the high-dose Lomecel-B arm versus the change in placebo ($P < 0.0049$). A trending increase was seen in the low-dose Lomecel-B arm. F, IL-10 significantly increased in the low-dose Lomecel-B arm versus placebo ($P < 0.0349$). G, IL-12 significantly increased in the low-dose Lomecel-B arm versus placebo ($P < 0.0015$). A to G, *P* values indicate analysis of variance evaluation of change from baseline through week 26 for the indicated comparisons. Plotted are the means ± standard error of the mean. H, The left hippocampus showed an increase in volume in the high-dose Lomecel-B group versus the change in placebo at week 13 post-treatment (*, $P < 0.0311$)

phenotype, and in turn neurogenesis and oligodendrogenesis,[21] and positively correlates with left subiculum volume in patients with mild cognitive impairment.[22] IL-4 injection in the APP23 AD mice was also shown to reduce Aβ levels and significantly improve memory deficits.[23]

IL-6 also significantly decreased in the placebo arm versus the high-dose Lomecel-B ($P < 0.0014$) arm (Figure 1C). IL-6 is a pleiotropic cytokine that can have beneficial effects, such as under exercise conditions, has pro-angiogenic–osteogenic activity, and can protect from glucose toxicity via VEGF signaling.[24,25] These accord with evidence that exercise can be disease modifying for AD.[26] We note that IL-6 levels in this study were also far below levels indicative of a cytokine storm, such as occurs with COVID-19, and also that baseline levels in the placebo arm were >twice those of the Lomecel-B arms, which could confound interpretation of results. Nevertheless, these observations support the pro-vascular hypothesis of Lomecel-B MOA for AD.

Post-treatment anti-inflammatory serum biomarkers were significantly higher in the Lomecel-B arms versus placebo, which included soluble IL-2 receptor α (sIL-2Rα), IL-10, and IL-12, in addition to IL-4 discussed above. sIL-2Rα significantly increased in the high-dose Lomecel-B arm versus placebo ($P < 0.0049$; Figure 1E). The low-dose Lomecel-B arm had significantly increased IL-10 ($P < 0.0349$; Figure 1F) and IL-12 ($P < 0.0015$) versus placebo (Figure 1G). IL-10 has well-documented anti-inflammatory properties.[27] IL-12 has anti-inflammatory and pro-inflammatory activities that are contextual dependent, and induces IL-10 expression as part of its anti-inflammatory roles.[28] IL-12 is also reported to be markedly lower in the cerebrospinal fluid of AD patients.[29] Similarly, the anti-inflammatory effects of sIL-2Rα are contextually dependent.[30] Coupled with the anti-inflammatory roles of IL-4,[18] these results support the potential of Lomecel-B to promote an anti-inflammatory milieu.

Brain magnetic resonance imaging (MRI) was used to obtain preliminary evidence on whether Lomecel-B may positively impact structural brain changes. Hippocampus volume and neurogenesis significantly decline in AD.[31] We found a significant transient increase in left hippocampal volume in the high-dose Lomecel-B arm versus the change in placebo at week 13 ($P = 0.0311$; Figure 1H). In contrast, neither the low-dose Lomecel-B arm showed significant changes, nor did the right hippocampus in any of the arms (Figure S2 in supporting information). Given the absence of observed brain edema, the left hippocampal volume increase would be consistent with an increase in neurogenesis, and if verified, would support pro-regenerative MOAs of Lomecel-B.

The widely used neurocognitive assessment, Mini–Mental State Examination (MMSE),[32] showed worsening (lower score) in the placebo arm, reaching significance from baseline at week 13 by 2.99 ± 1.12 points ($P = 0.0337$; two-sided 95% confidence interval [CI] −5.84 to −0.31). In contrast, the low-dose Lomecel-B arm showed no significant changes, and was significantly better than placebo at week 13 by 2.69 ± 1.39 points ($P = 0.0182$; two-sided 95% CI 0.51 to 4.97; Figure 2A). The high-dose Lomecel-B arm showed no significant changes from baseline or versus placebo. A decreases of one to three points on the MMSE is considered clinically meaningful,[33] and a 6-month minimally clinically important difference (MCID) has been calculated to be ≈1.4.[34,35] The MMSE difference between the low-dose Lomecel-B and placebo arms of nearly three points at week 13, and nearly four points at Week 26, is consistent with a potential clinically meaningful positive treatment effect of Lomecel-B.

The Alzheimer's Disease Assessment Scale–Cognitive subscale-11 (ADAS-Cog-11), often considered the gold standard for evaluating dementia,[36] showed no significant changes in any arm. However, a worsening trend (increased score) was observed in the placebo arm (Figure 2B), whereas the Lomecel-B arms appeared more stable. A change of ≥4 has been recommended by a consensus committee of the Food and Drug Administration (FDA) as clinically meaningful.[37] The difference at week 26 between placebo and low-dose Lomecel-B arms, while not significant, was 5.68 ± 3.66 ($P = 0.3359$; 95% CI −3.77 to 10.46) and if replicated in larger studies, would meet this requirement.

The Quality of Life (QOL) in Alzheimer's Disease assessment, QOL-AD,[38] showed significant improvement in the low-dose Lomecel-B arm versus placebo at week 26 by 3.85 ± 1.943 points ($P = 0.0444$; two-sided 95% CI 0.13 to 9.12; Figure 2C). There was no significant difference in the high-dose Lomecel-B arms versus placebo, and no significant changes in these arms from baseline.

2 | CONCLUSION

2.1 | Cell therapy for AD: a testable hypothesis

Here we present a first-in-human phase I clinical trial of Lomecel-B in AD. The infusions were well tolerated in participants, and did not produce excess SAEs or ARIA. In addition, we obtained preliminary effectiveness, the findings of which inform decisions on design of subsequent large clinical trials. Given the caveat that this trial was not powered for efficacy, our findings nevertheless support the potential of this cell therapy approach for AD, and are consistent with findings in AD animal models.[12–15] Specifically, we observed significant changes in multiple biomarkers and clinical outcome measures. Importantly, when significant changes occurred, these were almost uniformly favorable for the Lomecel-B arms versus placebo (see the Supplementary File for additional measures). The use of a placebo control was critical in this study, as it revealed the potential of Lomecel-B to stabilize decline for a number of biomarkers and clinical assessments (e.g., MMSE).

These preliminary biomarker results are consistent with our hypothesized mechanism(s) of action for Lomecel-B in AD, namely exerting pro-vascular, anti-inflammatory, and pro-regenerative effects.[7,8] Indeed, the significant changes we found in multiple pro-vascular and anti-inflammatory biomarkers suggests synergistic activities, and support the pleiotropic MOAs of Lomecel-B.

A missing component in almost all AD trials is overcoming the intrinsic inability of the central nervous system to regenerate; and a priori, it seems improbable that targeting a single pathological feature of AD, for example, Aβ, would substantially induce regenerative mechanisms. A cell therapy approach, such as with Lomecel-B, has the potential to stimulate intrinsic regenerative responses that might otherwise not occur. In the context of a human clinical trial, directly evaluating regeneration is not practical, and would require *post mortem* histological examination to rigorously evaluate. However, based on the transient increase in hippocampal volume we observed (and lack of accompanying ARIA), it is attractive to speculate that Lomecel-B could create a milieu conducive to enhanced neurogenesis.

In short, results from this Phase I trial support the pathophysiologic rationale of addressing the neuroinflammatory and vascular impairment contributing to AD pathogenesis,[3] and raise important questions leading to hypotheses to test in next-phase trials powered for efficacy.

- Hypothesis 1: Lomecel-B improves neurovascular functioning in a dose-dependent manner.
- Hypothesis 2: Lomecel-B reduces neuroinflammation in a dose-dependent manner.
- Hypothesis 3: Lomecel-B promotes neurogenesis in a dose-dependent manner.

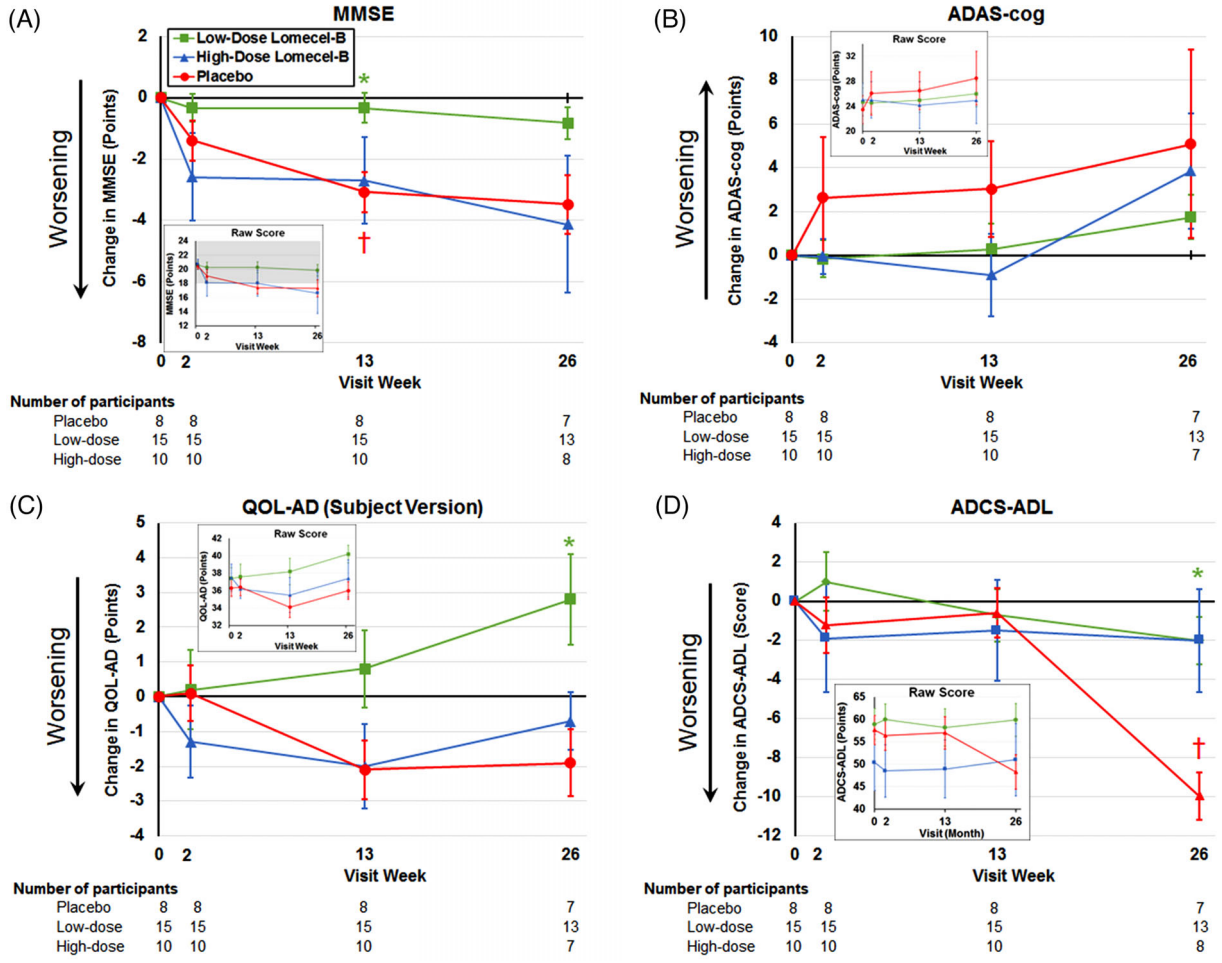

FIGURE 2 Secondary endpoints: changes in neurocognitive and neuropsychiatric assessments. A, The low-dose Lomecel-B arm showed significantly slower decline on the Mini-Mental State Examination (MMSE) versus placebo. The MMSE showed no significant decline in the low-dose Lomecel-B arms versus baseline, whereas the placebo arm showed a significant decline (†, $P = 0.034$ at week 13). This change from baseline in placebo versus the low-dose Lomecel-B arm was also significant (*, $P < 0.019$ at week 13). In contrast, the high-dose Lomecel-B arm showed no significant change versus placebo. B, The Alzheimer's Disease Assessment Scale–Cognitive subscale-11 (ADAS-Cog-11) showed no significant changes between any of the groups, although the placebo arm showed a worsening trend. C, The low-dose Lomecel-B arm improved on the patient version of the Quality of Life in Alzheimer's Disease (QOL-AD) assessment. The QOL-AD significantly increased from baseline in the low-dose Lomecel-B arm versus the change in placebo at week 26 (*, $P = 0.0444$). There was no significant difference between the high-dose Lomecel-B and placebo arms. D, At week 26, the placebo arm had significantly declined from baseline on the Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL; †, $P = 0.0211$), and was statistically significant versus the change in the low-dose Lomecel-B arm (*, $P = 0.0118$). Neither Lomecel-B arm showed a significant change from baseline. Plotted are the means ± standard error of the mean (SEM) for change from baseline for each arm. The inset on each graph shows the raw means ± SEM

- Hypothesis 4: Lomecel-B improves neurocognitive function in a dose-dependent manner.
- Hypothesis 5: Additive benefits are obtained from more frequent treatments with Lomecel-B.

2.2 | Roadmap for phase II trials

The major new findings of this placebo-controlled trial are that intravenous infusion of Lomecel-B in patients with mild AD is safe and well tolerated, and potentially produces biologically meaningful beneficial changes in serum biomarkers to improve neurocognition and quality of life in treated patients.[2] The results of this trial pave the way for future larger clinical trials powered to detect clinical efficacy to address a number of important issues. First, it is essential to establish the dose-responsiveness to Lomecel-B,[39] and determine whether the inconsistencies between the biomarkers results (the high dose generally being superior) and dementia assessments (the low dose generally showing better benefits) was due to under-powering or otherwise verifiable phenomena. In this regard, it is noteworthy that a lack of dose-response relationships for MSCs on cognitive/behavioral testing was also observed in preclinical models,[13] and furthermore, that nonlinear, inverted "U-shaped" dose-response phenomena is not uncommon in learning and memory models.[40] Second, the pharmocokinetics

from a single infusion of Lomecel-B must be clearly established. Third, investigation of additive or sustained effects with additional dosing of Lomecel-B must be performed. Other related indications, such as more advanced AD or mild cognitive impairment (MCI), as well use of Lomecel-B as part of a potential combinatorial treatment, also merit exploration.

Next-step phase II trials will need to be conducted to rigorously address all these issues. Biomarkers in multiple domains will be critical components to understand MOAs, dose-response effects, and pharmacokinetics to inform when follow-up treatment will be necessary because these presumably will be leading indicators of clinical dementia changes. Specifically, changes in neurovascular function could be non-invasively directly assessed through evaluation of vascular reactivity, which indicates changes in vascular health, and arterial spin labeling (ASL) via MRI to measure cerebral blood flow (CBF). These could then be correlated with changes in serum-based biomarkers of vascular function to validate these biomarkers as surrogate readouts. We have now begun a phase IIa multi-dose trial with an emphasis on biomarkers to address many of the hypotheses generated from this pilot study.

Likewise, neuroinflammation could be directly assessed via MRI through diffusion tensor imaging (DTI). This could then be correlated to changes in serum-based anti-inflammatory biomarkers to validate these as surrogate readouts. Similarly, volumetric analyses, particular of the whole hippocampus and dentate gyrus of the hippocampus, could be used to support the hypothesized pro-regenerative MOAs of Lomecel-B—one of the most difficult MOAs to investigate in patients. Potentially, these could be supported by evaluation of circulating pro-neuroregenerative biomarkers, such as BDNF.

2.3 | Limitations and further interpretations

Several limitations warrant mention. This study had a limited sample size and might have introduced imbalances in patient characteristics across treatment arms. While most biomarkers had similar baseline values, patients in the low-dose Lomecel-B arm had higher levels of Aβ isoforms versus the other arms. Similarly, both Lomecel-B arms had higher baseline total tau versus placebo. For MRIs, limited scans were amenable for volumetric analyses (75.7% of the baseline and week 13 scans, and 72.3% at week 26), and were not amendable to subregion analyses. Nonetheless, the results of this study are internally consistent, biologically plausible, and support rigorous evaluation in the next wave of clinical trials.

We also note that caregiver-completed assessments about the patient yielded mixed results (Figure 2D and Figure S3 in supporting information). These could be due to limitations in sensitivity and specificity that may make these not ideally suitable for a small interventional trial (see the Consolidated Methods and Results section and Supplementary File). Related to these and other patient assessments, such as the Trail-Making Test Parts A and B (TMT-A and TMT-B), many patients were near the best possible scores achievable at baseline (Figure S4 in supporting information). These may have imposed floor or ceiling effects (assessment-dependent) on potential improvements, which could have limited ability to observe significant effect changes within this relatively small trial.

With regard to biomarkers, those that changed generally conformed to a dose-response relationship to Lomecel-B, with the high dose showing the largest changes. This contrasts with the observation that the low-dose Lomecel-B arm generally showed better performance on clinical assessments. There are several explanations for this apparent discrepancy. Most importantly, this trial was not powered for effect, and thus the limited sample size could confound interpretation of results. Biomarkers were expected to be the more sensitive measures, and changes therein predicted to precede those of clinical assessments. Thus, the trial may have been sufficiently powered to detect biomarker changes, but less so for dementia assessment changes, particularly in the high-dose Lomecel-B arm, which had the smallest sample size. Another possibility is that the high dose could potentially have diminished anti-inflammatory properties at target sites. Such a phenomenon has been reported in ligament repair, in which enhanced pro-inflammatory biomarkers (e.g., IL-1β, IL-1α, IL-2) appear induced by a high MSC dose, with diminished ligament repair.[41] In a murine transient cortical ischemia stroke model, VEGF antagonism reduces ischemia/reperfusion-related brain edema and injury.[42] While edema was not found in this study, the possibility exists that the increased VEGF, IL-6, and hemostatic marker D-dimer (Figure 1D), the lack of significant difference from placebo on the anti-inflammatory cytokines IL-10 and IL-12, and the transient increase in hippocampal volume with the high dose of Lomecel-B, may be due to transient tissue reaction, such as inflammatory or tissue edema, and could explain the disparity with the clinical assessments results. This will be important to clarify in future studies.

Together the findings presented here support the safety of this novel therapeutic approach, and provide much needed hypothesis-generating clinical findings. Overall, these trial results are provocative, and provide a rationale for the initiation of a larger clinical trial powered to detect clinical efficacy.

3 | CONSOLIDATED METHODS AND RESULTS

3.1 | Trial design

This phase I double-blind, randomized, and placebo-controlled trial (Figure S1 in supporting information) was under oversight by a single institutional review board, data and safety monitoring board (DSMB), pharmacovigilance group, clinical monitors, and FDA under an Investigation New Drug Application (IND). Subjects and caregivers were consented for participation. Screening was three-tiered via initial clinical evaluation for probable mild AD, brain MRI to exclude confounding issues, and amyloid tracer (Neuraceq: Life Molecular Imaging) positron emission tomography (PET) to confirm the AD diagnosis. Randomized subjects received a single infusion of low- (2.0×10^7 cells) or high-dose Lomecel-B (1.0×10^8 cells), or placebo, similar to as previously

described.[43] Infusion day was defined as time 0. Follow-ups were at weeks 2, 4, 13, 26, 39, and 52 post-infusion.

3.2 | Statistical analysis

Third-party statisticians performed unblinded analyses (Statistics and Data Corp. and M2Gen). Sample size was chosen to yield a 79% probability of detecting SAEs that occur at a rate of ≥5%, and was calculated for each dose of Lomecel-B versus placebo separately. The primary endpoint was triggering of a Bayesian motivated safety stopping rule for frequency of TE-SAEs within 30 days after treatment. Calculated boundaries assumed a TE-SAE rate of 10.0%, and a TE-SAE rate >40% would trigger the stopping rule. The stopping rule had a 19% chance of Type I error, and was 91% powered.

Powering for the efficacy was not performed as this was a first-in-human study not designed for a definitive approvable outcome. Any proof-of-concept outcomes were considered hypothesis-generating to inform subsequent trials powered for effect.[44] Accordingly, alpha-spending for type 1 error rate was not performed. Two-sided tests were used with 0.05 significance level, and 95% CI calculated.[45] Effects of Lomecel-B versus placebo was via two-way analysis of variance, with significance defined as $P < 0.05$. Plots depict means and standard error of the mean. Serum biomarker expression values were normalized to the expression at time 0 for all patients, and outliers were removed by quantile approach. The *P*-values were adjusted for multiple test correction by the algorithm BH to control false discovery rate.

Follow-up compliance was 100% through week 13, and 85% through week 26 (13/15 for the low-dose Lomecel-B arm, 8/10 for the high-dose Lomecel-B arm, and 7/8 for placebo). Thereafter, it dropped such that five patients (33%) for the low-dose Lomecel-B arm, six (60%) for high-dose Lomecel-B, and two (13%) for placebo withdrew before week 52 (61% overall compliance). Six withdrawals (46%) occurred during the COVID-19 pandemic. As such, efficacy is only presented through week 26.

3.3 | Study population

This trial was conducted at four sites between November 2016 and September 2020. Of 50 screened subjects, 33 (66%) were enrolled and randomized between November 3, 2016 and September 19, 2019 (Figure 3). Leading reasons for screen failure included negative amyloid-tracer PET (52%) and MRI findings (29%). Baseline demographics are presented in Table 2. The mean age was 71.2 ± 8.4 years, and 48.5% (16/33) were female.

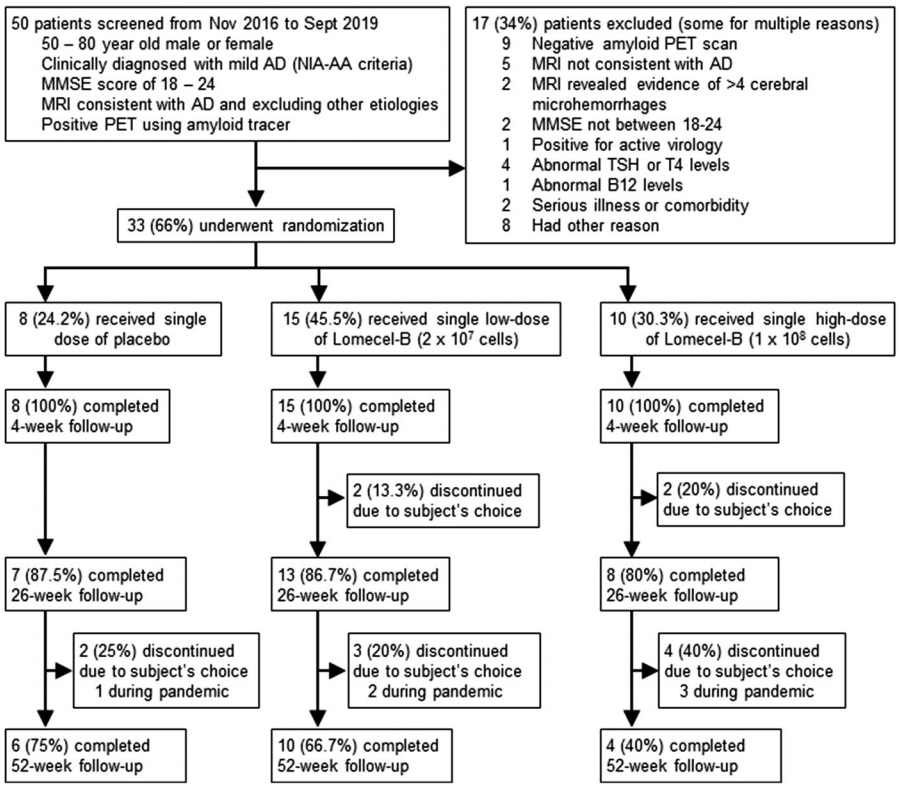

FIGURE 3 Consort diagram for trial enrollment, randomization, and trial completion. Subject screening consisted of a three-tiered process starting with a clinical assessment for probable mild Alzheimer's disease (AD), followed by magnetic resonance imaging (MRI) to rule out other potential etiologies and confounding complications, and finally a positron emission tomography (PET) scan using an amyloid tracer to confirm the mild AD diagnosis. Six of the withdrawals after the 26 week follow-up visit occurred during the COVID-19 pandemic. One subject in the placebo arm was unable to make the 26 week follow-up due to COVID-19 self-isolation, but returned for the other follow-ups. MMSE, Mini-Mental State Examination; NIA-AA, National Institute on Aging–Alzheimer's Association; TSH, thyroid stimulating hormone

TABLE 2 Baseline demographics

Variable	Placebo ($n = 8$)	20 M Lomecel-B ($n = 15$)	100 M Lomecel-B ($n = 10$)
Age (years) mean ± SD	75.9 ± 5.03	70.1 ± 9.49	69.3 ± 8.08
Female sex [*n* (%)]	6 (75.0)	4 (26.7)	6 (60.0)
Ethnicity and race [*n* (%)]			
Hispanic or Latino	1 (12.5)	3 (20)	2 (20.0)
Not Hispanic or Latino	7 (87.5)	12 (80)	8 (80.0)
White	6 (75)	13 (86.7)	10 (100.0)
Black/African American	2 (29)	1 (6.7)	0
More than one race	0	1 (6.7)	0
APOE genotype [*n* (%)]			
ε2/ε3	2 (25.0)	0	0
ε3/ε3	1 (12.5)	6 (40.0)	3 (30.0)
ε3/ε4	4 (50.0)	7 (46.7)	5 (50.0)
ε4/ε4	1 (12.5)	1 (6.65)	1 (10.0)
Unknown	0	1 (6.65)	1 (10.0)
Clinical assessment (points) mean ± SD (range)			
MMSE	20.45 ± 1.46 (18.0–22.0)	20.60 ± 2.06 (18.0–23.0)	20.70 ± 2.26 (18.0–24.0)
ADAS-Cog-11	23.46 ± 6.34 (15.7–37.7)	24.71 ± 8.49 (12.7–43.3)	25.07 ± 8.30 (12.7–38.7)
ADCS-ADL	57.60 ± 11.2 (44.0–74.0)	58.93 ± 13.3 (31.0–73.0)	50.40 ± 19.9 (20.0–73.0)
ADRQL	78.3 ± 17.5 (46.3–98.0)	89.1 ± 10.7 (69.0–100.0)	82.2 ± 16.8 (42.0–97.5)
GDS	2.8 ± 2.6 (0–7)	1.1 ± 1.4 (0–4)	1.5 ± 1.3 (0–4)
NPI	36.6 ± 39.7 (1–125)	13.1 ± 14.2 (0–46)	25.5 ± 27.2 (2–94)
QOL-AD (patient version)	36.3 ± 7.3 (25–44)	37.4 ± 4.8 (30–46)	37.5 ± 4.9 (30–45)
QOL-AD (caregiver version)	28.80 ± 4.59 (24–37)	32.50 ± 6.85 (20–42)	31.00 ± 7.02 (22–43)
Trail Making Test Part A	119.4 ± 64.0 (46–232)	101.6 ± 78.2 (28–300)	189.8 ± 111.3 (46–300)
Trail Making Test Part B	263.1 ± 104.3 (5–300)	260.0 ± 64.1 (100–300)	279.6 ± 61.8 (104–300)
Plasma biomarkers mean ± SD (range)			
IL-4 (pg/mL)	0.08 ± 0.04 (0.04–0.12)	0.13 ± 0.10 (0.04–0.34)	0.10 ± 0.06 (0.04–0.23)
IL-6 (pg/mL)	4.52 ± 8.21 (0.76–24.79)	1.94 ± 1.85 (0.71–6.98)	1.68 ± 1.15 (0.82–4.80)
IL-10 (pg/mL)	0.73 ± 0.89 (0.19–2.90)	0.51 ± 0.23 (0.15–1.16)	0.46 ± 0.20 (0.19–0.91)
sIL-2Rα (pg/mL)	589.4 ± 284.9 (268.0, 972.0)	536.1 ± 466.1 (9.0, 1809.0)	407.7 ± 247.5 (28.0, 774.0)
D-dimer (μg/mL) *	1.97 ± 2.25*** (0.29–5.75)	0.55 ± 0.41 (0.27–1.65)	0.46 ± 0.32 (0.27–1.06)

(Continues)

TABLE 2 (Continued)

Variable	Placebo ($n = 8$)	20 M Lomecel-B ($n = 15$)	100 M Lomecel-B ($n = 10$)
VEGF (pg/mL)	52.1 ± 20.3 (32–86)	42.8 ± 28.6 (11–126)	60.9 ± 39.1 (15–129)
$A\beta_{38}$ (pg/mL) **	143.9 ± 228.7 (26.6–630.2)	37,573.2 ± 136662.1**** (26.6–53,1361.8)	40.1 ± 28.6 (26.6–95.5)
$A\beta_{40}$ (pg/mL) *	65.1 ± 60.4 (12.1–189.2)	2,457.1 ± 7,676.0**** (21.0–29,952.8)	82.2 ± 47.8 (26.6–159.5)
$A\beta_{42}$ (pg/mL) **	12.9 ± 11.2 (5.2–39.7)	1,061.4 ± 3,520.2**** (7.9–13,756.2)	11.5 ± 7.2 (5.2–28.8)
NfL (pg/mL)	91.8 ± 24.2 (73.0–147.0)	101.0 ± 58.3 (26.2–270.0)	79.9 ± 33.3 (40.0–134.9)
Total tau (pg/mL)	44.6 ± 42.3 (16.5–124.7)	48268.4 ± 174172.4 (16.5–677236.6)	94135.0 ± 278841.3 (16.5–837665.9)

*$P < 0.05$ and **$P < 0.01$ for overall Kruskal–Wallace. ***$p < 0.05$ versus low- and high-dose Lomecel-B groups. ****P < 0.05 versus placebo and high-dose Lomecel-B group.

Abbreviations: Aβ, amyloid beta; ADAS-Cog-11, Alzheimer's Disease Assessment Scale–Cognitive subscale-11; ADCS-ADL, Alzheimer's Disease Cooperative Study-Activities of Daily Living; ADRQL, Alzheimer's Disease Cooperative Study-Activities of Daily Living; *APOE*, apolipoprotein E; GDS, Geriatric Depression Scale; IL, interleukin; MMSE, Mini-Mental State Examination; NfL, neurofilament light; NPI, Neuropsychiatric Inventory; QOL-AD, Quality of Life in Alzheimer's Disease; SD, standard deviation; sIL-R2α, soluble interleukin-2 receptor α; VEGF, vascular endothelial growth factor.

3.4 | Safety measures and outcomes

TE-AEs and TE-SAEs were defined as AEs or SAEs, respectively, which occurred on trial after infusion began through the end of study for each patient (week 52 follow-up visit). The TE-SAE stopping rule was never triggered, meeting the primary safety endpoint (Table 1 and Table S2).

3.5 | Biomarkers

Blood-based biomarkers were analyzed by a central laboratory (Cenetron Diagnostics) or Longeveron using the MESO QuickPlex SQ 120 system (Meso Scale Diagnostics, LLC). While pro-vascular and anti-inflammatory biomarkers showed significant increases versus placebo, no significant changes were found for any of the neuronal-related serum biomarkers examined, and interpretation of results was confounded by the large baseline differences between patients and arms. Serum levels of $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ trended higher in the Lomecel-B arms versus placebo (Table S5 in supporting information). Because plasma $A\beta_{42}$ is moderately decreased in preclinical/prodromal AD stages, and $A\beta_{40}$ and $A\beta_{42}$ show even greater significant decreases in AD,[46] these trends, if confirmed in future studies, would be consistent with improved BBB functioning to clear Aβ peptides from the brain parenchyma.

Brain MRI was performed quarterly for safety, and further used to evaluate hippocampus changes using FreeSurfer 6.0.[47] For this analysis, hippocampal size was normalized to the hippocampal fissure volume as an alternative to whole-brain normalization.

3.6 | Clinical assessments

Efficacy assessments performed on the patients were the MMSE,[32] ADAS-Cog-11,[36] QOL-AD,[38] TMT-A and TMT-B (Reitan Neuropsychology Laboratory), and Geriatric Depression Scale (GDS). Consistent with the MMSE, ADAS-Cog-11, and QOL-AD results, the TMT-A, TMT-B, and GDS showed potential trending improvements over placebo, but these did not reach significance (Figure S4).

Caregiver assessments of the patients included the Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL),[48] QOL-AD, Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADRQL),[49] and Neuropsychiatric Inventory (NPI),[50] and yielded mixed results (Figure 2D and Figure S3). The ADCS-ADL, which assesses patient abilities to perform activities of daily living, showed a significant and precipitous decline at week 26 in the placebo arm (Figure 2D), which was inconsistent with prior trajectory (6.95 ± 3.46 point change difference between the low-dose Lomecel-B and placebo arms: $P = 0.0118$; 95% CI 1.99 to 13.94). The placebo group showed trending improvements on the ADRQL and QOL-AD caregiver-version. The NPI was a particular outlier, in which the placebo showed an unexpected improvement that reached significance versus the low-dose Lomecel-B arm. Because improvement over time is unexpected for AD patients, it is possible these assessments may have lacked the sensitivity and specificity for this small interventional trial.

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CONFLICTS OF INTEREST

This research was supported by two grants from the Alzheimer's Association awarded to Longeveron (AAO, principal investigator). AAO, BH, LM-M, KR, and JH received partial salary support from these grants. MB, MA, BJH, and BB were clinical investigators on the clinical trial of this study, to which institutional payments were made by Longeveron, partially using funds from these grants. TW received a consulting fee from Longeveron that was pre-approved by his institution to perform MRI analyses, and paid in part by one of these grants. FS, BV, SB, MB, MA, and BJH serve as members of Longeveron's Alzheimer's Disease Program Steering Committee, and for which no support was made from these grants. AAO, BH, LM-M, KR, and JH are affiliated with Longeveron Inc., and receive salary support and stock options from Longeveron. In addition, the authors report the following over the past 36 months. MB has contracted with Biogen, Lilly Cerevel, UCB, AgeneBio, Cortexyme, Genentech, Roche, NIH, Cognito, BioIVT, Athira, Novartis, EISAI, ABBVIE, Cassava, Biohaven, Athira, Alector, GreenValley, Janssen, SAMUS, Vaccinex (institutional payments made). He also serves on advisory boards for both Lilly and Biogen, and has been a paid consultant for Biogen. MA has contracted with Biogen and Mededicus, and is President-elect (2022) of the American Association for Geriatric Psychiatry. SB has received (in the form of institutional support) grants from the Alzheimer's Association (Zenith award), NICHD (2 center grants, 1 network), NIA (multi-site, site PI), NIDA (multi-site, site PI), and NIMH (multi-site; site PI), which were not used for this study. Her institution has also received payments for patent from MS Cohen on a signal process algorithm unrelated to the current paper, and receives direct consulting fees on MRI research from the Pacific Neuroscience Institute, and received a speaker honorarium from the Society of Biological Psychiatry. GS has contracted or consulted for the AARP, Acadia, Allergan, Avanir, Biogen, Genentech, Gerontological Society of America, Handok, Herbalife, Home Care Assistance, Longeveron, McCormick Spice Institute, Medscape, Reckitt Benckiser, Roche, Theravalues, and WebMD. He has also received grant support from NIH (Columbia University Multi-site Clinical Trials), and is on a UCLA patent for FDDNP-PET. BH is a co-investigator on a Maryland Stem Cell Research Fund grant unrelated to this study, and is a co-inventor on a provisional patent related to Lomecel-B. BV has received grants from the Ton Institution, Lilly, Pfizer, and Pierre Fabre Merci outside of the presented work and provided to the institution, has received consulting fees from Lily, Biogen, Roche, and Longeveron outside of this work. FS has received consulting fees from Indiana University, and from the Fisabio Conferences, all paid directly to him. JMH is a co-founder, Chief Science Officer, and board member of Longeveron Inc. This relationship is reported to the University of Miami, and a management plan is in place. JMH is inventor of intellectual property licensed to Longeveron. Longeveron has paid license fees to the University of Miami through an Exclusive license fee, and as a university faculty member, he has received an institutionally designated share of these fees. He is a named co-inventor on provisional patents submitted by Longeveron, and an investigator on grants from the National Institutes of Health and Department of Defense unrelated to this research (institutional payments made). He has also served on two DSMBs (one for the National Institutes of Aging; the other for the National Eye Institute), has received support for presentation at the American Heart Association (covered by an NIH grant to University of Miami), and is affiliated with the Heart Failure Society of America. AAO has received grant support as principal investigator from the National Institute of Aging (two awards) and Maryland Stem Cell Research Fund (three awards) that were unrelated to this research (institutional payments made). He has received travel/accommodations support from the National Institutes of Health for the Geroscience Summit III, and from the University of the West Indies (The Bahamas) for presentations (paid directly to him). He is also a co-inventor on provisional patents submitted by Longeveron. The University of Miami is an equity owner in Longeveron, which has licensed intellectual property from the University of Miami.

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