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Original Research

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

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Abstract

BACKGROUND: Frailty in older adults is a rapidly growing unmet medical need. It is an aging-related syndrome characterized by physical decline leading to higher risk of adverse health outcomes.

OBJECTIVES: To evaluate the efficacy of Lomecel-B, an allogeneic medicinal signaling cell (MSC) formulation, in older adults with frailty.

DESIGN: This multicenter, randomized, parallel-arm, double-blinded, and placebo-controlled phase 2b trial is designed to evaluate dose-range effects of Lomecel-B for frailty on physical functioning, patient-reported outcomes (PROs), frailty status, and biomarkers.

SETTING: Eight enrolling clinical research centers, including the Miami Veterans Affairs Medical Center.

PARTICIPANTS: Target enrollment is 150 subjects aged 70-85 years of any race, ethnicity, or gender. Enrollment criteria include a Clinical Frailty Score of 5 ("mild") or 6 ("moderate"), a 6MWT of 200-400 m, and serum tumor necrosis factor-alpha (TNF-α) ≥2.5 pg/mL.

INTERVENTION: A single intravenous infusion of Lomecel-B (25, 50, 100, or 200 million cells) or placebo (N=30/arm). Patients are followed for 365 days for safety, and the efficacy assessments performed at 90, 180, and 270 days.

MEASUREMENTS: The primary endpoint is change in 6MWT in the Lomecel-B-treated arms versus placebo at 180 days post-infusion. Secondary and exploratory endpoints include change in: 6MWT and other physical function measures at all time points; PROs; frailty status; cognitive status; and an inflammatory biomarkers panel. A pre-specified sub-study examines vascular/endothelial biomarkers. Safety is evaluated throughout the trial.

RESULTS: The trial is conducted under a Food and Drug Administration Investigational New Drug (IND), with Institutional Review Board approval, and monitoring by an NIH-appointed independent Data Safety Monitoring Board.

CONCLUSION: This clinical trial investigates the use of a regenerative medicine strategy for frailty in older adults. The results will further the understanding of the potential for Lomecel-B in the geriatric condition of frailty.

Key words: Frailty, medicinal signaling cell, mesenchymal stem cells, physical function, 6-minute walk test.

Introduction

Frailty is an increasingly prevalent age-related multidimensional syndrome, which manifests with heterogeneous physical and cognitive symptoms rendering affected older adults at higher risk for adverse health outcomes and substantial socioeconomic consequences (1, 2). Due to the high prevalence of frailty in older adults and the lack of approved medical treatments or standards of care, developing appropriate therapies for frailty represents an important unmet medical need (3, 4).

Mechanistically, inflammaging, which is defined as an age-associated low-grade chronic inflammatory state (5), reduced regenerative capacity (6), and vascular endothelial dysfunction (7) are among the important biological underpinnings of the pathophysiology of frailty (8). Countering these pathological pathways presents a potential therapeutic approach and warrants the development of novel therapeutic strategies. There has been a significant increase in investigation of cell-based therapies for age-related chronic conditions including physical frailty and cognitive impairment. Medicinal signaling cells, also known as mesenchymal stem/stromal cells (MSCs), are multipotent culture-expanded cells with pleiotropic mechanisms of action (9, 10). MSCs have cellular and humoral immunomodulatory and pro-vascular properties, migrate to sites of inflammation and injury, regulate host stem cell niches through paracrine effects and heterocellular coupling, and can stimulate inherent regenerative and reparative mechanisms (7, 9, 10). Moreover, MSCs are immunoprivileged/immunoevasive, and are not subject to rejection by the receiving host, allowing the use of allogeneic products that have well-documented safety profiles (11).

The first human trial investigating the application of allogeneic MSCs in older adults with frailty was the CRATUS study that demonstrated acceptable safety and tolerability results of allogeneic MSCs (12, 13). The CRATUS study also provided promising efficacy findings which allowed the powering of the current study to be determined. The phase I CRATUS trial was an open label study investigating safety and efficacy of a single intravenous infusion of allogeneic

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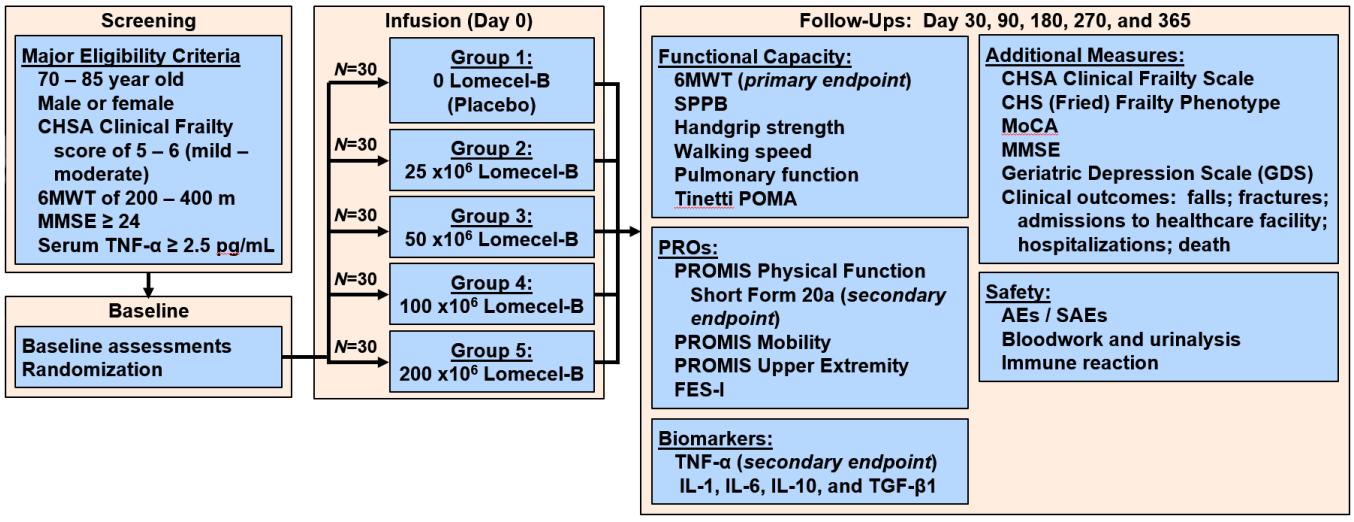
Figure 1. Schematic of the Trial Design



Target enrollment is 150 subjects aged 70-85 years of any race, ethnicity, or gender. Enrollment criteria includes a Clinical Frailty Score of 5 ("mild") or 6 ("moderate"), a six-minute walk test (6MWT) of 200-400 m, a Mini Mental Status Examination (MMSE) score ≥24, and a serum tumor necrosis factor-alpha (TNF-α) level of ≥2.5 pg/mL. Eligible subjects are randomized (1:1:1:1:1) to receive a single intravenous infusion of 25, 50, 100, or 200 million Lomecel-B, or Placebo (N=30/arm). The infusion day is defined as Day 0. Safety and efficacy assessments are conducted at 30-, 90-, 180-, and 270-days post-infusion. A follow-up telephone call at 12 months post-infusion is performed for clinical outcomes and adverse event data collection.

MSCs (20, 100, or 200 million cells/infusion, N=5 patients/arm). This study met its primary safety objective, and no treatment-emergent serious adverse events (TE-SAEs) were attributed to the study intervention. Moreover, the six-minute walk test (6MWT) distance (6MWD) increased at 3- and 6-months post-treatment in the allogeneic MSC groups (up to 76.6 m from baseline in Phase 1, and 64.8 m in Phase 2), and circulating TNF-α levels decreased at 6 months after infusion in all treatment groups. The randomized placebo-controlled phase 2 portion of CRATUS examined safety of 100 or 200 million allogeneic MSCs versus placebo in 30 frail participants (N=10/arm). In addition, this study was designed to obtain provisional efficacy data to inform a larger next-stage trial powered for efficacy. This study supported the excellent safety profile of allogeneic MSC infusions in frail older individuals, with no TE-SAEs or other safety concerns, meeting the primary endpoint. Additionally, the Phase 2 results were consistent with the results from Phase 1 in that the allogeneic MSC groups showed improvements in 6MWT, respiratory function, female sexual quality of life, and serum TNF-α. These findings provided support for further investigation of allogeneic MSCs as a therapeutic strategy for frail older adults.

As the next-step in this program, we designed the randomized, double-blinded and placebo-controlled phase 2b trial presented here. This trial is powered to detect changes in 6MWT, and further designed to evaluate dose-response to an allogeneic MSC formulation, called Lomecel-B, in the target population. The subject population consists of older adults with mild to moderate physical frailty, as determined by: age; clinically-assessed mild to moderate frailty; a 6MWD of 200–400 meters, which is supportive of the frailty status; and whom had an underlying pro-inflammatory state, a characteristic of frailty (5). The subject population is also cognitively intact to minimized related confounding issues, e.g., inability to follow assessment instructions. Study powering is based on the 6MWT, used as the primary endpoint. The trial is designed to evaluate changes in important indicators of frailty status in the domains of physical functioning, patient-reported outcomes (PROs), biomarkers, and frailty status. We hope to objectively gain important insights into the use of Lomecel-B as a biological therapy to improve health status in those with frailty.

Methods

Trial Design

The title of this trial is "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", and is registered with ClinicalTrials.gov (NCT03169231). This Phase 2b trial is double-blinded, randomized, and placebo-controlled with parallel arms at doses ranging from 0 to 200M cells of Lomecel-B. Oversight is by a single IRB (Western IRB: Puyallup, WA), independent pharmacovigilance group (ProPharma Group: Washington, DC), independent DSMB, and independent clinical monitors (Syneos/Joulé Inc.: Edison, NJ). The study and manufacturing of investigational product are under Food and Drug Administration (FDA) oversight as an Investigational New Drug Application (IND). IQVIA (Durham, NC) is selected as the CRO for this study.

Target enrollment is 150 males and females aged 70-85, who provide written informed consent, with a Clinical Frailty Score of 5 ("mild") or 6 ("moderate") (14), a six minute walk test (6MWT) of 200-400 meters, a score ≥ 24 on the Mini Mental Status Examination (MMSE), and a serum tumor necrosis factor-alpha (TNF-α) of ≥ 2.5 pg/mL at screening. The rationale for these selection criteria is detailed in the Discussion section.

Table 1. Enrollment Criteria

Inclusion Criteria
• Be willing and able to provide written informed consent and comply with all procedures required by the Protocol.
• Be >70 and < 85 years of age at the time of signing the Informed Consent Form.
• Have a CSHA Clinical Frailty Scale score of 5 “mildly frail” or 6 “moderately frail”.
• Have a 6-minute walk distance of ≥ 200m and ≤ 400 m. Distances of two 6MWTs (performed with at least 60 minutes interval) are to be within 15% of each other.
• Have a serum TNF-α level of ≥ 2.5 pg/mL.
Exclusion Criteria
• Be unwilling or unable to perform any of the assessments required by the Protocol.
• 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 diagnosis of dementia.
• Have a score ≤ 24 on the Mini Mental State Examination (MMSE).
• Have poorly controlled blood glucose levels (HbA1c >8.0%).
• 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 curatively-treated basal cell carcinoma, squamous cell carcinoma, melanoma in situ or cervical carcinoma if recurrence occurs.
• Have any condition that in the opinion of the Principal Investigator limits lifespan to < 1 year.
• Have autoimmune disease with the exception of psoriasis (e.g., rheumatoid arthritis, systemic lupus erythematosus).
• Be currently taking corticosteroids or similar powerful steroidal anti-inflammatory medication (e.g., Prednisone, TNF-α antagonists) on a regular basis (exceptions allowed include regular use of steroidal nasal sprays, topical steroids, and estrogen-replacement therapy).
• Test positive for hepatitis B virus - 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.
• Test positive for viremic Hepatitis C virus, HIV1/2, or syphilis.
• Have a resting blood oxygen saturation of <93% (measured by pulse oximetry).
• Known or suspected alcohol or drug abuse within three years preceding Screening.
• Have a known hypersensitivity to dimethyl sulfoxide (DMSO).
• Be an organ transplant recipient (other than transplantation for corneal, bone, skin, ligament, or tendon).
• Be actively listed (or expected future listing) for transplant of any organ (other than corneal transplant).
• Have any clinically important abnormal screening laboratory values, including, but not limited to: - Hemoglobin <10.0 g/dL, white blood cell <2,500/uL, or platelet count <100,000/uL - Liver dysfunction evidenced by enzymes (AST and ALT) > 3 times the ULN - Coagulopathy (INR>1.3) not due to a reversible cause (e.g., warfarin and/or Factor Xa inhibitors).
• Uncontrolled hypertension (resting systolic blood pressure >180 mm Hg or diastolic blood pressure of >110 mm Hg at Screening).
• Have unstable angina pectoris, uncontrolled or severe peripheral artery disease within the previous 3 months.
• Have congestive heart failure defined by NYHS (New York Heart Association) Class III or IV, or an ejection fraction of <25%.
• Have coronary artery bypass surgery, angioplasty, peripheral vascular disease revascularization, or a myocardial infarction within previous 3 months.
• Have severe pulmonary dysfunction: acute exacerbation of chronic obstructive lung disease stage III or IV (Gold classification), and/or PaO2 levels <60 mmHg.
• Have a partial ileal gastric bypass, or other significant intestinal malabsorption.
• Have documented advanced hepatic or renal disease.
• Have cognitive or language barriers that prohibit obtaining informed consent or any study elements.
• Be currently hospitalized or living in a long-term care facility (e.g., nursing home).
• Be currently participating (or participated within the previous 30 days of consent) in an investigational therapeutic or device trial.
• 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.

The complete inclusion and exclusion criteria are listed in Table 1. Subjects are randomized (1:1:1:1:1) within each investigational site to receive a single intravenous infusion of 25, 50, 100, or 200 million Lomecel-B, or Placebo (N=30/arm) as shown in Figure 1. The infusion day is defined as Day 0. Safety and efficacy assessments are conducted at 30, 90, 180, and 270 days after infusion. A follow up telephone call at 12 months post-infusion is performed for clinical outcomes and adverse event data collection.

Lomecel-B and Placebo

Lomecel-B is a formulation of allogeneic MSCs, prepared by Longeveron under an FDA IND Chemistry, Manufacturing, and Controls (CMC) section. The starting material is sourced from healthy young adult donors in compliance with the Code of Federal Regulations, specifically 21 CFR part 1271, and culture-expanded using current Good Manufacturing Practices (cGMP). The placebo is the vehicle used for resuspension of

Table 2. Schedule of events

VISIT		Screening Visit	Baseline Visit	Infusion Visit	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 (Follow up Phone Call)
DAY		-21 ± 21	-14 ± 14	0	30 ± 7	90 ± 14	180 ± 14	270 ± 14	365 ± 14
Informed consent		x							
Medical history		x							
Physical examination		x	x	x	x	x	x	x	
12-lead electrocardiogram		x							
Concomitant medications		x	x	x	x	x	x	x	x
Randomization			x						
Study drug infusion				x					
Review of adverse events			x	x	x	x	x	x	x
Frailty Assessments	CSHA Clinical Frailty Scale assessment	x				x	x	x	
	CHS Frailty Phenotype assessment		x			x	x	x	
Cognitive Assessments	Mini Mental State Exam (MMSE)	x					x		
	Montreal Cognitive Assessment (MoCA)		x			x	x	x	
Physical Function/ Performance Assessments	Grip strength		x			x	x	x	
	Six-Minute Walk Test (6MWT)	x	x			x	x	x	
	Short Physical Performance Battery (SPPB)		x			x	x	x	
	Tinetti-Performance Oriented Mobility (POMA) Assessment		x			x	x	x	
	Spirometry		x			x	x	x	
Patient-Reported Outcomes/ Questionnaires	PROMIS—Physical function—Short Form 20a	x			x		x	x	
	PROMIS—Physical Function—Mobility	x			x	x	x		
	PROMIS—Physical Function—Upper Extremity	x			x	x	x		
	Falls Efficacy Scale-International (FES-I)	x			x	x	x		
	Geriatric Depression Scale Short Form (GDS-SF)		x			x	x	x	
	Sexual Quality of Life-Female (SQOL-F)		x			x	x	x	
	International Index of Erectile Function (IIEF)		x			x	x	x	
Clinical Laboratory Evaluations	Blood/serum samples for Central Laboratory	x		x	x	x	x	x	
	Blood/serum samples for biomarker analysis	x		x	x	x	x	x	
	Serology for communicable diseases	x							
	Urinalysis	x			x	x	x	x	

Lomecel-B (PlasmaLyte-A with 1.0% human serum albumin). Lomecel-B and placebo are prepared in identically appearing infusion bags bearing identical appearing labels and delivered via peripheral intravenous infusion in an out-patient setting.

Study Measures and Outcomes

A timetable of the study assessments and activities is presented in Table 2.

Efficacy Measures

Clinical assessments for efficacy are performed at baseline, and at 90-, 180- and 270-days post infusion, except for the MMSE which is only performed at the screening visit as an inclusion criterion and at 180 days post-infusion. Additionally, a CSHA Clinical Frailty Scale assessment and 6MWT are also performed at the screening visit as inclusion criteria in addition to the other visits. These assessments include:

Physical Function/Performance Assessments:

- The Six-Minute Walk Test (6MWT) serves as the primary endpoint of the trial, using the administration guidelines established by the American Thoracic Society (15). The 6MWT measures the distance the subject can walk in six minutes, and is an integrated measure of physical capacity and mobility (16). Another well established and commonly used physical function measurement is the 400m walk test (17), however the 6MWT is designed to stress the cardiorespiratory capacities to estimate the physiological reserves and exercise tolerance. It measures the capacity to cope with challenging and prolonged stressors.
- Grip strength, tested via dynamometer, is a validated diagnostic marker of frailty with high sensitivity, specificity, and accuracy (18, 19). Both dominant and non-dominant hands are measured.
- The Short Physical Performance Battery (SPPB) is an objective assessment of balance, lower body strength, and mobility (20, 21). It entails a 4-Meter Walk Gait Speed Test, five chair-stand test, and a balance test. The SPPB is predictive of important health outcomes in older adults, including disability, hospitalization, institutionalization, and mortality (17).

- The Tinetti-Performance Oriented Mobility Assessment (POMA) assesses gait and balance ability, and is used to evaluate a person's risk for falling within the next year (22). Higher scores indicate better the performance, to a maximum score of 28 points. In general, scores < 19 points indicate a high risk for falling. POMA scores are significantly lower in frailty, and can differentiate older adults with frailty from those who are pre-frail (23).
- Forced Expiratory Volume in the first second (FEV1) is measured by spirometry to evaluate respiratory capacity. Reduced FEV1 correlates with reduced gait speed in older adults with mobility limitations (24).

Frailty Assessments:

- The Canadian Study of Health and Aging (CSHA) derived and validated Clinical Frailty Scale (CFS) is an instrument for measuring frailty status based on clinical judgment (14). The instruments ranks the subject between a CFS of 1 (very fit) to 9 (terminally ill). In this study, enrolled subjects must have a screening CFS of 5 (mildly frail) or 6 (moderately frail).
- Frailty Phenotype Assessment (FPA) using Cardiovascular Health Study (CHS) criteria is an operationalized phenotype definition developed and validated by Fried and colleagues (4). The CHS Frailty Phenotype is based on five criteria for evaluating specific signs and symptoms associated with frailty that are often slightly modified for differences in population, geographic region, and physiology (25-28). In this trial, the specific criteria evaluated are as follows:
  - Unintentional weight loss of greater than 10 pounds or more than 5% of body weight in the past year (as self-reported at screening for baseline) or compared to baseline at follow-up visits.
  - Endurance and energy measured by self-reported exhaustion and fatigue based on the following two questions from Center for Epidemiologic Studies Depression Scale (CES-D) (29). A score of "1" is given if responses to both questions are either "moderate amount of time" or "most of the time". A score of "0" is given if the responses to both questions are either "rarely or none of the time" or "some or little of the time":
    - I felt that everything I did was an effort in the last week:
      - Rarely or none of the time (<1 day)
      - Some or little of the time (1 to 2 days)
      - Moderate amount of the time (3 to 4 days)
      - Most of the time
    - I could not get going in the last week:
      - Rarely or none of the time (<1 day)
      - Some or little of the time (1 to 2 days)
      - Moderate amount of the time (3 to 4 days)
      - Most of the time
  - Physical activity level as self-reported in the past 3 month: weight bearing physical activity was not performed, more than four hours per day were spent sitting, and went for a short walk once per month or less (Adapted from (30, 31)).
  - Weakness as measured by an average dominant hand grip strength of ≤30 kg for men and ≤18 kg for women in the grip strength test (Adapted from (30)).
  - Slowness as assessed by a time of ≥ 6 seconds in the 4-meter gait speed test (performed as part of the SPPB assessment).

For scoring purposes, in each of these five subsections a score of 0 (subject did not meet the specific criterion) or 1 (subject met the specific criterion) is assigned, and then the final frailty score is obtained as the sum of all the five items. Accordingly, this generates a 6-level ordinal variable ranging from 0 to 5, which is categorized into a 3-level variable representing robustness (none of the criteria are met), pre-frailty (one or two criteria are met) and frailty (3 or more criteria are met).

PROs and Quality-of-Life (QOL) assessments:

- The Patient-Reported Outcomes Measurement Information System (PROMIS) is a set of validated measures for evaluating physical, mental and social health in adults and children across all conditions (32). Each questionnaire yields a summed raw score, which is then converted into standardized T-scores. The adult PROMIS Physical function—Short Form 20a was selected as a secondary endpoint in this study since it has shown strong test-retest reliability, and showed a minimally important difference of 2 points (~0.20 SD) (33). The adult PROMIS Mobility and PROMIS Upper Extremity are used as exploratory PROs.
- The Falls Efficacy Scale-International (FES-I) is a short, easy to administer tool that measures the level of concern about falling on a four-point Likert scale (1=not at all concerned to 4=very concerned (34). The FES-I was developed in a collaborative effort with members of the Prevention of Falls Network Europe (ProFaNE), European Committee focused on fall prevention and the psychology of falling. The FES-I has excellent internal validity (Cronbach's alpha=0.96) and test-retest reliability (ICC=0.96).
- Geriatric Depression Scale Short Form (GDS-SF) is a 15-item screening tool used to identify depression in older adults. Higher GDS-SF scores indicating increased depression, are seen in older adults with frailty compared to individuals who are pre-frail (23).
- Sexual quality of life is measured by the Sexual Quality of Life-Female (SQOL-F) (35) and International Index of Erectile Function (IIEF) (36). These measure are chosen as sexual functioning is reduced, and related stress is increased, in older adults with frailty (37).

Cognitive Assessments:

- To evaluate global cognitive function the Mini Mental State Exam (MMSE) and Montreal Cognitive Assessment (MoCA) are performed to assess the cognitive component of frailty (23). The MMSE is also part of the inclusion criteria.

Safety Measures

Safety assessments are conducted throughout the trial as described in the schedule of events (Table 2) as follows. Blinded attributions of any potential changes to the infusion product is made by the clinical investigator.

- Physical examination: Vital signs are assessed at each study visit at least once, including weight; height; temperature; heart rate; blood pressure; respiratory rate; pulse oximetry; general appearance; skin and nails; limbs; lymph nodes; head, eyes, ears, nose, throat, and neck; respiratory; cardiovascular; and abdominal.
- Electrocardiogram (ECG): 12-lead ECG is to assess for any clinically significant cardiac changes over the course of the trial.
- Laboratory tests: Hematology, blood chemistry, coagulation, and urinalysis are to assess for any significant laboratory changes over the course of the trial. Shift tables in each category are generated and reviewed by the Medical Monitor to capture clinically significant changes.
- Anti-HLA allo-antibody production: Panel reactive antibody (PRA) tests are used to for signs of graft rejection.
- Medical history: Comprehensive past, social, and family history is obtained at the screening visit. The medical history is obtained via an interview and by medical records.
- All adverse events and serious adverse events occurring at any time during the trial will be collected, documented, and reported by the investigator. For each event, the investigator will provide the date of onset and resolution, intensity, treatment required, outcome, seriousness, and potential causality with regards to the study product or infusion procedure.
- Concomitant medications: Current medications, including prescription and over-the-counter medications, are recorded.

Laboratory Evaluations and Biomarkers

Blood (Screening, Day 0, and at each follow-up) and urine (Screening, and at each study visit post-infusion) samples collection is for safety and efficacy evaluations. As part of the enrollment criteria, serology for communicable diseases is performed at screening visit. High-sensitivity immunoassays are performed for quantification of blood-based biomarkers including: interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α, interferon-gamma (IFN-γ), Vascular Endothelial Growth Factor (VEGF)-A, VEGF-C, VEGF-D, VEGF receptor 1 (VEGFR-1), D-dimer, basic Fibroblast Growth Factor (bFGF), placental growth factor (PlGF), Tie-2, and Transforming growth factor-β (TGF-β). Q2 Solutions (Morrisville, NC) was contracted as the independent central laboratory for this study.

Study Endpoints

Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline in 6MWT compared to placebo at 180 days post-infusion. The primary endpoint is analyzed in two ways. First, an analysis is performed to examine whether any individual dose of Lomecel-B differs from placebo. Second, an analysis assesses for the presence of a dose-response effect between amount of cell product infused and distance walked.

Secondary Efficacy Endpoints

The secondary endpoints are the following compared to placebo at 180 days post-infusion.

1. Change in PROMIS—Physical Function—Short Form 20a total score
2. Change in serum TNF-α

Exploratory Endpoints

The key exploratory endpoints expand upon the primary and secondary endpoints. First, the primary endpoint analysis is expanded to evaluate whether there is a difference between active Lomecel-B and placebo at any or across all time-points, and whether a dose-response curve is evident at other time points. Second, the secondary endpoint analysis is expanded for the PROMIS questionnaire to explore other questionnaires. Changes in patient self-reported outcomes will be correlated with changes in 6MWT. Thirdly, biomarker analysis is explored using a panel of inflammatory and vascular-endothelial biomarkers.

Exploratory analysis covers changes across visits for all other clinical assessments not included in the list of primary and secondary endpoints, and blood-based biomarkers.

In addition, a pre-specified sub-study evaluates biomarkers of the metabolic syndrome. These include glucagon, leptin, C-peptide, gastric inhibitory polypeptide (GIP) (active), glucagon-like peptide 1 (GLP-1) (active), insulin, and pancreatic polypeptide (PP).

Clinical events

The incidence of the following clinical outcomes is assessed through 365 days post-infusion: falls, fractures, admissions to healthcare facility (e.g., assisted-living facility, nursing home, long-term care facility, etc.), hospitalizations, and death.

Sample Size and Statistical Analysis

Statistical analysis is performed by an independent party (Pharma Data Associates, NJ). The sample size calculation is based on the primary endpoint, the change from baseline in 6MWT at 6 months (Δ6MWT). Thirty (30) subjects per treatment arm provides approximately 80% power to

demonstrate an effect size of 0.75 (treatment difference of each dose vs placebo in change from baseline in 6MWT divided by the common standard deviation) using a one-sided α=0.025. Assuming the common SD of 75 m, this sample size provides 50% and 80% power for the between treatment difference of 39 m and 56 m, respectively. These distances exceed prior calculated minimal clinically important differences (see Discussion) and are less than the changes seen in the CRATUS study (up to 76.6 m) (12, 13).

The analysis of the primary efficacy endpoint is performed on the modified intent to treat (MITT) population which includes all randomized subjects who have received an infusion and have at least one post-baseline assessment for the primary efficacy endpoint. Each dose group is compared to the placebo group in pairwise comparisons using a Mixed-Effect Model Repeated Measure (MMRM) method. The treatment effect is analyzed using MMRM including change from baseline at each post-treatment time point up to month 9 as the response variable, treatment, visit, interaction between treatment and visit as fixed factors, baseline distance as covariate, and patient as repeated measure unit. An unstructured (US) variance-covariance matrix is used to model the correlation among repeated measurements. To account for multiple testing of the different dose groups versus placebo, the step-up Hochberg (1988) procedure is used for the primary analysis of the primary endpoint. For the secondary analysis of the primary endpoint (dose-response effect), MCP-Mod (multiple comparison procedure – modeling) method is used.

The MCP-Mod method is a hybrid approach combining hypothesis testing and modeling in a structured manner to analyze phase 2 dose-ranging studies with the purpose of finding suitable dose(s) for confirmatory phase 3 trials (38, 39). The first step of the procedure (MCP-step) is used to assess presence of a dose-response signal using a trend test deducted from a set of pre-specified candidate models. The second step (Mod-step) relies on parametric modeling or model averaging to find the "optimal" dose for confirmatory trials.

In the first step, linear, quadratic, Emax and Sigmoid Emax dose-response models are used for the trend test in Δ6MWT. In the second step, the model mean of the dose-response curve is plotted with 95% confidence interval.

Statistical testing for the key secondary endpoint PROMIS—Physical Function—Short Form 20a and all other secondary/exploratory endpoints uses MMRM method without adjusting for multiple testing. The Fisher's exact test is used to analyze subject incidences of falls, fractures, admission to healthcare facility, hospitalizations, and deaths.

To assess the relationship between outcome variables, correlations are calculated for the absolute values as well as the changes from baseline between the primary endpoint 6MWT and the secondary/exploratory PRO endpoints, and other biomarkers. In addition, regression analyses are conducted to evaluate whether these specific secondary/exploratory endpoints and PRO instruments can predict the clinical outcome measured as 6MWT in this population.

Safety assessments are based mainly on the nature, frequency, relationship, and severity of adverse events (AEs). AEs are coded by primary system organ class (SOC) and preferred term (PT) according to the Medical Dictionary for Regulatory Activities. The treatment-emergent adverse events (TE-AEs) are summarized by the number and percentage (n and %) of subjects in each SOC and PT. For summaries by relationship to study drug and relationship to study infusion, "Definitely/Probably/Possibly related" are combined, and "Unlikely/Unrelated" are combined. When multiple AEs are 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.

Discussion

This study is designed to assess the effect of 4 doses of Lomecel-B compared to placebo on mobility and exercise tolerance, patient-reported physical function, and biomarkers for inflammation and vascular-endothelial function, in older adults with frailty. Frailty is a common and important geriatric syndrome characterized by age-associated declines in physiologic reserve and function across multi-organ systems, leading to increased vulnerability to stressors and a higher risk for adverse health outcomes (40). When exposed to stressors such as acute or chronic illness (e.g. myocardial infarction), a new drug, a "minor" infection, or iatrogenically (e.g. surgery), frail patients are at risk for marked and often disproportionate decompensation, adverse events, procedural complications, prolonged recovery, functional decline, disability and mortality (4, 40-44).

Proposed factors underlying the biology of frailty include chronic systemic inflammation (45-47), vascular-endothelial dysfunction, endocrine dysfunction, neuropsychological impairment, cardio- and cerebrovascular disease, and malnutrition (44, 48, 49). In frailty, muscle breakdown exceeds muscle synthesis, leading to a progressive decline in muscle mass, strength, and function, and sarcopenia. Under stressed conditions, subclinical impairments are unmasked, and a vicious cycle ensues with physical inactivity and malnutrition leading to further decline. The clinical manifestations of frailty can present as a constellation of signs and symptoms, which can vary to a degree among individuals (4, 41, 42). Common clinical indicators of frailty are sarcopenia, fatigue, weakness, exhaustion, poor endurance, low physical activity, poor balance, slow gait speed, falls, anorexia, malnutrition, and weight loss. Improving mobility and physical functionality may result either concurrently or subsequently in improvements in other associated signs and symptoms.

Study Population

The study population for this trial consists of older adults with mild to moderate frailty. The choice of this population was informed in part by results from the CRATUS trial (12, 13), and improved study powering by reducing baseline variability, given the relatively modest sample size. The CRATUS study enrolled subjects with CFS scores ranging

from 4 ("vulnerable") to 6 ("moderate frailty"). In CRATUS study, it was shown that those with a CFS score of 4 performed relatively well on the 6MWT (most >400 m) and raised concerns that this could impose a ceiling effect on potential improvement in more robust patients, and thereby confound interpretation of the primary endpoint if such patients were included in this study (Oliva et al., unpublished). On the other hand, we were also concerned that subjects with more severe frailty (CFS score ≥ 7) might not respond as effectively to a single infusion of Lomecel-B, which again could confound the data. From our analyses of CRATUS, we found that 83% of subjects with baseline CFS scores ≥ 5 (i.e., frail) had 6MWT distances < 400 m, and 85% of those subjects with baseline 6MWT distances >400 m had CFS scores < 5 (i.e., predisposition to frailty). Most subjects with a CFS score or 5-6 also had a 6MWD >200 m. Thus, we narrowed the population to those with a 6MWD of 200-400 m, which would be mutually confirmatory of the clinician-assessed CFS score of 5 – 6.

The 6MWT has a documented learning curve that is effectively overcome after 2 trials (50). Thus, 6MWT is performed twice at screening to overcome this learning curve that might otherwise be misconstrued as placebo-effect. The third 6MWT, performed at baseline, acts as the formal baseline measure for this study.

Frailty in older adults is underlined by a chronic low-level pro-inflammatory state, in which other groups have found significant correlations to serum levels of TNF-α and other pro-inflammatory cytokines (5). In CRATUS, we found that subjects with baseline serum TNF-α ≥ 2.5 pg/mL appeared to have greater responses to a single dose of cells (unpublished results) compared with those below 2.5 pg/mL. We thus incorporated a serum TNF-α ≥ 2.5 pg/mL as part of the enrollment criteria to capture a potentially more responsive patient population. Given the anti-inflammatory potential of Lomecel-B, regular use of TNF-α antagonists or powerful steroidal anti-inflammatory medication (e.g., Prednisone) is an exclusion criteria to reduce potential confounding issues introduced by such medications.

Finally, the trial incorporates exclusion criteria intended to minimize confounding effects of severe comorbidities. These include exclusions for poorly controlled diabetes or hypertension, advanced hepatic, cardiac, pulmonary, or renal disease. Exclusions are also made for neurological conditions and cognitive disorders (e.g., dementia), to enrich for a population with physical, but not cognitive, frailty. Subjects with any physical disability that would impact their mobility are also excluded.

Dosing Selection Rationale

To gain clarity on potential optimal dosing, this Phase 2b trial is designed to evaluate dose-response using dosages that double from 25M to 200M cells, versus placebo. This dose range was chosen based on provisional efficacy data from the CRATUS trial, which suggested an optimal dose at 100 million cells, with minimal additional benefits at higher dosing (12). This is also in accord with other studies using allogeneic MSCs for other aging-related indications, e.g., cardiomyopathy (51-53). We thus chose a dose-range around the 100M cell dosage in order to understand what might represent a minimal clinically effective dose.

The maximum dosing level is also guided by previous trials, which showed the safety of allogeneic MSCs at the maximum dose evaluated of 200M cells (12). This safety profile is consistent with other clinical results, which demonstrate the general high safety profile of allogeneic MSCs (9, 11).

Study Endpoints

Six-minute Walk Test (6MWT) as Primary Endpoint

The 6MWT is a validated test that is a reliable indicator of frailty status in older adults (54, 55) which is also shown applicable in several other conditions (56-59). The 6MWT is applicable to frailty because the test is an integrated global assessment of cardiac, respiratory, circulatory and muscular capacity. The 6MWT is predominately a test of aerobic capacity, and is a reflection of a patient's ability to perform activities of daily living (ADLs) (15). In a study evaluating the distance on the 6MWT as a frailty indicator in 60 older adults with heart failure, a positive correlation was found between patients considered to have low endurance (6MWT of <300 m) and a CHS frailty phenotype score of ≥ 3 ($p<0.001$) (54). There was also a positive correlation for subjects with high endurance (6MWT>300 meters) and non-frailty ($p<0.001$) (54). Enright et al. (60) reported strong correlations between low body mass and 6MWT, indicating that frail subjects with sarcopenia perform poorly on 6MWT.

Frailty results in impaired mobility leading to dependence on others for ADLs. Typical ADLs that involve walking, such as shopping, require average walking distances of 200-600 meters (61). Such distances included walking to the post office (52 meters), bank (57.1 meters), medical office building (65.8 meters), pharmacy (206 meters), department store (346 meters), and a grocery store (380 meters) (62). National Surveys indicate that at least 40% of community-dwelling adults over 74 have some difficulty walking a quarter mile (400 meters) (63). According to the US Census Bureau, difficulty walking a quarter mile is a standard criteria used to assess disability in the physical domain which is also "the most common lower body functional limitation for adults" (64). Furthermore, slow walking speed is a strong predictor of mortality in older adults (65).

Several studies have determined minimal clinically important differences (MCIDs) in the 6MWT. These range from 17.8 – 20 m in older adults (66, 67), to 32 m for patients with congestive heart failure (68), whereas a decline of 30 m correlates with a 19% increase in mortality for patients with congestive heart failure (69).

Strengths, Limitations, and Risk-Benefits

The trial is powered for efficacy, and endpoints evaluate changes in multiple domains for assessing frailty. It is also rigorously designed to evaluate dose-response relationship of Lomecel-B, centered around a dose that appeared most effective in prior trials (100M cells). This study refines a target patient population based on data from prior trials, which could enhance study powering through reduction in baseline variability.

A general limitation is the lack of interventional trials using an investigational products for frailty, and thus no precedence has been established for registrational endpoints. Subjects in this trial are given a single dose of Lomecel-B or placebo and followed for 9 months. While this dosing frequency and study duration could potentially be limiting to observing an effect, the prior clinical trials support potential efficacy with a single dose over this time-window.

Finally, there are no approved medical therapeutics for frailty in older adults. Allogeneic MSCs have a well-documented high safety profile (11), were shown to be safe and tolerable in successfully completed phase 1 and phase 2 trials for this indication (12, 13) and also showed provisional efficacy in those trials. Given the major and growing unmet medical need of frailty in older adults, and the demonstrated safety and provisional efficacy of this approach, the risk-benefit ratio strongly justifies conducting this next-phase trial.

Summary

This study evaluates several efficacy domains of Lomecel-B for frailty in older adults and represents the first clinical trial powered for efficacy and dose-response establishment of a regenerative medicine product for this growing unmet medical need. The results of this trial will have important implications in an emerging area of geroscience and could lead to novel approaches to enhance healthspan in older individuals thereby reducing the socio-economic societal burden imposed by frailty in the aging population.

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Conflicts of Interest: AAO, BH, LM-M, KNR, KY, LD, GAG and JMH are affiliated with Longeveron Inc. JMH is a co-founder, board member and paid consultant of Longeveron Inc. JMH is also inventor of technology licensed to Longeveron Inc. This relationship is reported to the University of Miami, and a management plan is in place. EV and JW are members of Longeveron's Science Advisory Board, for which they receive personal fees (honoraria). The University of Miami is an equity owner in Longeveron, which has licensed intellectual property from the University of Miami.

Ethical standard: Informed consent is obtained from all the participants involved in the study. Ethics approval for this study is provided by the Institutional Review Board (WIRB).

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