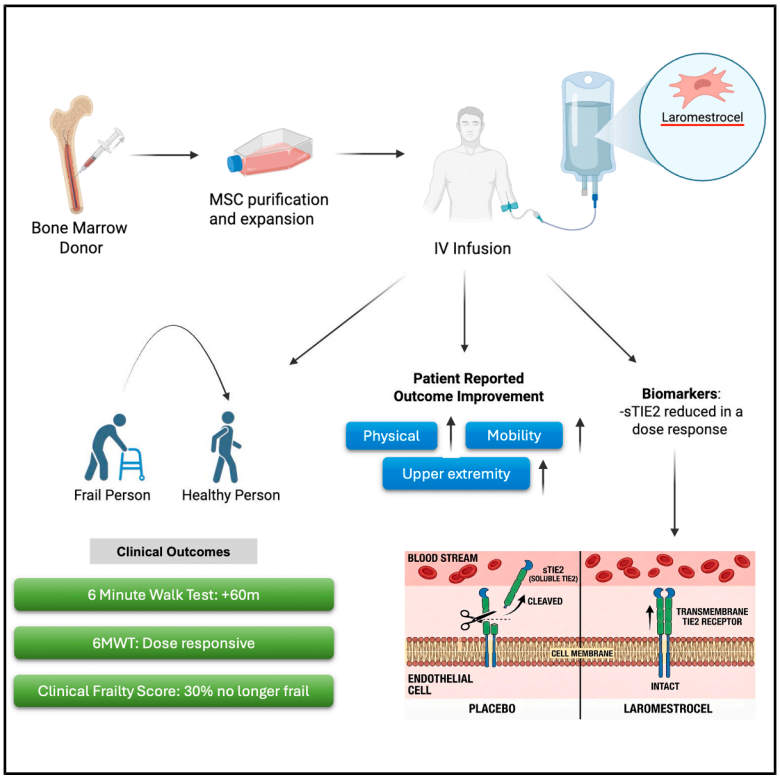
Cell Stem Cell

Clinical and Translational Report

Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty

Graphical abstract



Highlights

- Performance on the 6-minute walk test improved in a dose-response fashion
- Improved 6-minute walk test distance correlated with patient-reported outcomes
- The percentage of study subjects classified as frail decreased by month 9
- Decreased soluble TIE2 in blood may reflect improved vascular function and inflammaging

Authors

Jorge G. Ruiz, Anthony A. Oliva, Jr., Kevin N. Ramdas, ..., Zarin Zainul, Brian G. Rash, Joshua M. Hare

Correspondence

jhare@longeveron.com

In brief

Ruiz et al. present results of a phase 2 randomized trial of intravenous laromestrocel, a mesenchymal stem cell product, which improved the physical condition of patients with age-related clinical frailty after 9 months, compared with placebo. Reduced sTIE2 in blood provides a mechanistic link to improved vascular function and inflammaging.

Ruiz et al., 2026, Cell Stem Cell *33*, 1–12
March 5, 2026
https://doi.org/10.1016/j.stem.2026.01.017

CellPress

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

Cell Stem Cell

CellPress

Clinical and Translational Report

Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty

Jorge G. Ruiz,[1,2,17] Anthony A. Oliva, Jr.,[3,17] Kevin N. Ramdas,[3] Julian Javier,[4] Jeffrey Rosen,[5] Robert Perry,[6] Antonio Blanco,[7] Pedro Ylisastigui,[8] Jeremy Walston,[9,18] Hidenori Arai,[10] Elena Volpi,[11] Anne B. Newman,[12] Brittany Varnado,[3] Lisa McClain-Moss,[3] Eric Naioti,[3] Danial Mehranfard,[3] Dan Gincel,[3,18] Chao Wang,[13] Michael J. Mintzer,[14] Joel Danisi,[14] Geoff A. Green,[3] Jeffrey Botbyl,[15] Zarin Zainul,[3] Brian G. Rash,[3] and Joshua M. Hare[3,16,19,*]

[1]Memorial Healthcare System, Hollywood, FL, USA
[2]Florida Atlantic University Schmidt College of Medicine, Boca Raton, FL, USA
[3]Longeveron Inc., Miami, FL, USA
[4]Advanced Research for Health Improvement, LLC, Naples, FL, USA
[5]Clinical Research of South Florida, Coral Gables, FL, USA
[6]Panax Clinical Research, Miami Lakes, FL, USA
[7]Vista Health Research, LLC, Miami, FL, USA
[8]Clinical Physiology Associates, Ft. Myers, FL, USA
[9]Johns Hopkins University, Baltimore, MD, USA
[10]National Center for Geriatrics and Gerontology, Obu, Japan
[11]Sealy Center on Aging, University of Texas Medical Branch, Galveston, TX, USA
[12]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA
[13]Pharma Data Associates LLC, Piscataway, NJ, USA
[14]Miami VA Healthcare System Geriatric Research, Education and Clinical Center (GRECC), Miami, FL 33125, USA
[15]Provonix, Sewell, NJ 08080, USA
[16]Interdisciplinary Stem Cell Institute, University of Miami Miller School of Medicine, Miami, FL, USA
[17]These authors contributed equally
[18]Deceased
[19]Lead contact
*Correspondence: jhare@longeveron.com
https://doi.org/10.1016/j.stem.2026.01.017

SUMMARY

Frailty, a syndrome that decreases healthspan in older individuals, lacks effective therapies. We conducted a randomized, dose-finding clinical trial to test whether human bone marrow-derived allogeneic mesenchymal stem cells (MSCs; laromestrocel) improve physical functioning and patient self-reported outcomes in ambulatory individuals with frailty (ClinicalTrials.gov #NCT03169231; $N = 148$). Laromestrocel infusion results in clinically meaningful, dose- and time-dependent increases in the 6-min walk test (6MWT; primary endpoint) compared with placebo: 63.4 m (95% confidence interval [CI]: 17.1–109.6 m; $p = 0.0077$) at month 9 and 41.3 m (95% CI: −2.4–84.9 m; $p = 0.0635$) at month 6. Increased 6MWT distance correlates with PROMIS Physical Function score, and increasing doses of laromestrocel are associated with decreases in soluble (degraded) tyrosine kinase with immunoglobulin and epidermal growth factor homology domains (TIE2), the cognate receptor for the angiopoietins, identifying a potential biomarker of laromestrocel responsiveness. These findings identify a stem cell therapy approach for the management of patients with hypomobility and other features of aging frailty.

INTRODUCTION

Aging-related frailty is defined as a state of heightened vulnerability to stressors, resulting in disproportionate rates of adverse health care outcomes.[1] As a result, older individuals with frailty are at increased risk for disability, mobility impairment, dementia, hospitalization, institutionalization, and death; disproportionately consume healthcare resources; and have diminished healthspan.[2,3] An estimated 12%–24% of people 65 years and older are frail, with the proportion increasing significantly with advancing age.[4,5]

A key manifestation of frailty is a diminution in physical functioning that affects both strength and endurance.[6–8] Consequently, affected individuals have limited life space due to reduced mobility, reduced quality of life (QOL), and impairment in activities of daily living (ADLs).[9–11] There is increasing

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

recognition that measuring and modifying hypomobility is of both clinical and policy importance, particularly with aging populations.[6,10,11] For this study, we used the 6-min walk test (6MWT) as an integrated assessment of multiple physiological systems for strength, mobility, and endurance.[12] The 6MWT is a valid and reliable indicator of the ability to perform basic ADLs and to assess frailty status and captures meaningful life-space measures, e.g., the ability to walk around a block.[13]

Frailty is a multisystemic, degenerative state independent of normal aging that may be reversible and potentially responsive to treatment.[14] Among the many underlying causes and mechanisms for frailty[15] are low-grade chronic inflammation, termed "inflammaging," characterized by increased levels of pro-inflammatory cytokines.[16–19] Another contributor, which is linked to and can be caused by inflammaging, is vascular-endothelial dysfunction, coupled with atherosclerosis, vasomotor changes, and further inflammation.[20] Vascular biomarkers are therefore a subject of key interest in the frailty space. Furthermore, frailty is associated with abnormal skeletal muscle function[21] and reduction in the number and renewal capacity of multiple types of stem cells,[22–25] including muscular, neural, and hematopoetic.[26] Therefore, therapeutic strategies targeting underlying mechanisms of frailty, including stem cell restoration, might show clinically measurable benefits.

Here, we report the results of a phase 2b dose-finding trial using an allogeneic mesenchymal stem cell (MSC) formulation, called laromestrocel (Lomecel-B), for aging-related frailty (individuals enrolled were 70 to 85 years of age) without dementia. Laromestrocel has multi-modal mechanisms of action (MOAs) that have the potential to address multiple biological targets in frailty, including inflammaging, vascular dysfunction/vessel restoration, and depressed skeletal muscle energetics.[27,28] These multipotent stem cells offer potential as an allogeneic, readily available therapeutic with a high safety profile demonstrated in previous clinical studies,[29–31] including phase 1/2 studies conducted in older adults with aging-related frailty[32–34] and Alzheimer's disease (AD)[35,36]—diseases that both feature inflammation as a core driver of pathology. Remarkably, the studies of laromestrocel in AD treatment showed reduced brain atrophy, improved cognitive performance and QOL, and signs of reduced inflammation in the brain with laromestrocel infusion, compared with placebo.[35] This study on aging frailty was powered for effect based on changes in the 6MWT in previous exploratory studies[33,37] and designed to evaluate the dose-response relationship of laromestrocel to 6MWT, explore patient-reported outcomes (PROs), and examine candidate serum biomarkers that could impact vascular function and angiogenesis. We identified soluble tyrosine kinase with immunoglobulin and epidermal growth factor homology domain (sTIE2)—a receptor activated through binding of the angiopoietins, ANGPT1 and ANGPT2—as a candidate biomarker for vascular function and inflammation that appears to have a dose-response relationship with laromestrocel infusion quantity.

RESULTS

Between August 2017 and February 2020, 365 patients were screened, and 155 met all inclusion/exclusion criteria (Table 1) and were randomized (Figure 1). Reasons for screen failure were TNF-α < 2.5 pg/mL (n = 57; 26.9%), hepatitis B virus positivity (n = 28; 13.2%), 6MWT distance out of range (n = 19; 9.0%), HbA1c > 8.0% (n = 19; 9.0%), and various other reasons (each < 9%). Seven subjects were excluded from the analyses due to withdrawal prior to infusion. The remaining 148 subjects received a single infusion of study product (laromestrocel or placebo) and were included in the safety population analyses. Of these, 137 completed the trial (95.8%); 5 discontinued by choice (3.4%), 4 were lost to follow-up (2.7%), and 2 died on study (1.4%). Of the safety population, 143 had at least 1 follow-up visit and made up the modified intention-to-treat (mITT) population for efficacy analysis: group 1, placebo (n = 29); group 2, 25 million (25M) laromestrocel (n = 35); group 3, 50M laromestrocel (n = 30); group 4, 100M laromestrocel (n = 33); and group 5, 200M laromestrocel (n = 16). Table 2 depicts well-balanced baseline characteristics between the 5 groups. The mean participant age was 74.3 to 76.8 years, with 20.6% to 53.3% female, and generally mild to moderate frailty with a mean Canadian Study of Health and Aging (CSHA) Clinical Frailty Scale (CFS) score of 5.1. Potency characteristics of conditioned medium from all lots of laromestrocel used in the study are shown in Table S2.

Dose-dependent increase in 6MWT distance

Baseline 6MWT distances were comparable for all arms, approximately 300 meters in all groups (Table 2). The first component of the primary endpoint was change in 6MWT for an individual dose of laromestrocel relative to placebo at 6 months post-infusion, which was numerically improved but did not reach statistical significance (Figure 2A; Table S3). A formal dose-response analysis—the second, pre-specified component of the primary endpoint—showed a statistically significant relationship of increasing laromestrocel dosage to increased change in 6MWT distance at 6 months (Figure 2B). All 5 dose-response candidate models tested were statistically significant ($p < 0.05$ for each), in which the dose-response was best modeled by the linear curve, which has a significant p-value (p = 0.0321) and the smallest Akaike Information Criterion (AIC) within the dose range tested. The difference in 6MWT between the highest dose of laromestrocel (2×10^8 cells: 200M) and placebo was 41.3 m (95% confidence interval [CI]: −2.4–84.9 m; p = 0.0635) at month 6. We next assessed the full dose-response relationship at all time points. When differences at month 9 were analyzed, the change in laromestrocel for 50M and 200M doses did reach statistical significance, reflecting ongoing time-dependent separation between active treatment arms and placebo (Figure 2A; Table S3). At month 9 post-infusion, the difference in change between the 200M laromestrocel arm and placebo was 63.4 m (95% CI [17.1, 109.6] m; p = 0.0077). At 9 months, the 50M laromestrocel arm 6MWT distance was also improved by 49.2 m (95% CI [10.9, 87.5] m; p = 0.0122). In addition, the changes in 6MWT from baseline are presented (Figure 2A; Table S3). Changes from baseline in 6MWT were significantly improved for 3 of 4 laromestrocel groups at 6 months (50M: p = 0.0053; 100M: p = 0.0443; 200M: p = 0.0065) and 2 of 4 laromestrocel groups at 9 months (50M: p = 0.0125; 200M: p = 0.0115), but not statistically significant for placebo at any time point.

To explore any important demographic impact upon the response to laromestrocel, we examined responses stratified for patient sex and body mass index (BMI). There was no

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

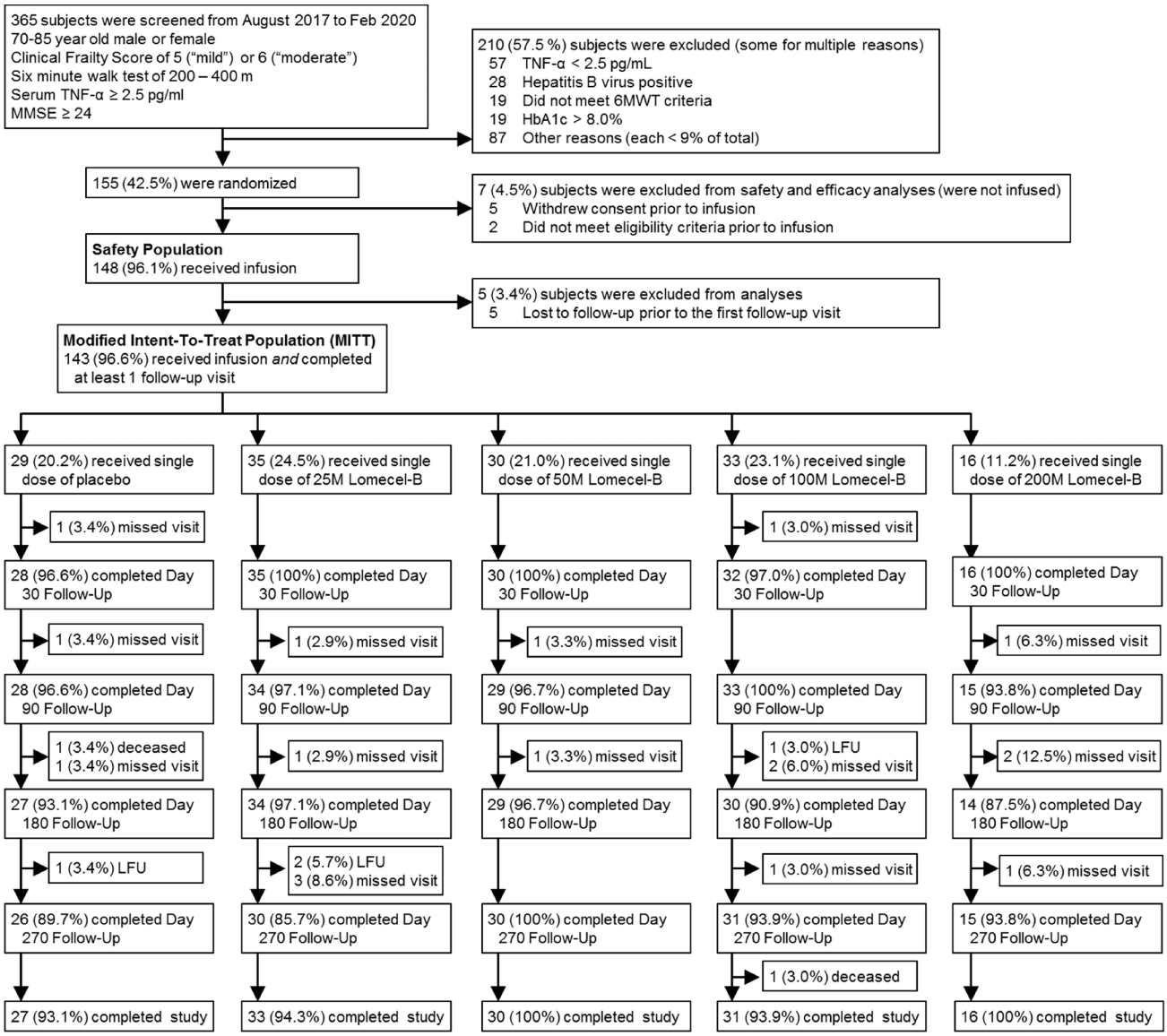


Figure 1. CONSORT diagram for trial enrollment, randomization, and trial completion

Of 365 screened subjects, 155 were enrolled that ranged in age from 70 to 85 years, had a CFS score of 5 ("mild") or 6 ("moderate"), a 6MWT distance of 200–400 m, and serum TNF-α ≥ 2.5 pg/mL. Seven subjects withdrew prior to infusion and were excluded from further analyses. Of the 148 patients receiving infusion (defined as the Safety Population), 143 completed at least one follow-up visit, which was defined as the modified Intent-To-Treat (mITT) population for efficacy analyses. Of these, 137 (95.8%) completed the trial. Withdrawals and missed visits were due to various causes, including the COVID-19 pandemic.
LFU, lost to follow-up.

treatment interaction with sex affecting the response to laromestrocel ($p = 0.675$ treatment by sex). To address BMI, we included it as a covariate in our model and found that it also did not impact study results ($p = 0.740$ treatment by BMI interaction).

Patient-Reported Outcomes

The Patient-Reported Outcomes Measurement Information System (PROMIS) Physical Function SF20 was used as a secondary endpoint to evaluate patient-perceived changes in overall physical functioning. Similarly, the PROMIS Mobility and PROMIS Upper Extremity were used to evaluate mobility and upper body function, respectively, as prospectively tested pre-specified exploratory endpoints. Although we did not detect a statistically significant change compared with placebo in the PROMIS scores, the change in 6MWT and PROMIS Physical Function SF20 were correlated. The month 6 post-infusion Pearson Correlation Coefficient for all treatment groups combined with placebo was R = 0.3124 ($p = 0.0002$) (Figure 3**A**), and for raw 6MWT versus PROMIS R = 0.44 ($p < 0.0001$). There was a trending correlation between change from baseline in 6MWT and PROMIS for placebo alone, but it did not reach statistical significance (R = 0.35; $p = 0.0752$). Likewise, the month 6 post-infusion correlation coefficient between the 6MWT and PROMIS Mobility

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

Table 1. Inclusion/exclusion criteria

Inclusion criteria	
1	Be willing and able to provide written informed consent and comply with all procedures required by the protocol.
2	Be >70 and <85 years of age at the time of signing the Informed Consent Form.
3	Have a CSHA Clinical Frailty Scale score of 5 "mildly frail" or 6 "moderately frail."
4	Have a 6-min walk distance of >200 m and <400 m. The distances of two 6MWTs are to be within 15% of each other (see 9.1.1.2).
5	Have a serum TNF-α level > 2.5 pg/mL.
Exclusion criteria	
1	Be unwilling or unable to perform any of the assessments required by the protocol.
2	Have a diagnosis of any disabling neurologic disorder, including, but not limited to, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, cerebrovascular accident with residual deficits (e.g., muscle weakness or gait disorder), or the diagnosis of dementia.
3	Have a score ≤24 on the Mini Mental State Examination (MMSE).
4	Have poorly controlled blood glucose levels (HbA1c >8.0%).
5	Have a clinical history of malignancy within 2.5 years (i.e., subjects with prior malignancy must be cancer free for 2.5 years), except for curatively treated basal cell carcinoma, squamous cell carcinoma, melanoma *in situ*, or cervical carcinoma if recurrence occurs.
6	Have any condition that in the opinion of the principal investigator limits lifespan to <1 year.
7	Have an autoimmune disease with the exception of psoriasis (e.g., rheumatoid arthritis, systemic lupus erythematosus).
8	Be currently taking corticosteroids or similar powerful steroidal anti-inflammatory medication (e.g., prednisone and TNF-α antagonists) on a regular basis (exceptions allowed include regular use of steroidal nasal sprays, topical steroids, and estrogen-replacement therapy).
9	Test positive for hepatitis B virus: a. If the subject tests positive for anti-HBc or anti-HBs, they must be currently receiving treatment for hepatitis B prior to infusion and remain on treatment throughout the study.
10	Test positive for viremic Hepatitis C virus, HIV1/2, or syphilis.
11	Have a resting blood oxygen saturation of <93% (measured by pulse oximetry).
12	Known or suspected alcohol or drug abuse within three years preceding Screening.
13	Have a known hypersensitivity to dimethyl sulfoxide (DMSO).
14	Be an organ transplant recipient (other than transplantation for corneal, bone, skin, ligament, or tendon).
15	Be actively listed (or expected future listing) for transplant of any organ (other than corneal transplant).
16	Have any clinically important abnormal screening laboratory values, including, but not limited to: a. Hemoglobin <10.0 g/dL, b. White blood cell <2,500/μl or platelet count <100,000/μl. c. Liver dysfunction evidenced by enzymes (AST and ALT) > 3 times the ULN d. Coagulopathy (INR > 1.3) not due to a reversible cause (e.g., warfarin and/or factor Xa inhibitors).
17	Uncontrolled hypertension (resting systolic blood pressure >180 mm Hg or diastolic blood pressure of >110 mm Hg at screening).
18	Have unstable angina pectoris, uncontrolled, or severe peripheral artery disease within the previous 3 months.
19	Have congestive heart failure defined by NYHA (New York Heart Association) Class III or IV, or an ejection fraction of <25%.
20	Have coronary artery bypass surgery, angioplasty, peripheral vascular disease revascularization, or a myocardial infarction within the previous 3 months.
21	Have severe pulmonary dysfunction: acute exacerbation of chronic obstructive lung disease stage III or IV (Gold classification), and/or PaO_2 levels <60 mmHg.
22	Have a partial ileal gastric bypass or other significant intestinal malabsorption.
23	Have documented advanced hepatic or renal disease.
24	Have cognitive or language barriers that prohibit obtaining informed consent or any study elements.
25	Be currently hospitalized or living in a long-term care facility (e.g., nursing home).
26	Be currently participating (or have participated within the previous 30 days of consent) in an investigational therapeutic or device trial.
27	Have a history or current evidence of any condition, therapy, or clinically significant laboratory abnormality, including urinalysis, or other circumstance that, in the opinion of the investigator, might confound the results of the study or interfere with his or her participation for the full duration of the study.

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

Table 2. Screening/baseline demographics (safety population; *N* = 148)

Treatment group	Placebo	25M	50M	100M	200M
Number of subjects (*n*)	30	37	31	34	16
Age (Mean years ± SD)	74.3 ± 4.1	76.8 ± 3.8	74.8 ± 3.7	75.8 ± 3.8	75.8 ± 4.0
Female sex [*n* (%)]	16 (53.3)	16 (43.2)	14 (45.2)	7 (20.6)	7 (43.8)
Ethnicity [*n* (%)]					
Hispanic/Latino	15 (50.0)	21 (56.8)	14 (45.2)	23 (67.6)	5 (31.3)
Race [*n* (%)]					
White	28 (93.3)	36 (97.3)	29 (93.5)	33 (97.1)	16 (100.0)
Black/African American	2 (6.7)	1 (2.7)	2 (6.5)	1 (2.9)	0
6MWT (meters; mean ± SD)[a]	314.7 ± 64.9	304.9 ± 59.0	316.8 ± 59.2	311.6 ± 55.4	308.1 ± 63.1
TNF-α (Mean pg/mL ± SD)[b]	3.77 ± 1.19	3.90 ± 1.65	3.88 ± 0.83	3.54 ± 0.98	3.79 ± 1.16
PROMIS Physical Function SF-20 (Mean score ± SD)[a]	41.4 ± 5.7	41.4 ± 7.4	42.9 ± 8.8	42.7 ± 8.5	40.9 ± 6.1
PROMIS Mobility (Mean score ± SD)[a]	41.4 ± 6.9	41.4 ± 8.7	42.4 ± 9.1	42.2 ± 8.4	40.1 ± 8.3
PROMIS Extremity (Mean score ± SD)[a]	45.1 ± 8.6	43.5 ± 10.0	46.6 ± 11.1	45.8 ± 10.1	42.3 ± 9.1
MMSE [Mean points ± SD]	28.8 ± 1.5, (25–30)	28.7 ± 1.4, (25–30)	28.8 ± 1.4, (25–30)	28.8 ± 1.4, (25–30)	29.1 ± 0.8, (28–30)
TIE2 [Mean ng/L ± SD][a]	4,089.0 ± 2,374.5	3,473.3 ± 1,372.8	4,422.1 ± 1,643.9	3,765.0 ± 1,722.1	3,600.0 ± 1,675.4
CSHA Clinical Frailty Scale (CSF) (Mean score ± SD)[a]	5.1 ± 0.3	5.1 ± 0.3	5.1 ± 0.3	5.1 ± 0.3	5.1 ± 0.3
CHS Frailty "Fried" Phenotype (Mean score ± SD)[a]	1.4 ± 1.1	1.5 ± 1.1	1.4 ± 1.1	1.5 ± 1.2	1.1 ± 0.9

[a]Efficacy baseline assessment from the mITT population (N = 143).
[b]TNF-α values are from the screening visit.

was 0.3046 (p = 0.0003), and for the 6MWT and PROMIS Upper Extremities was 0.2318 (p = 0.0070).

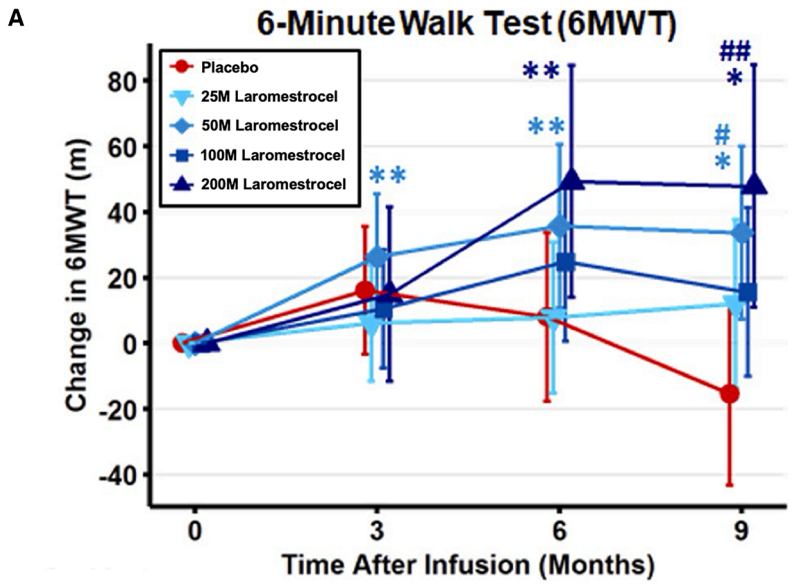
We next examined whether laromestrocel influenced the change in the CSHA-CFS. Categorization of CFS scores into bins (CFS 2–3, CFS 4–5, or CFS 6) accordingly showed greater percentages of subjects who improved after laromestrocel treatment compared with placebo and correspondingly higher rates of moderate frailty (CFS 6) at 9 months in the placebo arm. While only 14.8% (95% CI: 4.19–33.73%) of placebo subjects improved to CFS grade 2–3 (defined as being well, with or without treated co-morbidities) at 9 months, 30.8% (95% CI: 22.27–40.5%) of subjects receiving laromestrocel improved to grade 2–3 (Figure S2; Table S4), suggesting the potential for a clinical benefit of laromestrocel in aging frailty.

sTIE2 as a biomarker of activity

A prespecified exploratory goal of this clinical trial was to identify a blood biomarker with the potential to predict a functional outcome of laromestrocel treatment and that exhibited a laromestrocel-related dose-response change. To address this, we screened 8 biomarkers using a vascular/angiogenesis meso scale discovery (MSD) panel plus C-reactive protein. Of all of the screened potential biomarkers explored in this study, soluble TIE2 (sTIE2) was identified as a candidate biomarker that met these criteria. TIE2 (also known as TEK) is a tyrosine kinase receptor present on microvascular endothelium and endothelial precursor cells and is activated through binding of the angiopoietins, ANGPT1 and ANGPT2, which act in a generally antagonistic fashion to balance downstream signaling related to vascular health and angiogenesis[38] and also regulate inflammatory responses.[39,40] TIE2 is thus a key receptor at the confluence of major angiogenic/vascular health and inflammatory pathways. TIE2 is proteolytically cleaved by matrix metalloproteases (MMPs),[41] which are inhibited by tissue inhibitors of metalloproteases (TIMP)s,[8] and as such, increasing levels of sTIE2 in the circulation reflect degradation of this key cell-surface signaling pathway receptor. Numerous groups have identified elevated circulating levels of sTIE2 in diverse pathophysiological states notably involving the vasculature, ranging from peripheral vascular disease,[42] myocardial ischemia,[43] sepsis,[44,45] and central nervous system/psychiatric illnesses.[46–48] We confirmed that TIMP2 is secreted in large amounts by laromestrocel[35] (see TIMP2 testing results for this trial in Table S2), and we found that sTIE2 was significantly decreased in the 100M laromestrocel group compared with placebo (n = 27) at 6 months post-infusion (Figure 4A), (−400.0 pg/mL; 95% CI [−762.58, −37.51] pg/mL; n = 28, p = 0.021). At 9 months post-infusion, the 100M laromestrocel group was lower compared with placebo (n = 26) by −562.6 pg/mL (95% CI [−898.58, −226.69] pg/mL; n = 29, p = 0.001). An Emax model best described the dose-response (p = 0.006), with a plateau evident at the 50M laromestrocel dose (Figure 4B).

There were changes in other vascular and inflammatory biomarkers, but none met the criteria for a dose-response relationship. These included placenta-derived growth factor (PlGF), basic fibroblast growth factor (bFGF), Fms-related tyrosine kinase 1 (FLT1), and vascular endothelial growth factors (VEGF)-A, -C, and -D (Figure S1). Baseline values for all serum

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017



	Baseline (*n*)	Month 3 (*n*)	Month 6 (*n*)	Month 9 (*n*)
Placebo	29	28	27	26
25M	35	34	34	30
50M	30	29	29	30
100M	33	33	30	31
200M	16	15	14	15

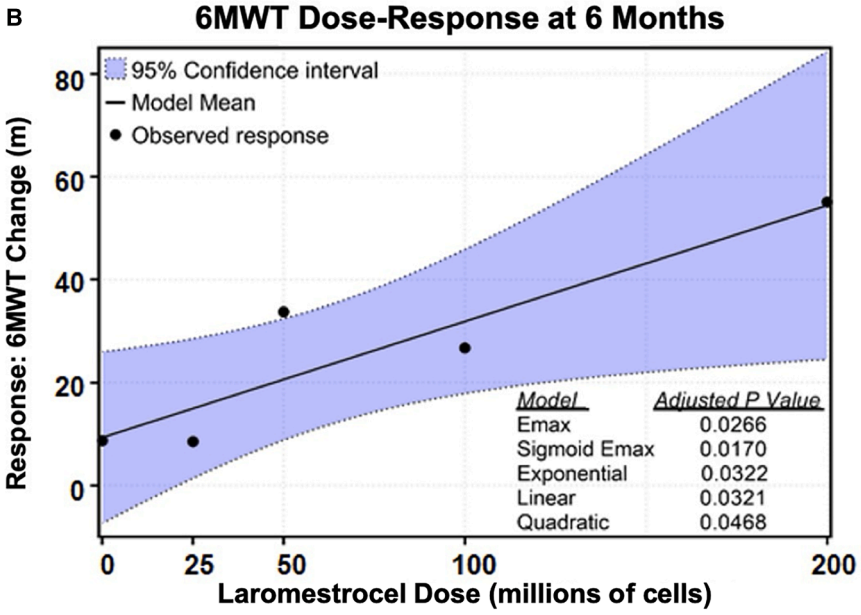


Figure 2. Dose-dependent increases in 6MWT in the laromestrocel arms versus placebo

(A) Change in 6MWT distance versus time in the mITT population. All four laromestrocel arms showed trending or significant increases in walk distance, in which the 200M dose showed the greatest change from baseline. By contrast, the placebo arm showed a trending decrease by 9 months post-treatment. $^{*}p < 0.05$ for change from baseline. $^{**}p < 0.01$ for change from baseline. $^{\#}p < 0.05$ for change from baseline of the laromestrocel arm versus change from baseline in the placebo. $^{\#\#}p < 0.01$ for change from baseline of the laromestrocel arm versus change from baseline in the placebo. Error bars represent ± 95% confidence interval.

(B) The dose-response effect at 6 months was calculated using the multiple comparison procedure-modeling (MCP-Mod) method. All five models were statistically significant, although the linear model yielded the smallest AIC value, suggesting it was the most parsimonious predictive model.

See also Table S3.

biomarkers are provided in Table S5. Potential beneficial changes in key ligands for vascular growth and health were observed, including significantly increased PlGF at months 1 (25M, $p = 0.031$), 3 (25M, $p = 0.030$), and 6 (50M, $p = 0.015$), and numeric but not statistically significant increases in bFGF and VEGF-D for all but one treatment group compared with placebo at months 3 and 9, and one group meeting statistical significance at month 9 (200M VEGF-D; $p = 0.017$) (Figure S1). C-reactive protein (CRP), a marker for systemic inflammation, did not show statistically significant changes with respect to placebo at any time point (Figure S1).

Safety and clinical events

No safety concerns on this study were raised by either the National Institute of Aging (NIA)-appointed Data and Safety Monitoring Board (DSMB) or pharmacovigilance monitor. Overall, the proportion of subjects with treatment-emergent serious adverse events (TE-SAEs) was comparable across the different study arms (Table S6). There were 2 deaths in the study: a pulmonary embolism occurring 296 days post-infusion in the 100M laromestrocel arm and a combined cerebral arteriosclerosis/coronary artery disease/aspiration pneumonia occurring 167 days post-infusion in the placebo arm. There were no SAEs attributed to the study product. Two infusions were temporarily interrupted (both in the 25M laromestrocel arm) but continued to completion, and both subjects completed follow-up visits. All adverse events (AEs) that occurred during the infusion were considered by investigators and the DSMB as not product related. There were no statistically significant differences in the rates of falls, fractures, hospitalizations, and admissions to healthcare facilities in any of the laromestrocel arms versus placebo.

To determine whether variations in laromestrocel potency could account for differential group responses, mixed-effects

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

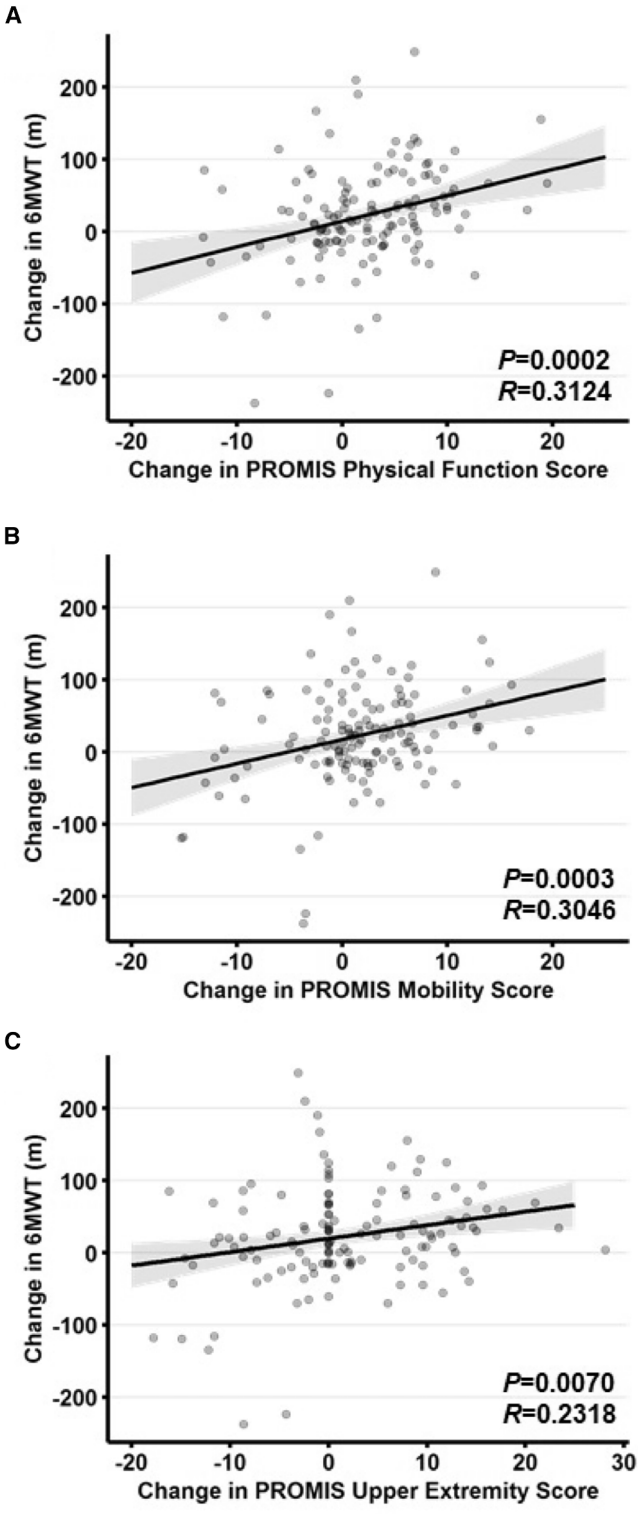


Figure 3. Changes in PROMIS assessments correlated to changes in 6MWT distance

(A) Change in the PROMIS Physical Function SF20 at 6 months post-treatment in the mITT population, chosen as a secondary endpoint, significantly correlated to the change in 6MWT.

(B and C) Change in the PROMIS Mobility and PROMIS Upper Extremity also both significantly correlated to the change in 6MWT at 6 months post-treatment.

models for repeated measures (MMRMs), including lot and the lot-by-treatment interaction, were fitted for 6MWT, CFS, and PROMIS to assess the potential influence of lot on treatment response. Neither the main effect of the lot nor the lot-by-treatment interaction was statistically significant in any model.

DISCUSSION

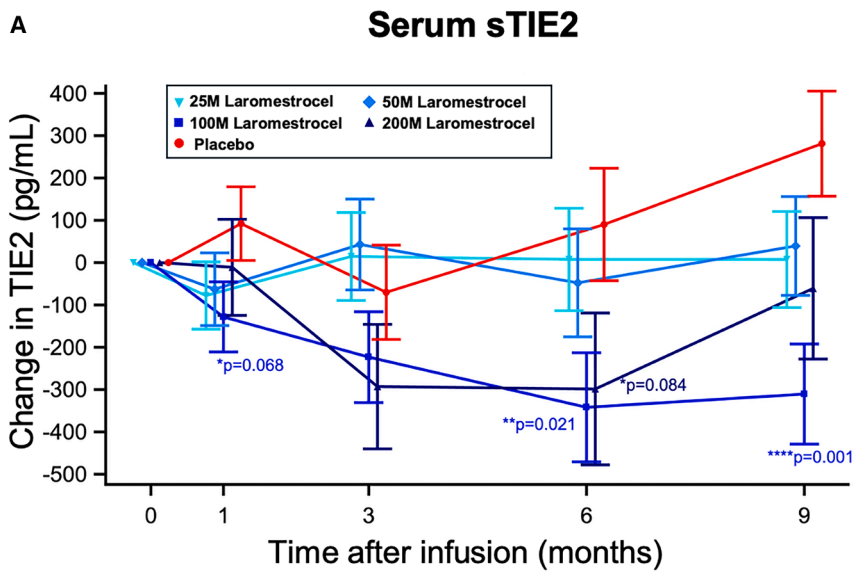
A principal finding of this study is that a single infusion of laromestrocel, when compared with placebo, led to a dose-dependent increase in walking distance in older adults with mild to moderate frailty. The increases seen in the highest dose groups exceeded minimally significant clinical thresholds[49–51] and correlated with improvements in patient self-reported outcomes. Finally, serum levels of sTIE2, a factor shown to increase in the circulation in association with impaired vascular function (Figure 5), also improved (decreased levels) in a dose-dependent fashion with laromestrocel treatment. These findings are consistent with the potential that laromestrocel may treat frailty and lead to improvements in the patient's QOL and mobility, thereby increasing life space, reducing dependency on others, and leading to improved healthspan.

Our findings extend previous studies in patients with frailty, in which a single infusion of allogeneic MSCs suggested improved walk distance.[32,33] An additional pre-specified objective of this trial was to establish whether laromestrocel manifests a dose-response effect. Indeed, a clear and significant dose-response relationship to an increase in 6MWT was evident at 6 months post-treatment, supporting evidence of bioactivity. Furthermore, at 9 months post-infusion, improvement was sustained and reached statistical significance versus placebo, which had begun to decline from baseline (Figure 2A). The stronger difference between the treatment and placebo groups observed at 9 months may indicate ongoing and sustained bioactivity, or it could indicate an indirect downstream effect, such as fulfillment of prerequisite angiogenesis in muscle or attenuation of inflammaging processes. As such, future studies should monitor the response to treatment for longer than 6 months.

The 6MWT is designed to stress cardiorespiratory capacities to estimate physiological reserves and exercise tolerance. It measures the capacity to cope with challenging and prolonged stressors, for which those with frailty have significantly diminished capacity. Walking distance has been previously shown to be associated with adverse clinical outcomes in numerous disease states, particularly heart failure and frailty.[52] The clinical importance of the change in 6MWT[53] is supported by the magnitude of a ~60 m increase in 6MWT relative to placebo sustained over 6–9 months, with a dose-response effect. This increase exceeds minimal clinically meaningful increases for 6MWT, which range from 17.8 m in individuals with frailty and a fear of falling[50] to 32.4 m in outpatients with chronic heart failure[49] and 20–30 m in older individuals diagnosed with mobility disabilities.[51,53] These results strongly support laromestrocel as a therapeutic candidate for treating hypomobility in older adults with frailty, and possibly for frailty at any age due to other causes, e.g., chemotherapy.

CFS and PROMIS measures offer broader insights into the effects of frailty on function and mobility, which are critical for patient-centered clinical decisions. As part of this study, we sought

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017



	Baseline (*n*)	Month 1 (*n*)	Month 3 (*n*)	Month 6 (*n*)	Month 9 (*n*)
Placebo	29	27	28	27	26
25M	35	34	32	33	31
50M	31	27	30	29	30
100M	31	31	30	28	29
200M	16	16	16	14	14

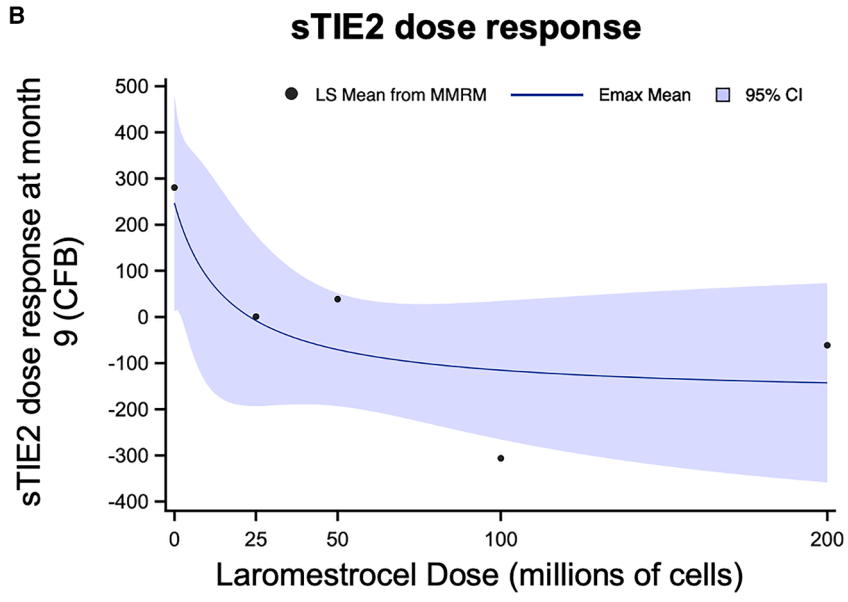


Figure 4. Dose-dependent changes in sTIE2 (mITT)

(A) Soluble TIE2 (sTIE2) decreased compared with placebo in the 100M laromestrocel group at 6 and 9 months post-infusion ($p = 0.021$, $n = 28$, and $p = 0.001$, $n = 29$, respectively). At 9 months post-infusion, the difference between the 200M laromestrocel group and placebo was statistically significant. *$p < 0.1$, **$p < 0.05$, and ***$p < 0.01$ for change from baseline of laromestrocel arm versus change from baseline in the placebo. The number of subjects per group per time point is indicated. Error bars represent ± standard error of the mean (SEM).

(B) The dose-response effect was calculated using the MCP-Mod method, which was best modeled with the Sigmoidal Emax ($p = 0.0175$).

to determine a measure of patient perception of improved physical functioning. Indeed, the PROMIS Physical Function SF20, an assessment of overall physical functioning, showed improvement that significantly correlated with increased 6MWT distance (Figure 3A). Moreover, the PROMIS Mobility showed an even stronger significant correlation (Figure 3B). These results indicate that the positive effects of laromestrocel were not just limited to objective physical measures but generalized to older individuals' perceptions of functional improvement. Survival and hospitalization rates have been shown to be substantially impacted by CFS severity grade.[54] The improvement in CFS severity grade observed in this study further indicates the potential for a positive effect on a caregiver-assessed frailty score. Future studies of laromestrocel are planned to include an analysis of these outcomes with extended follow-up duration up to 2 years.

Another goal of this study was to identify a biomarker correlated with bioactivity. Among a panel of potential biomarkers, we found that decreasing sTIE2 levels over time were associated with laromestrocel infusion in a dose-response fashion. TIE2 is a key receptor tyrosine kinase present on microvascular endothelium and endothelial precursor cells and is activated through binding of the angiopoietins, ANGPT1 and ANGPT2.[55] Cleavage of the extracellular domain of TIE2 (sTIE2), mediated by MMP14,[41] can be detected in the serum, in which increasing levels are indicative of endothelial dysfunction or vascular inflammation. In the context of laromestrocel MOAs, the decreased levels of sTIE2 support a pro-vascular activity in frailty and are consistent with the suggestive pro-vascular and anti-inflammatory activity of laromestrocel for AD.[36] Importantly, we found reduced sTIE2 levels compared with placebo in response to laromestrocel administration in our phase 2a CLEAR MIND clinical trial in AD.[35] The prevention of TIE2 cleavage is a biologically plausible action of laromestrocel. In this regard, laromestrocel secretes high levels of tissue inhibitors of MMPs (TIMPs).[56] Future studies are needed to confirm and extend these findings, including assessments of other vascular biomarkers, measures of

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

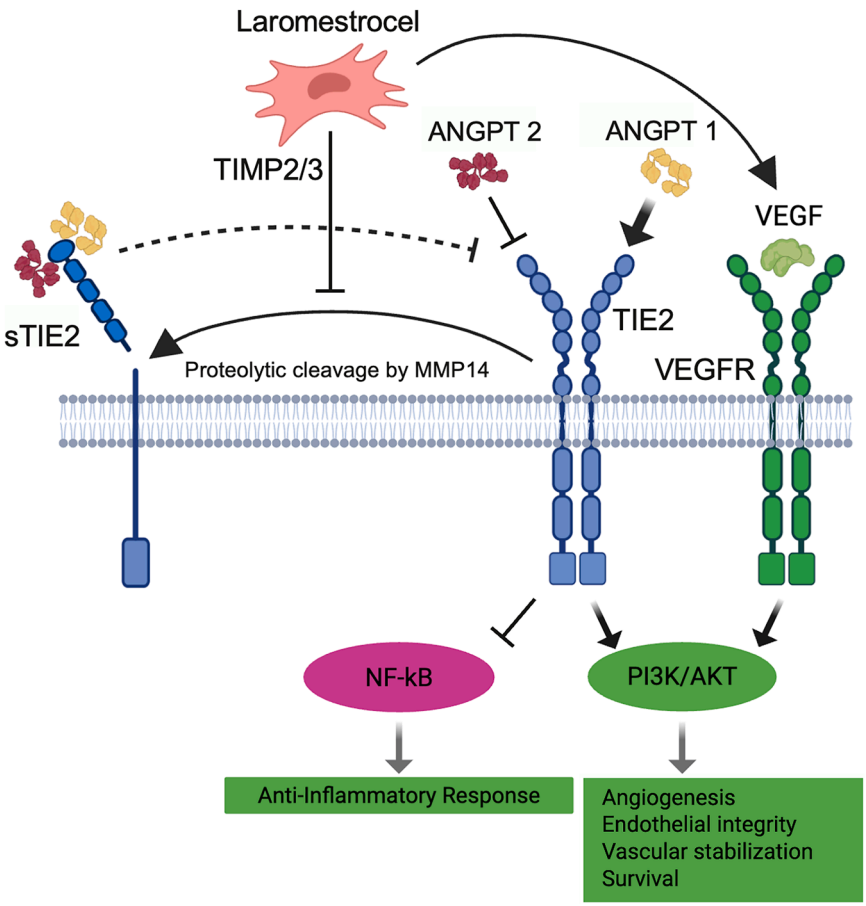


Figure 5. Potential pro-vascular and anti-inflammatory MOA of laromestrocel

Laromestrocel secretes key factors regulating the angiopoietin and VEGF pathways. Secretion of TIMP2 attenuates proteolytic cleavage of the TIE2 receptor by MMP14, preserving the balance between ANGPT 1 and ANGPT 2 signaling cascades. Together with the production of VEGF and downstream signaling through the AK strain transforming (AKT) pathway, these pathways act synergistically to promote angiogenesis, epithelial cell homeostasis and integrity, vascular health, and other pro-vascular effects. Anti-inflammatory potential by preserving TIE2 signaling may therefore influence inflammaging.
Created with BioRender.com.

endothelial function, and other potential mechanisms such as peripheral immune involvement through reported efferocytosis of MSCs and subsequent anti-inflammatory macrophage activity.[57–59]

It is noteworthy that although significant dose-dependent improvements were observed with the 6MWT, other physical function measures, such as the 4-meter gait speed test (4MGST; Table S7) and grip strength (Table S8), did not show significant improvements. It is tempting to speculate that this could be due to the different muscle fibers involved and the activities of laromestrocel. The 6MWT is an endurance measure, which would primarily involve Type I muscle fibers, which are aerobic, highly vascularized, and mitochondria-dense. By contrast, strength primarily involves Type II fibers that are larger, poorly vascularized, and anaerobically driven. Our cumulative data support the hypothesis that Type I muscle fiber activity improves in response to laromestrocel, which may be directly attributable to the pro-vascular activity of laromestrocel. Future studies to examine this hypothesis further are merited.

Limitations of the study

One limitation of this study was the relatively small sample size per group. Nevertheless, this study was powered for the primary endpoint (6MWT) based on results of an earlier phase 1/2 trial[33] and indeed revealed a dose-response relationship to increased 6MWT and numeric improvement on the CFS at 9 months. Although our study assesses a primary endpoint of mobility in frail adults, which can be associated with reduced life space, the condition of frailty encompasses additional dimensions such as cognitive performance that we have not explored in depth here. Frailty can be measured using other scales such as the deficit accumulation index (DAI),[60] which will be addressed in future trials. Our study specifically excluded subjects with dementia. Additionally, females were under-represented in the 100M arm relative to the others (roughly 21% versus over 40%). The number of subjects in the 200M laromestrocel arm was smaller than in the other arms. This was due to the addition of this arm after study enrollment had begun. Nevertheless, the results obtained from this arm were consistent with the dose-response of laromestrocel and were aligned with observations obtained from the other arms. TNF-α measurements were deemed unreliable due to degradation during storage of samples and are, therefore, not reported. Subject socioeconomic status and gender identity were also not assessed.

RESOURCE AVAILABILITY

Lead contact

Requests for information and resource access should be directed to and will be fulfilled by the lead contact and corresponding author, Dr. Joshua M. Hare (jhare@longeveron.com).

Materials availability

No new, unique reagents were generated by this study.

Data and code availability

SAS v.9.4 and R 4.5.1 were used for all statistical analyses included in the manuscript. Custom SAS and R code for the study statistical analyses are available via the online repository at FigShare: 10.6084/m9.figshare.31171648. Trial data will be made available upon institutional review board (IRB)-approved research requests and will be provided by the lead contact. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

We acknowledge Shirley Arroyo Vizcarrondo for her excellent technical assistance in potency-related MSD experiments. Funding: this study was funded by grant R44AG062015 from the National Institute of Aging (NIA) of the National Institutes of Health (NIH) to Longeveron Inc.

AUTHOR CONTRIBUTIONS

Statistical analysis was performed by C.W., E.N., and J.B. Authors with unrestricted access to all data included J.G.R., A.A.O. Jr., K.N.R., C.W., E.N., J.B., B.G.R., and J.M.H. J.G.R., A.A.O. Jr., B.G.R., and J.M.H. analyzed data and drafted the manuscript. B.G.R. and J.M.H. reviewed and revised the manuscript. Z.Z. performed ELISA and MSD secreted analyte analysis experiments for laromestrocel-conditioned medium and performed data analysis for all potency assays. B.V. performed MSD experiments for blood biomarker assessments. All authors have agreed to submit the manuscript and have approved the final draft.

DECLARATION OF INTERESTS

J.M.H. reports having a patent for cardiac cell-based therapy and holds equity in Vestion Inc. and maintains 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. J.M.H. is the Chief Scientific Officer, a compensated consultant, and a board member for Longeveron Inc. 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 Inc., which has licensed intellectual property from the University of Miami.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- METHOD DETAILS
  - Trial Conduct and Oversight
  - Laromestrocel and Placebo
  - Patient Reported Outcomes (PRO)
  - Biomarkers
  - Identification of potential effector proteins
  - Safety Assessments
- QUANTIFICATION AND STATISTICAL ANALYSIS
- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.stem.2026.01.017.

Received: September 7, 2025
Revised: December 12, 2025
Accepted: January 28, 2026

REFERENCES

1. Hoogendijk, E.O., Afilalo, J., Ensrud, K.E., Kowal, P., Onder, G., and Fried, L.P. (2019). Frailty: implications for clinical practice and public health. Lancet *394*, 1365–1375. https://doi.org/10.1016/S0140-6736(19)31786-6.
2. Kim, D.H., and Rockwood, K. (2024). Frailty in Older Adults. N. Engl. J. Med. *391*, 538–548. https://doi.org/10.1056/NEJMra2301292.
3. Suo, X., Li, L., Guo, J., Zhu, Y., Xu, M., Nie, J., Liu, Y., Liu, F., Zhang, J., Wang, Q., et al. (2025). Association of Frailty With Dementia and the Mediating Role of Brain Structure and Immunometabolic Signatures. Neurology *105*, e214199. https://doi.org/10.1212/WNL.0000000000214199.
4. O'Caoimh, R., Sezgin, D., O'Donovan, M.R., Molloy, D.W., Clegg, A., Rockwood, K., and Liew, A. (2021). Prevalence of frailty in 62 countries across the world: a systematic review and meta-analysis of population-level studies. Age Ageing *50*, 96–104. https://doi.org/10.1093/ageing/afaa219.
5. Howlett, S.E., Rutenberg, A.D., and Rockwood, K. (2021). The degree of frailty as a translational measure of health in aging. Nat. Aging *1*, 651–665. https://doi.org/10.1038/s43587-021-00099-3.
6. Boyle, P.A., Buchman, A.S., Barnes, L.L., James, B.D., and Bennett, D.A. (2010). Association between life space and risk of mortality in advanced age. J. Am. Geriatr. Soc. *58*, 1925–1930. https://doi.org/10.1111/j.1532-5415.2010.03058.x.
7. Xue, Q.L., Fried, L.P., Glass, T.A., Laffan, A., and Chaves, P.H.M. (2008). Life-space constriction, development of frailty, and the competing risk of mortality: the Women's Health And Aging Study I. Am. J. Epidemiol. *167*, 240–248. https://doi.org/10.1093/aje/kwm270.
8. May, D., Nayak, U.S., and Isaacs, B. (1985). The life-space diary: a measure of mobility in old people at home. Int. Rehabil. Med. *7*, 182–186. https://doi.org/10.3109/03790798509165993.
9. Cohen, H.J., Smith, D., Sun, C.L., Tew, W., Mohile, S.G., Owusu, C., Klepin, H.D., Gross, C.P., Lichtman, S.M., Gajra, A., et al. (2016). Frailty as determined by a comprehensive geriatric assessment-derived deficit-accumulation index in older patients with cancer who receive chemotherapy. Cancer *122*, 3865–3872. https://doi.org/10.1002/cncr.30269.
10. Mümken, S.A., Alonso-Perez, E., Haeger, C., O'Sullivan, J.L., Xue, Q.L., Lech, S., Herrmann, W.J., and Gellert, P. (2024). Prevention of frailty in relation with social out-of-home activities in older adults: results from the Survey of Health, Ageing, and Retirement in Europe. Eur. J. Ageing *21*, 35. https://doi.org/10.1007/s10433-024-00829-7.
11. Ankuda, C.K., Freedman, V.A., Covinsky, K.E., and Kelley, A.S. (2021). Population-Based Screening for Functional Disability in Older Adults. Innov. Aging *5*, igaa065. https://doi.org/10.1093/geroni/igaa065.
12. Boxer, R., Kleppinger, A., Ahmad, A., Annis, K., Hager, D., and Kenny, A. (2010). The 6-minute walk is associated with frailty and predicts mortality in older adults with heart failure. Congest. Heart Fail. *16*, 208–213. https://doi.org/10.1111/j.1751-7133.2010.00151.x.
13. Harada, N.D., Chiu, V., and Stewart, A.L. (1999). Mobility-related function in older adults: assessment with a 6-minute walk test. Arch. Phys. Med. Rehabil. *80*, 837–841. https://doi.org/10.1016/S0003-9993(99)90236-8.
14. Walston, J., Bandeen-Roche, K., Buta, B., Bergman, H., Gill, T.M., Morley, J.E., Fried, L.P., Robinson, T.N., Afilalo, J., Newman, A.B., et al. (2019). Moving Frailty Toward Clinical Practice: NIA Intramural Frailty Science Symposium Summary. J. Am. Geriatr. Soc. *67*, 1559–1564. https://doi.org/10.1111/jgs.15928.
15. Bisset, E.S., and Howlett, S.E. (2019). The biology of frailty in humans and animals: Understanding frailty and promoting translation. Aging Med. (Milton) *2*, 27–34. https://doi.org/10.1002/agm2.12058.
16. Franceschi, C., and Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J. Gerontol. A Biol. Sci. Med. Sci. *69*, S4–S9. https://doi.org/10.1093/gerona/glu057.

17. Ferrucci, L., and Fabbri, E. (2018). Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat. Rev. Cardiol. *15*, 505–522. https://doi.org/10.1038/s41569-018-0064-2.

18. Li, X., Li, C., Zhang, W., Wang, Y., Qian, P., and Huang, H. (2023). Inflammation and aging: signaling pathways and intervention therapies. Signal Transduct. Target. Ther. *8*, 239. https://doi.org/10.1038/s41392-023-01502-8.

19. Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., and Santoro, A. (2018). Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat. Rev. Endocrinol. *14*, 576–590. https://doi.org/10.1038/s41574-018-0059-4.

20. Damluji, A.A., Chung, S.E., Xue, Q.L., Hasan, R.K., Moscucci, M., Forman, D.E., Bandeen-Roche, K., Batchelor, W., Walston, J.D., Resar, J.R., et al. (2021). Frailty and cardiovascular outcomes in the National Health and Aging Trends Study. Eur. Heart J. *42*, 3856–3865. https://doi.org/10.1093/eurheartj/ehab468.

21. Buch, A., Carmeli, E., Boker, L.K., Marcus, Y., Shefer, G., Kis, O., Berner, Y., and Stern, N. (2016). Muscle function and fat content in relation to sarcopenia, obesity and frailty of old age–An overview. Exp. Gerontol. *76*, 25–32. https://doi.org/10.1016/j.exger.2016.01.008.

22. Liu, L., and Rando, T.A. (2011). Manifestations and mechanisms of stem cell aging. J. Cell Biol. *193*, 257–266. https://doi.org/10.1083/jcb.201010131.

23. Matteini, F., Montserrat-Vazquez, S., and Florian, M.C. (2024). Rejuvenating aged stem cells: therapeutic strategies to extend health and lifespan. FEBS Lett. *598*, 2776–2787. https://doi.org/10.1002/1873-3468.14865.

24. Ferrón, S., Mira, H., Franco, S., Cano-Jaimez, M., Bellmunt, E., Ramírez, C., Fariñas, I., and Blasco, M.A. (2004). Telomere shortening and chromosomal instability abrogates proliferation of adult but not embryonic neural stem cells. Development *131*, 4059–4070. https://doi.org/10.1242/dev.01215.

25. Cai, Y., Xiong, M., Xin, Z., Liu, C., Ren, J., Yang, X., Lei, J., Li, W., Liu, F., Chu, Q., et al. (2023). Decoding aging-dependent regenerative decline across tissues at single-cell resolution. Cell Stem Cell *30*, 1674–1691.e8. https://doi.org/10.1016/j.stem.2023.09.014.

26. Chambers, S.M., Shaw, C.A., Gatza, C., Fisk, C.J., Donehower, L.A., and Goodell, M.A. (2007). Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. PLOS Biol. *5*, e201. https://doi.org/10.1371/journal.pbio.0050201.

27. Pittenger, M.F., Discher, D.E., Péault, B.M., Phinney, D.G., Hare, J.M., and Caplan, A.I. (2019). Mesenchymal stem cell perspective: cell biology to clinical progress. NPJ Regen. Med. *4*, 22. https://doi.org/10.1038/s41536-019-0083-6.

28. Oliva, A.A., McClain-Moss, L., Pena, A., Drouillard, A., and Hare, J.M. (2019). Allogeneic mesenchymal stem cell therapy: A regenerative medicine approach to geroscience. Aging Med. (Milton) *2*, 142–146. https://doi.org/10.1002/agm2.12079.

29. Thompson, M., Mei, S.H.J., Wolfe, D., Champagne, J., Fergusson, D., Stewart, D.J., Sullivan, K.J., Doxtator, E., Lalu, M., English, S.W., et al. (2020). Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: An updated systematic review and meta-analysis. EClinicalmedicine *19*, 100249. https://doi.org/10.1016/j.eclinm.2019.100249.

30. Hare, J.M., Traverse, J.H., Henry, T.D., Dib, N., Strumpf, R.K., Schulman, S.P., Gerstenblith, G., DeMaria, A.N., Denktas, A.E., Gammon, R.S., et al. (2009). A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J. Am. Coll. Cardiol. *54*, 2277–2286. https://doi.org/10.1016/j.jacc.2009.06.055.

31. Golpanian, S., El-Khorazaty, J., Mendizabal, A., DiFede, D.L., Suncion, V.Y., Karantalis, V., Fishman, J.E., Ghersin, E., Balkan, W., and Hare, J.M. (2015). Effect of aging on human mesenchymal stem cell therapy in ischemic cardiomyopathy patients. J. Am. Coll. Cardiol. *65*, 125–132. https://doi.org/10.1016/j.jacc.2014.10.040.

32. Golpanian, S., DiFede, D.L., Khan, A., Schulman, I.H., Landin, A.M., Tompkins, B.A., Heldman, A.W., Miki, R., Goldstein, B.J., Mushtaq, M., et al. (2017). Allogeneic Human Mesenchymal Stem Cell Infusions for Aging Frailty. J. Gerontol. A Biol. Sci. Med. Sci. *72*, 1505–1512. https://doi.org/10.1093/gerona/glx056.

33. Tompkins, B.A., DiFede, D.L., Khan, A., Landin, A.M., Schulman, I.H., Pujol, M.V., Heldman, A.W., Miki, R., Goldschmidt-Clermont, P.J., Goldstein, B.J., et al. (2017). Allogeneic Mesenchymal Stem Cells Ameliorate Aging Frailty: A Phase II Randomized, Double-Blind, Placebo-Controlled Clinical Trial. J. Gerontol. A Biol. Sci. Med. Sci. *72*, 1513–1522. https://doi.org/10.1093/gerona/glx137.

34. Golpanian, S., DiFede, D.L., Pujol, M.V., Lowery, M.H., Levis-Dusseau, S., Goldstein, B.J., Schulman, I.H., Longsomboon, B., Wolf, A., Khan, A., et al. (2016). Rationale and design of the allogeneiC human mesenchymal stem cells (hMSC) in patients with aging fRAilTy via intravenoUS delivery (CRATUS) study: A phase I/II, randomized, blinded and placebo controlled trial to evaluate the safety and potential efficacy of allogeneic human mesenchymal stem cell infusion in patients with aging frailty. Oncotarget *7*, 11899–11912. https://doi.org/10.18632/oncotarget.7727.

35. Rash, B.G., Ramdas, K.N., Agafonova, N., Naioti, E., McClain-Moss, L., Zainul, Z., Varnado, B., Peterson, K., Brown, M., Leal, T., et al. (2025). Allogeneic mesenchymal stem cell therapy with laromestrocel in mild Alzheimer's disease: a randomized controlled phase 2a trial. Nat. Med. *31*, 1257–1266. https://doi.org/10.1038/s41591-025-03559-0.

36. Brody, M., Agronin, M., Herskowitz, B.J., Bookheimer, S.Y., Small, G.W., Hitchinson, B., Ramdas, K., Wishard, T., McInerney, K.F., Vellas, B., et al. (2023). Results and insights from a phase I clinical trial of Lomecel-B for Alzheimer's disease. Alzheimers Dement. *19*, 261–273. https://doi.org/10.1002/alz.12651.

37. Yousefi, K., Ramdas, K.N., Ruiz, J.G., Walston, J., Arai, H., Volpi, E., Newman, A.B., Wang, C., Hitchinson, B., McClain-Moss, L., et al. (2022). The Design and Rationale of a Phase 2b, Randomized, Double-Blinded, and Placebo-Controlled Trial to Evaluate the Safety and Efficacy of Lomecel-B in Older Adults with Frailty. J. Frailty Aging *11*, 214–223. https://doi.org/10.14283/jfa.2022.2.

38. Yuan, H.T., Khankin, E.V., Karumanchi, S.A., and Parikh, S.M. (2009). Angiopoietin 2 is a partial agonist/antagonist of Tie2 signaling in the endothelium. Mol. Cell. Biol. *29*, 2011–2022. https://doi.org/10.1128/MCB.01472-08.

39. Fiedler, U., Reiss, Y., Scharpfenecker, M., Grunow, V., Koidl, S., Thurston, G., Gale, N.W., Witzenrath, M., Rosseau, S., Suttorp, N., et al. (2006). Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat. Med. *12*, 235–239. https://doi.org/10.1038/nm1351.

40. Fiedler, U., Krissl, T., Koidl, S., Weiss, C., Koblizek, T., Deutsch, U., Martiny-Baron, G., Marmé, D., and Augustin, H.G. (2003). Angiopoietin-1 and angiopoietin-2 share the same binding domains in the Tie-2 receptor involving the first Ig-like loop and the epidermal growth factor-like repeats. J. Biol. Chem. *278*, 1721–1727. https://doi.org/10.1074/jbc.M208550200.

41. Idowu, T.O., Etzrodt, V., Seeliger, B., Bolanos-Palmieri, P., Thamm, K., Haller, H., and David, S. (2020). Identification of specific Tie2 cleavage sites and therapeutic modulation in experimental sepsis. eLife *9*, e59520. https://doi.org/10.7554/eLife.59520.

42. Findley, C.M., Mitchell, R.G., Duscha, B.D., Kontos, C.D., Annex, B.H., and Kontos, C.D. (2008). Plasma levels of soluble Tie2 and vascular endothelial growth factor distinguish critical limb ischemia from intermittent claudication in patients with peripheral arterial disease. J. Am. Coll. Cardiol. *52*, 387–393. https://doi.org/10.1016/j.jacc.2008.02.045.

43. Lee, K.W., Lip, G.Y.H., and Blann, A.D. (2004). Plasma angiopoietin-1, angiopoietin-2, angiopoietin receptor tie-2, and vascular endothelial growth factor levels in acute coronary syndromes. Circulation *110*, 2355–2360. https://doi.org/10.1161/01.Cir.0000138112.90641.7f.

44. Parikh, S.M. (2016). Targeting Tie2 and the host vascular response in sepsis. Sci. Transl. Med. *8*, 8. https://doi.org/10.1126/scitranslmed.aaf5537.

45. Han, S., Lee, S.J., Kim, K.E., Lee, H.S., Oh, N., Park, I., Ko, E., Oh, S.J., Lee, Y.S., Kim, D., et al. (2016). Amelioration of sepsis by TIE2 activation-induced vascular protection. Sci. Transl. Med. *8*, 335ra55. https://doi.org/10.1126/scitranslmed.aad9260.

46. Jiang, L., Hu, X., Feng, Y., Wang, Z., Tang, H., Lin, Q., Shen, Y., Zhu, Y., Xu, Q., and Li, X. (2024). Reduction of renal interstitial fibrosis by targeting Tie2 in vascular endothelial cells. Pediatr. Res. *95*, 959–965. https://doi.org/10.1038/s41390-023-02893-8.

47. Yan, F., Meng, X., Cheng, X., Pei, W., Chen, Y., Chen, L., Zheng, M., Shi, L., Zhu, C., and Zhang, X. (2023). Potential role between inflammatory cytokines and Tie-2 receptor levels and clinical symptoms in patients with first-episode schizophrenia. BMC Psychiatry *23*, 538. https://doi.org/10.1186/s12888-023-04913-7.

48. Scheufler, K.M., Drevs, J., van Velthoven, V., Reusch, P., Klisch, J., Augustin, H.G., Zentner, J., and Marme, D. (2003). Implications of vascular endothelial growth factor, sFlt-1, and sTie-2 in plasma, serum and cerebrospinal fluid during cerebral ischemia in man. J. Cereb. Blood Flow Metab. *23*, 99–110. https://doi.org/10.1097/01.Wcb.0000037547.46809.83.

49. Shoemaker, M.J., Curtis, A.B., Vangsnes, E., and Dickinson, M.G. (2013). Clinically meaningful change estimates for the six-minute walk test and daily activity in individuals with chronic heart failure. Cardiopulm. Phys. Ther. J. *24*, 21–29. https://doi.org/10.1097/01823246-201324030-00004.

50. Kwok, B.C., Pua, Y.H., Mamun, K., and Wong, W.P. (2013). The minimal clinically important difference of six-minute walk in Asian older adults. BMC Geriatr. *13*, 23. https://doi.org/10.1186/1471-2318-13-23.

51. Perera, S., Mody, S.H., Woodman, R.C., and Studenski, S.A. (2006). Meaningful change and responsiveness in common physical performance measures in older adults. J. Am. Geriatr. Soc. *54*, 743–749. https://doi.org/10.1111/j.1532-5415.2006.00701.x.

52. Yazdanyar, A., Aziz, M.M., Enright, P.L., Edmundowicz, D., Boudreau, R., Sutton-Tyrell, K., Kuller, L., and Newman, A.B. (2014). Association Between 6-Minute Walk Test and All-Cause Mortality, Coronary Heart Disease-Specific Mortality, and Incident Coronary Heart Disease. J. Aging Health *26*, 583–599. https://doi.org/10.1177/0898264314525665.

53. Bohannon, R.W., and Crouch, R. (2017). Minimal clinically important difference for change in 6-minute walk test distance of adults with pathology: a systematic review. J. Eval. Clin. Pract. *23*, 377–381. https://doi.org/10.1111/jep.12629.

54. Rockwood, K., Song, X., MacKnight, C., Bergman, H., Hogan, D.B., McDowell, I., and Mitnitski, A. (2005). A global clinical measure of fitness and frailty in elderly people. CMAJ *173*, 489–495. https://doi.org/10.1503/cmaj.050051.

55. Sack, K.D., Kellum, J.A., and Parikh, S.M. (2020). The Angiopoietin-Tie2 Pathway in Critical Illness. Crit. Care Clin. *36*, 201–216. https://doi.org/10.1016/j.ccc.2019.12.003.

56. Lozito, T.P., Jackson, W.M., Nesti, L.J., and Tuan, R.S. (2014). Human mesenchymal stem cells generate a distinct pericellular zone of MMP activities via binding of MMPs and secretion of high levels of TIMPs. Matrix Biol. *34*, 132–143. https://doi.org/10.1016/j.matbio.2013.10.003.

57. Elliott, M.R., Koster, K.M., and Murphy, P.S. (2017). Efferocytosis Signaling in the Regulation of Macrophage Inflammatory Responses. J. Immunol. *198*, 1387–1394. https://doi.org/10.4049/jimmunol.1601520.

58. Cheung, T.S., Giacomini, C., Cereda, M., Avivar-Valderas, A., Capece, D., Bertolino, G.M., delaRosa, O., Hicks, R., Ciccocioppo, R., Franzoso, G., et al. (2023). Apoptosis in mesenchymal stromal cells activates an immunosuppressive secretome predicting clinical response in Crohn's disease. Mol. Ther. *31*, 3531–3544. https://doi.org/10.1016/j.ymthe.2023.10.004.

59. Galleu, A., Riffo-Vasquez, Y., Trento, C., Lomas, C., Dolcetti, L., Cheung, T.S., von Bonin, M., Barbieri, L., Halai, K., Ward, S., et al. (2017). Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Sci. Transl. Med. *9*, eaam7828. https://doi.org/10.1126/scitranslmed.aam7828.

60. Rockwood, K., and Mitnitski, A. (2011). Frailty defined by deficit accumulation and geriatric medicine defined by frailty. Clin. Geriatr. Med. *27*, 17–26. https://doi.org/10.1016/j.cger.2010.08.008.

61. Juma, S., Taabazuing, M.M., and Montero-Odasso, M. (2016). Clinical Frailty Scale in an Acute Medicine Unit: a Simple Tool That Predicts Length of Stay. Can. Geriatr. J. *19*, 34–39. https://doi.org/10.5770/cgj.19.196.

62. Ritt, M., Ritt, J.I., Sieber, C.C., and Gaßmann, K.G. (2017). Comparing the predictive accuracy of frailty, comorbidity, and disability for mortality: a 1-year follow-up in patients hospitalized in geriatric wards. Clin. Interv. Aging *12*, 293–304. https://doi.org/10.2147/CIA.S124342.

63. Cesari, M., Bernabei, R., Vellas, B., Fielding, R.A., Rooks, D., Azzolino, D., Mariani, J., Oliva, A.A., Bhasin, S., and Rolland, Y. (2022). Challenges in the Development of Drugs for Sarcopenia and Frailty - Report from the International Conference on Frailty and Sarcopenia Research (ICFSR) Task Force. J. Frailty Aging *11*, 135–142. https://doi.org/10.14283/jfa.2022.30.

64. Egorin, M.J., Rosen, D.M., Sridhara, R., Sensenbrenner, L., and Cottler-Fox, M. (1998). Plasma concentrations and pharmacokinetics of dimethylsulfoxide and its metabolites in patients undergoing peripheral-blood stem-cell transplants. J. Clin. Oncol. *16*, 610–615. https://doi.org/10.1200/JCO.1998.16.2.610.

65. Cella, D., Yount, S., Rothrock, N., Gershon, R., Cook, K., Reeve, B., Ader, D., Fries, J.F., Bruce, B., Rose, M., et al. (2007). The Patient-Reported Outcomes Measurement Information System (PROMIS): progress of an NIH Roadmap cooperative group during its first two years. Med. Care *45*, S3–S11. https://doi.org/10.1097/01.mlr.0000258615.42478.55.

66. Born, J., Lange, T., Hansen, K., Mölle, M., and Fehm, H.L. (1997). Effects of sleep and circadian rhythm on human circulating immune cells. J. Immunol. *158*, 4454–4464. https://doi.org/10.4049/jimmunol.158.9.4454.

67. Bretz, F., Pinheiro, J.C., and Branson, M. (2005). Combining multiple comparisons and modeling techniques in dose-response studies. Biometrics *61*, 738–748. https://doi.org/10.1111/j.1541-0420.2005.00344.x.

68. Menon, S.M., and Zink, R.C. (2015). Modern Approaches to Clinical Trials Using SAS: Classical, Adaptive, and Bayesian Methods (SAS Institute).

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
PE Mouse anti-human CD11b	BD Biosciences	Cat# 561001; RRID: AB_10563205
Mouse Anti-CD19 PE Monoclonal Antibody	BD Biosciences	Cat# 555413; RRID: AB_395813; Cat# 561741; RRID: AB_10893795
PE Anti-human CD34	BD Biosciences	Cat# 560941; RRID: AB_10563214
Mouse Anti-CD45 FITC Conjugated	BD Biosciences	Cat# 555482; RRID: AB_395874; Cat# 560976; RRID: AB_10563935
Mouse Anti-CD90 Monoclonal Antibody, FITC	BD Biosciences	Cat# 555595; RRID: AB_395969
FITC anti-human CD90 (Thy1)	BioLegend	Cat# 328108; RRID: AB_803429
APC Mouse Anti-human CD73	BD Biosciences	Cat# 560847; RRID: AB_10612019
APC Mouse Anti-human CD73 (Ecto-5'-nucleotidase)	BioLegend	Cat# 344005; RRID: AB_1877158; Cat# 344006; RRID: AB_1877157
Mouse Anti-human CD105 PE	BD Biosciences	Cat# 560839; RRID: AB_2033932
Biological samples		
Bone marrow aspirate for lot LMSC012	AllCells®	LMSC012
Bone marrow aspirate for lot LMSC013	AllCells®	LMSC013
Bone marrow aspirate for lot LMSC014	AllCells®	LMSC014
Bone marrow aspirate for lot LMSC016	AllCells®	LMSC016
Bone marrow aspirate for lot LMSC017	AllCells®	LMSC017
Bone marrow aspirate for lot LMSC019	AllCells®	LMSC019
Bone marrow aspirate for lot LMSC020	AllCells®	LMSC020
Bone marrow aspirate for lot LMSC023	AllCells®	LMSC023
Bone marrow aspirate for lot LMSC027	AllCells®	LMSC027
Bone marrow aspirate for lot LMSC037	Stem Express (now CGT Global)	LMSC037
Bone marrow aspirate for lot LMSC039	Oklahoma Blood Institute (now Our Blood Institute)	LMSC039
Bone marrow aspirate for lot LMSC040	Stem Express (now CGT Global)	LMSC040
Bone marrow aspirate for lot LMSC041	Stem Express (now CGT Global)	LMSC041
Chemicals, peptides, and recombinant proteins		
Alpha MEM w/L-glut	Thermo Fisher Scientific	12561-049
FBS	Corning	35-070-CV
0.05% Trypsin-EDTA	Corning	25-052-CI
CS5	Stem Cell Technologies	07933
Human Serum Albumin	Nova Biologics, Inc.	68982-0643-01
Phosphate Buffer Saline (PBS)	Corning	21-040-CV
Critical commercial assays		
Human TIMP2 ELISA kit	Abcam	Cat# AB100653
Human TIMP-2 ELISA kit	Thermo Fisher Scientific Inc.	Cat# EHTIMP2
V-PLEX Angiogenesis Panel 1 Human Kit	Meso Scale Diagnostics, LLC	Cat# K15190G; K15190D
V-PLEX Proinflammatory Panel 1 Human Kit	Meso Scale Diagnostics, LLC	Cat# K15049G; K15049D
V-PLEX Plus Human CRP Kit	Meso Scale Diagnostics, LLC	Cat# K151STG-1
Deposited data		
ClinicalTrials.gov	National Library of Medicine	NCT03169231
Software and algorithms		
GraphPad Prism 10.6.1	www.graphpad.com	RRID: SCR_002798

(*Continued on next page*)

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
DISCOVERY WORKBENCH (v4.0)	www.mesoscale.com	RRID: SCR_019192
Spectramax ID3 SoftMax software	www.moleculardevices.com	Version 1.2.0.0
SAS Version 9.4	www.sas.com	RRID: SCR_008567
R Project for Statistical Computing (v4.5.1)	https://www.r-project.org/	RRID: SCR_001905
Custom Analysis Code: Laromestrocel Trial	This paper; Figshare https://figshare.com	DOI: 10.6084/m9figshare.31171648
CytExpert Software	https://www.beckman.fr/flow-cytometry	RRID: SCR_017217
Microsoft Office 365	Microsoft Corp	Version 16.101.3 (25100321)

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

All study subjects provided written informed consent via a Western IRB approved consent form. IQVIA enrolled all subjects and performed the randomization. Subjects met enrollment criteria of being 70–85 years of age, being cognitively unimpaired (Mini-Mental Scale Exam score $\geq$ 24) and having mild to moderate frailty as assessed by the Canadian Study of Health and Aging (CSHA) Clinical Frailty Scale (CFS) (score of 5 or 6, representing mildly to moderately frail individuals, respectively).[61,62] In addition, each subject had a screening 6MWT distance between 200 and 400 m,[63] and a baseline serum tumor necrosis factor-alpha (TNF-α) $\geq$ 2.5 pg/ml. Full inclusion/exclusion criteria are listed in Table 1, and baseline characteristics of all study participants are provided in Table 2.

METHOD DETAILS

Trial Conduct and Oversight

This double-blind, randomized, parallel-arm, and placebo-controlled trial, entitled "A Phase 2b, Randomized, Blinded and Placebo-Controlled Trial to Evaluate the Safety and Efficacy of Longeveron Allogeneic Human Mesenchymal Stem Cells Infusion in Patients with Aging Frailty", is listed on clinicaltrials.gov (NCT03169231). The trial was carried out between 2018 and 2021 in clinical sites in Florida, Texas, Maryland, Pennsylvania, and New Jersey. Oversight was provided by a single Institutional Review Board (IRB) (Western IRB; Puyallup, WA), an independent pharmacovigilance group (ProPharma Group; Washington, DC), an independent DSMB (appointed by the National Institute of Aging (NIA)), and independent clinical monitors (Syneos/Joulé Inc.; Edison, NJ). The study and manufacturing of laromestrocel are under the oversight of the Food and Drug Administration (FDA) according to Investigational New Drug Application (IND) # 016644. IQVIA (Durham, NC) served as the CRO for this study.

Subjects received placebo, or laromestrocel at doses of 2.5 x 10^7 cells ("25M"), 5.0 x 10^7 cells ("50M"), 1.0 x 10^8 cells ("100M"), or 2.0 x 10^8 cells ("200M"). Subjects were initially randomized 1:1:1:1 each to placebo (n=29), or 25M (n=35), 50M (n=30), or 100M (n=33) laromestrocel using block sizes of 4. A 200M laromestrocel arm (n=16) was introduced after 92 patients were enrolled after new funding permitted the group addition. Accordingly, to balance out this new arm with the others within each investigator center, the randomization scheme was modified from central randomization to center-stratified randomization. For existing sites, the new allocation was site specific, taking into account the number of patients already randomized for each site. Under no circumstances was the probability of assigning the 200M group set to 1 for any new patients. The process was carried out by a statistician who a) was blinded to individual treatment assignment, b) was blinded for the allocation of the existing sites, and c) was not aware of when the new randomization schedule would be implemented. All study patients, caregivers and personnel who participated in the conduct and analysis of the study, including investigators, clinical site and sponsor staff, were blinded to treatment group. Only personnel preparing the infusions were unblinded to group identity.

Laromestrocel and Placebo

Laromestrocel and placebo were manufactured per the Chemistry, Manufacturing, and Controls (CMC) section of an Investigational New Drug Application (IND) as reported previously.[35] Allogeneic MSCs for laromestrocel were sourced from healthy young adult bone marrow donors aged 18-45 in compliance with the Codes of Federal Regulations (CFR) 1271, and culture-expanded using current Good Manufacturing Practices (cGMP). Marrow donor characteristics are listed in Table S1, and marrow sources are provided in the key resources table. Laromestrocel MSCs were isolated from bone marrow aspirates using density gradient separation and expanded in complete α-MEM medium supplemented with fetal bovine serum (FBS) within multilayer vessels until 85–90% confluent. At each passage, the pooled cells were trypsinized, counted, and assessed for viability (which must be $\geq$70%) before re-seeding. The same complete medium was used throughout the process. For collection and cryopreservation, conditioned medium (supernatant) samples were first collected and frozen at $-80\,^{\circ}$C. The cells were then pooled, assessed for count/viability, and filled into CS50 cryobags at specific concentrations. The cryoprotectant suspension contains Hespan, HSA, and DMSO. Cells are frozen using controlled-rate freezers and stored at $\leq -135\,^{\circ}$C in vapor-phase liquid nitrogen (LN_2) freezers. Each donor lot was tested to confirm MSC identity. Release criteria included the following: cell viability $\geq$ 70%; endotoxin $\leq$ 5 EU/mL; USP 71 Sterility negative/no growth; $\geq$ 95% positive for CD73, CD90, and CD105 by flow cytometry; $\leq$ 2 % positive for CD45, CD11b, and CD19; $\leq$ 5% positive CD34. The flow cytometry data were collected on a CytoFLEX V5-B5-R3 flow cytometer using CytExpert software (Beckman Coulter) and

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

analyzed by FlowJo software. Laromestrocel must be negative for mycoplasma, adventitious viruses via culture with three cell types, and also negative for Parvo B19, HIV 1, HIV 2, Hepatitis B, Hepatitis C, HTLV 1, HTLV 2, CMV and EBV by PCR. Each passing dose was cryopreserved in liquid nitrogen until needed for infusion. On the day of infusion, laromestrocel is thawed, washed and diluted, and prepared for administration by Longeveron or a qualified third-party facility as described previously.[35] After cell resuspension, any remaining DMSO in the cryoprotectant is present only as a trace, well below clinically meaningful levels.[64]

The placebo was PlasmaLyte-A with 1% human serum albumin, which was the vehicle used for the final formulation of laromestrocel as previously described.[35] Laromestrocel and placebo were delivered via peripheral intravenous infusion at ~2 mL/min (80 mL total volume administered over ~40 min) in an outpatient setting. To maintain blinding, laromestrocel and placebo were prepared in identically appearing infusion bags bearing indistinguishable labels. The details of key reagents are provided in the key resources table.

Patient Reported Outcomes (PRO)

The Patient-Reported Outcomes (PRO) Measurement Information System (PROMIS) is a set of NIH-developed validated PROs for evaluating physical, mental, and social health.[65] The adult PROMIS Physical function—Short Form 20a (SF20), used to evaluate patient-reported overall physical functioning, was used as a secondary endpoint since it has been shown to have strong test-retest reliability and a minimally clinically important difference of 2 points (~0.20 SD). The PROMIS Mobility and PROMIS Upper Extremity were used to evaluate mobility and upper body function, respectively, as pre-specified exploratory endpoints.

Biomarkers

Identification of candidate biomarkers correlating with laromestrocel dose was a prespecified exploratory endpoint of this trial. Blood collections were performed between 9 and 11 AM to minimize circadian rhythm fluctuations.[66] Serum and plasma samples were centrifuged on-site shortly after collection, aliquoted, snap-frozen, and cryostored until use. Blood biomarker analyses were performed in a blind fashion using serum samples. To the best extent possible, samples from all time-points for each patient were run in parallel to minimize inter-experimental variability. The central lab was Q^2 (an IQVIA company; Durham NC), which performed blood and urine safety analyses, and high-sensitivity electrochemiluminescent multiplex immunoassays on serum samples using a Meso QuickPlex system and V-Plex proinflammatory panels K151A9H and K15049D (Meso-Scale Discovery (MSD): Rockville, MD). Longeveron used a Meso QuickPlex system to run the V-Plex Angiogenesis Panel (K15190D) and the C-reactive protein (CRP) assay (K151STG-1). Standard curves for each analyte passed if no more than two of seven dilution points failed criteria of within 30% recovery and 30% coefficient of variation (CV).

Identification of potential effector proteins

Laromestrocel conditioned media was collected from all lots used in the study and analyzed for the presence of secreted factors. Presence of tissue inhibitor of metalloproteinase 2 (TIMP-2) was assessed using a TIMP-2 human enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions. All samples were diluted to 1:100, and plates read on a Spectramax ID3 spectrophotometer running SoftMax software version 1.2.0.0, and the standard curve was fit using either a four-parameter logistic (4PL) or a five-parameter logistic (5PL) model in GraphPad Prism 10. TIMP-2 concentrations were measured in ng/mL and normalized to cell density at the time of the laromestrocel harvest (cells/mL); the final TIMP-2 potency is reported in ng/cell. Additionally, the conditioned media were evaluated using the Meso Scale Discovery (MSD) platform for angiogenesis and proinflammatory-related markers using Human V-PLEX Angiogenesis Panel 1 (K15190G, K15190D) and Human V-PLEX Proinflammatory Panel 1 (K15049G), respectively. The MSD data Analyses were performed using a MESO QuickPlex SQ 120MM instrument running DISCOVERY WORKBENCH® 4.0 software. Standard curve calibrators were serially diluted from 1–7 according to the kit's manual. For each analyte, 5 of the 7 calibrators were required to meet the per cent recovery range of 70% to 130% and a coefficient of variation (CV) <30% for that specific run to be considered for further data analysis. In MSD experiments, analytes that were detected below the lower limit of quantification (LLOQ) value are marked as ND (not detected). Analytes that were detected above the LLOQ values (in pg/mL) were further normalized to the cell density (cell/mL) and the data are represented as pg/cell. All samples were run in duplicate in both ELISA and MSD experiments and means reported (Table S2). The details of the ELISA and MSD kits used in this study are provided in the key resources table.

Safety Assessments

Safety assessments included evaluation of adverse events (AEs) and serious AEs (SAEs) for frequency, severity, and blinded relationship to study product. AEs were coded by primary system organ class (SOC) and preferred term (PT) according to the Medical Dictionary for Regulatory Activities (MedDRA) 23.0. The treatment-emergent (TE-) AEs and TE-SAEs were summarized by the number and percentage (n and %) of subjects in each SOC and PT. When multiple AEs were reported with the same preferred term, the AE of the strongest relation is included in summary by relationship, and the AE of the most severe grade is included in the summary by severity table.

Please cite this article in press as: Ruiz et al., Randomized phase 2b dose-escalation trial of stem cell therapy with laromestrocel for aging frailty, Cell Stem Cell (2026), https://doi.org/10.1016/j.stem.2026.01.017

QUANTIFICATION AND STATISTICAL ANALYSIS

Unblinding and statistical analyses were performed by an independent statistician group (Pharma Data Associates, LLC: Piscataway, NJ). The sample size was calculated based on the primary endpoint of change from baseline in 6MWT.[36] Using a one-sided α=0.025 and effect size of 0.75 (calculated via the difference in change from baseline in 6MWT of the interventional arms versus placebo divided by the common standard deviation of 75 m), 30 subjects per arm provided approximately 80% power for a treatment difference of 56 m. This distance is less than the changes seen in the prior phase 1/2 study (up to 76.6 m).[32,33]

Efficacy endpoint and biomarker analyses were performed using the modified intent-to-treat (mITT) population, defined as all randomized subjects who received an infusion and completed at least one post-baseline assessment for the primary efficacy endpoint. Each laromestrocel group was compared to placebo pairwise using a Mixed-Model Repeated Measure (MMRM) model, and least squares (LS) means calculated for comparisons of changes in laromestrocel groups to changes in placebo. An unstructured variance-covariance matrix was used to model the correlation among repeated measurements. Dose-response effects were calculated using a multiple comparison procedure-modeling (MCP-Mod) method, which is a hybrid approach combining hypothesis testing and modeling to analyze phase 2 dose-ranging studies to find suitable dose(s) for confirmatory phase 3 trials.[67,68] The candidate models included linear, quadratic, exponential, Emax, and Sigmoid Emax dose-response models. In addition to the adjusted p-values, the Akaike Information Criterion (AIC) was used to evaluate the best parsimonious and predictive model. Smaller AIC means better model. The model means of the dose-response curve were plotted with 95% confidence intervals. The Safety Population, for evaluating safety, was defined as all subjects who received an infusion.

To account for multiple testing of the different dose groups versus placebo, the step-up Hochberg procedure was used for the primary analysis of the primary endpoint. Secondary analysis of the primary endpoint was dose-response effect via MCP-Mod method. Simple linear regressions and correlations were calculated for the absolute values and changes from baseline between the 6MWT and patient reported outcome questionnaire.

ADDITIONAL RESOURCES

This trial is registered in ClinicalTrials.gov as: #NCT03169231.