STATISTICAL ANALYSIS PLAN

Treatment of Central Retinal Vein Occlusion Using STem Cells Study (TRUST)

(Phase I/II Randomized, Prospective, Double-masked, Sham-controlled Study of Intravitreal Autologous Bone Marrow CD34+ Stem Cell Therapy for Central Retinal Vein Occlusion)

IND#: 17023

02 April 2024

Version 1.0

Prepared and distributed by:

TRUST Data Coordinating Center
The Emmes Company, LLC
Rockville, Maryland

SIGNATURE PAGE

Study Chair / Principal Investigator: Susanna Park, MD, PhD

Signature: Susanna Park Susanna Park Date: 03/Apr/2024 01:54 AM EDT

I am approving this document.

Data Coordinating Center Principal Investigator: Jennifer McCormack, MS

Signature: Jennifer McCormack Jennifer McCormack Date: 02/Apr/2024 12:24 PM EDT

I am approving this document.

Data Coordinating Center Lead Statistician: Jacquie King, MS

Signature: Jacquie King Jacquie King Date: 02/Apr/2024 12:45 PM EDT

I am approving this document.

TABLE OF CONTENTS

1.0 STUDY SYNOPSIS 1
2.0 STUDY OBJECTIVES 2
2.1 Primary Objective 2
2.2 Secondary Objective 2
2.3 Exploratory Objective 2
3.0 STUDY DESIGN 2
3.1 General Design and Plan 2
3.2 Sample Size Considerations 3
3.3 Randomization 3
3.4 Masking 4
3.4.1 Type of Masking 4
3.4.2 Maintenance of the Masking 4
3.5 Unmasking 5
3.6 Participant Discontinuation / Replacement 6
4.0 GENERAL ANALYSIS POPULATIONS, DEFINITIONS AND CONVENTIONS 7
4.1 Analysis Populations 7
4.1.1 Screened Population 7
4.1.2 Intent-to-Treat Population 7
4.1.3 Safety Population 7
4.1.4 Per Protocol Population 7
4.2 General Definitions 7
4.2.1 Study Periods 7
4.2.2 Baseline 7
4.2.3 Adverse Event 7
4.2.4 Serious Adverse Event 8
4.2.5 Post-treatment AEs 8
4.3 Tables, Figures and Listings Conventions 8
5.0 STUDY POPULATION 8
5.1 Eligibility Criteria for Selection of Study Population 9
5.1.1 Study Eye Inclusion/Exclusion Criteria 9
5.1.2 Participant-Level Inclusion/Exclusion Criteria 10
6.0 ANALYSIS OF PARTICIPANT CHARACTERISTICS 11
7.0 OUTCOME ANALYSIS 11
7.1 Primary Outcomes 11
7.1.1 Safety 11
7.1.2 Feasibility 12

7.1.3 Supportive Analysis for Primary Outcome 12
7.2 Patient Profiles 13
7.3 Secondary Efficacy Outcomes 13
7.3.1 Secondary Efficacy Outcome Analysis Methods 14
7.3.2 Secondary Efficacy Outcome Measures 14
7.4 Exploratory Outcomes 22
7.4.1 Advanced Retinal Imaging (Investigational OCT) 22
7.4.2 Cell Characterization 23
7.4.3 Participant Satisfaction 24
7.5 Methods for Handling Missing Data 24
8.0 DATA QUALITY 24
8.1 Data Audits 24
8.2 Protocol Deviations 24
9.0 SOFTWARE TO BE USED FOR ANALYSIS 24
10.0 UPDATES TO THE STATISTICAL ANALYSIS PLAN 25
11.0 LIST OF PROPOSED TABLES, LISTINGS AND FIGURES 26
12.0 REFERENCES 31
13.0 APPENDIX 32
13.1 Simulation Results 32

TABLES

Table 1: Masking of Treatment Assignment of Enrollment Center Personnel 5
Table 2: Participant Follow-up and Replacement Decisions 6
Table 3: Visual Acuity Outcomes 14
Table 4: Microperimetry Outcomes 15
Table 5: OCT/OCTA Outcomes 15
Table 6: Multifocal ERG Outcomes 18
Table 7: Full-field ERG Outcomes 19
Table 8: Autofluorescence Outcomes 20
Table 9: Fundus Photography and Fluorescein Angiography Outcomes 20
Table 10: Quality of Life Outcomes 21
Table 11: Advanced Retinal Imaging Outcomes 22
Table 12: Cell Characterization Outcomes 23
Table 13: Participant Satisfaction Outcomes 24

FIGURES

Figure 1: Power Curve for T-test 32
Figure 2: Power Curve with Differing Levels of Treatment Decay 33
Figure 3: Power Curve with Differing Levels of Period Effect 33

1.0 STUDY SYNOPSIS

The goal of this phase I/II prospective, randomized, sham-controlled, double-masked clinical trial is to determine whether intravitreal autologous CD34+ stem cell therapy is safe, feasible and potentially beneficial in eyes with vision loss from central retinal vein occlusion (CRVO). Retinal Vein Occlusion (RVO) is a leading retinal vascular cause of vision loss in the elderly. CD34+ stem cells in human bone marrow are mobilized into the circulation in response to tissue ischemia for tissue revascularization and repair. Since local delivery of CD34+ stem cells benefits ischemic tissue, intravitreal delivery of CD34+ stem cells may benefit vision and retinal ischemia in eyes with RVO. A pilot clinical trial has shown no major safety or feasibility concerns using intravitreal autologous CD34+ bone marrow stem cells. In this proposed expanded phase I/II study, approximately 20 participants (20 eyes) with persistent vision loss from CRVO will be enrolled and followed for 1 year. Only eyes with CRVO will be enrolled to maintain uniformity in the study population. Total study duration will be approximately 60 months. This single-center study will be conducted at the University of California Davis (Enrollment Center). The Data Coordinating Center will be Emmes. The Study Chair/Principal Investigator will be Dr. Susanna Park. The study will be conducted under an IND cleared from the FDA. A Data and Safety Monitoring Committee (DSMC) will oversee the study. Participants will be randomized 1:1 to immediate cell therapy/deferred sham therapy or immediate sham therapy/deferred cell therapy, where deferred therapy refers to the therapy administered at month 6 and immediate therapy refers to therapy administered at baseline (either sham or cellular). At month 6, the (immediate) cell treated eye will receive sham treatment and the (immediate) sham treated eye will receive cellular therapy. The cell therapy involves a bone marrow aspiration, isolation of CD34+ cells from the aspirate under Good Manufacturing Practice (GMP) conditions, and intravitreal injection of the isolated CD34+ cells. The sham therapy involves a sham bone marrow aspiration with penetration of the skin but with no penetration of the bone and a sham injection without penetrating the eye. The participant, examining ophthalmologist, visual acuity examiner, photographers and OCT, perimetry, and ERG technicians will remain masked to study treatment assignment for study duration. A comprehensive eye examination with ETDRS best-corrected visual acuity, optical coherence tomography (OCT) and OCT angiography (OCTA), autofluorescence, fundus photography, fluorescein angiography, microperimetry, and electroretinography will be performed at baseline and serially. A subset of participants with good fixation on microperimetry and clear media on exam and commercial-grade OCTA and who give consent will have ultra-high resolution cellular retinal imaging using research-grade OCT and OCTA and adaptive optics-OCT at baseline. These participants with high quality images at baseline will have repeat imaging at 1 month after receipt of cellular treatment, with at least 2 of the participants randomized to the immediate sham therapy/deferred cellular therapy arm also having imaging 1 month after sham therapy. Post-release flow cytometry characterization will be performed to determine the composition of the CD34+ enriched final product in terms of hematopoietic versus angiogenic stem cells based on cell surface markers (i.e., CD133(+)/CD45(+)/CD34(+) vs CD31(+)/VEGFR-2(+)/CD45(-)/CD34(+)) provided there are an adequate number of cells, as determined by the GMP lab. The primary outcome measures for the safety and feasibility of the stem cell therapy will be the incidence and severity of ocular and systemic adverse events reported during Study Period 1 (i.e., the day of the first treatment (Treatment 1) up to but not including the day of the second treatment (Treatment 2)) and the mean and range of number of CD34+ cells isolated and the number injected into the eye. The secondary safety outcome measures are the incidence and severity of ocular and systemic adverse events reported throughout study duration. The secondary efficacy outcome measures will be the changes in visual function, retinal perfusion and morphology following cell therapy. The long-term objective is to determine whether intravitreal autologous CD34+ cell therapy can minimize, or reverse vision loss associated with retinal ischemia without compromising safety.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the study is to assess the safety and feasibility of the stem cell therapy, which will be evaluated using the following primary outcome measures:

- Primary safety outcome: Incidence and severity of ocular and systemic adverse events reported during Treatment Period 1 (i.e., the day of the first treatment (Treatment 1) up to but not including the day of the second treatment (Treatment 2)).
- Feasibility outcome: The mean and range of number of CD34+ cells isolated from the bone marrow aspirate and number injected into the eye.
- Secondary safety outcome: Incidence and severity of ocular and systemic adverse events reported throughout study duration.

2.2 Secondary Objective

The secondary objective is to explore the effect of stem cell therapy on retinal function and morphology. Secondary efficacy outcomes include changes in best corrected visual acuity (BCVA), microperimetry, OCT/OCTA, electroretinography (ERG), fundus photography, fluorescein angiogram, autofluorescence and quality of life.

2.3 Exploratory Objective

Exploratory aims include descriptive analysis of changes in the retina and retinal vasculature at a cellular level using adaptive optics-OCT imaging, descriptive and quantitative analysis of changes in retinal vascular density and retinal morphology using ultrahigh resolution swept-source OCT and OCTA. An additional exploratory aim includes quantitative analysis of the hematopoietic and endothelial stem cell subcomponents and subcomponent of cells with VEGFR-2 in the harvested CD34+ population. A study survey will collect participants' experiences with various aspects of the study, including their impressions on when they received stem cell injection vs sham injection.

3.0 STUDY DESIGN

3.1 General Design and Plan

This is a phase I/II randomized, prospective, double-masked, sham-controlled study to determine the feasibility, safety and potential efficacy of intravitreal injection of autologous adult CD34+ stem cells from bone marrow (herein referred to as cellular therapy) in participants with vision loss from CRVO.

All potential participants diagnosed with CRVO will be screened by the PI or designee for study eligibility based on available clinical information. Those whose medical record suggests they may be eligible for the study will be asked to attend one or more screening visits at the UC Davis Eye Center. Informed consent will be obtained prior to conduct of any study procedures. Participants will undergo pre-treatment screening assessment and baseline testing to evaluate the extent of the condition and to confirm eligibility for the study. Once the participant is confirmed to be eligible, he/she will be randomized 1:1 to immediate cell therapy/deferred sham therapy or immediate sham therapy/deferred cell therapy.

Participants who are randomized to immediate cell therapy/deferred sham therapy will undergo a bone marrow aspiration followed by intravitreal injection of isolated CD34+ cells after baseline testing. Follow-up assessments will occur at 1 day, 1 week, 1 month, 3 months and 6 months post stem cell injection. Within 1 month of the 6-month follow-up study visit, participants will receive a sham bone marrow aspiration and sham injection. At least 1 day will separate the 6-month follow-

up study visit and the sham therapy. Participants will continue to be followed at 1 day, 1 week, 1 month, 3 months, and 6 months for a total follow-up of 12 months after the stem cell injection (and 6 months following the sham injection). For participants who did not complete the study satisfaction survey at the 12-month visit, an exit visit may occur following the 12-month visit to allow the survey to be completed.

Participants who are randomized to immediate sham therapy/deferred cell therapy will undergo a sham bone marrow aspiration followed by sham injection after baseline testing. Follow-up assessments will occur at 1 day, 1 week, 1 month, 3 months and 6 months post sham injection. Within 1 month of the 6-month follow-up study visit, participants will undergo a bone marrow aspiration followed by intravitreal injection of isolated CD34+ cells. At least 1 day will separate the 6-month follow-up study visit and the cellular therapy. Follow-up assessments will occur at 1 day, 1 week, 1 month, 3 months, and 6 months thereafter for a total follow-up of 12 months after the sham injection (and 6 months following the stem cell injection). For participants who did not complete the study satisfaction survey at the 12-month visit, an exit visit may occur following the 12-month visit to allow the survey to be completed. The study schema is provided in Section 2.0 of the protocol and Table 2 of the protocol details the schedule of evaluations.

3.2 Sample Size Considerations

There is no formal sample size calculation for this small phase I/II study and no formal hypothesis testing will be performed. A sample size of 20 participants was chosen to ensure that it is financially feasible to conduct the study and logistically possible to complete the study enrollment within the study period. The primary objective is to assess the safety and feasibility of the stem cell therapy; however, it is desired to obtain some gauge of potential efficacy of this cell therapy. Thus, participants will be randomized 1:1 to immediate cellular therapy/deferred sham or immediate sham/deferred cellular therapy. After their first treatment (cellular or sham), participants will be followed for 6 months, at which time participants who initially received sham treatment will be given stem cell treatment and vice versa. The small sample size is not ideal, but for practical purposes, it would be difficult to increase the sample size significantly without increasing the enrollment period for the study.

Although the study as designed may not be large enough to determine efficacy, some efficacy signals might be observed with the current study design. Such a signal may warrant further investigation of this therapy for CRVO. When a phase II/III randomized controlled trial is planned, appropriate sample size calculations will be performed.

It is recognized that the study has limited power to show efficacy; however, we hope to obtain some trends in change in visual function or retinal morphology or perfusion that may suggest efficacy to justify a larger randomized prospective clinical trial. Power calculations are explored in section 5.2.2 for the proposed method of analyzing the secondary outcomes.

3.3 Randomization

Randomization will occur after informed consent has been obtained and once a participant is confirmed to be eligible. Randomization will need to occur prior to the baseline visit for logistical and scheduling reasons. Much coordination among the hematologist, the Good Manufacturing Practices (GMP) facility, and the treating ophthalmologist needs to occur on days in which the cellular therapy is given. Therefore, knowing which treatment arm to which the participant has been randomized prior to the baseline visit is necessary. Great care will be taken to choose participants who are highly motivated to participate so as to prevent drop-out post-randomization.

A secure, internet-based eligibility, enrollment, and randomization system is integrated into the study to control and document the randomization assignments. Eligible participants will be randomized in a 1:1 ratio to immediate cellular therapy/deferred sham therapy or immediate

sham/deferred cellular therapy. Random assignments are generated and maintained by the Data Coordinating Center (DCC).

3.4 Masking

3.4.1 Type of Masking

This is a double-masked, sham-controlled trial, in which participants, visual acuity examiners, examining ophthalmologists, study photographers and technicians will remain masked to treatment assignment during study duration.

3.4.2 Maintenance of the Masking

To maintain the masking for the study duration of each participant's follow-up period, there will be separate ophthalmologists designated for administration of study therapy and study visit follow-up examination after study therapy (**TABLE 1**). The PI will remain masked as the examining ophthalmologist for the study duration. The treating ophthalmologist will be an experienced retinal specialist and faculty member of the UC Davis Eye Center. He/she will remain unmasked to therapy assignment and perform the eye examinations during the first week of each participant's follow-up after sham or cell therapy. An experienced retinal specialist from the Enrollment Center will be available as a backup unmasked treating ophthalmologist, and a separate experienced retinal specialist from the Enrollment Center will be available as a masked examining ophthalmologist. However, all efforts will be made to have the study therapy administered by the main treating ophthalmologist and all masked study examinations performed by the masked examining ophthalmologist (PI) to minimize confounding variables.

Study Coordinators from the UC Davis Eye Center Clinical Trials Team will be assigned to the study to manage the day-to-day activities regarding the study under the supervision of the PI, with the unmasked Study Coordinator being provided only general guidance from the PI. The unmasked Study Coordinator will coordinate and schedule all unmasked study visits and therapies.

The masked and unmasked Study Coordinators will be responsible for scheduling all study visits taking utmost care to preserve the masking. Prior to randomizing the participant, the masked Coordinator will schedule the Treatment 1 and Treatment 2 study visits working under the pretense that both procedures involve stem cell therapy. Once the participant is randomized, the unmasked Study Coordinator will provide both the study hematologist and the GMP laboratory with information regarding which Treatment study visit will require bone marrow/cellular therapy and which will require sham aspiration/sham injection. The unmasked Study Coordinator will also schedule the post Treatment Day 1 and Week 1 study visits for both Study Periods.

The visual acuity examiners and photographers and technicians obtaining fundus photographs, fluorescein angiograms, OCT/OCTA and autofluorescence imaging, microperimetry and electroretinography (ERG) will be masked to study treatment assignment to avoid bias in outcome assessment. The advanced retinal imaging team, however, will be unmasked for the duration of the study.

Table 1: Masking of Treatment Assignment of Enrollment Center Personnel	
	Masked Status
Principal Investigator	Masked
Examining Ophthalmologist	Masked
Masked Study Coordinator	Masked
Hematologist	Unmasked
Visual Acuity Examiner	Masked
Treating Ophthalmologist	Unmasked
Unmasked Study Coordinator	Unmasked
Photographers	Masked
OCT Technicians	Masked
Perimetry Technicians	Masked
ERG Technicians	Masked
Study Participants	Masked
Advanced Retinal Imaging Team	Unmasked
GMP Laboratory Team	Unmasked

All DCC staff will be unmasked to treatment assignment throughout a participant's follow-up. Knowledge of the treatment assignment by DCC staff will not affect assessment of therapy outcome as there will be little to no interaction of DCC staff with visual acuity examiners, examining ophthalmologists, study photographers or technicians, or study participants. Care will be taken when DCC staff are conversing with site personnel. DCC staff will be trained in the masked status of each site staff member especially the Study Coordinators to ensure that during regular communications information that may be unmasking is not inadvertently provided. The electronic data capture system will be designed in such a way that masked staff will not have access to any data related to therapy or adverse events reported during the first month following therapy as this information is potentially unmasking.

While the study is ongoing, site personnel, including the Study PI, will not have access to cumulative summaries of study findings broken down by treatment arm. Instead, these summaries will be presented only to the DSMC within a closed session in which only the DSMC members and select DCC staff (e.g., study statistician) are present. Although not fail-safe, restricting access in this way will help prevent unintentional changes in study implementation.

3.5 Unmasking

There are no anticipated circumstances in which it may be necessary to unmask a particular study participant before completion of follow-up. However, in the rare case that it becomes necessary, the request to unmask an individual participant will be made by the Study PI. Unmasking the participant should be made only in cases of medical emergency when knowledge of the treatment group assignment may be necessary for clinical management and decision making. The decision to unmask a participant will be made by the Study Principal Investigator and the DCC Medical Monitor.

3.6 Participant Discontinuation / Replacement

In the event that a participant is terminated from the study after randomization and prior to receipt of treatment (cellular or sham) at the Treatment 1 visit regardless of the reason, an additional participant will be accrued as a replacement. In the event that a participant receives the first treatment but opts not to receive the second treatment, an additional participant will be accrued as a replacement, but the original participant will be encouraged to return for all study visits to ensure the participant's safety is not compromised. The replacement participant will receive both treatments and will be followed for 12 months, the same as any other participant enrolled in the study. If a participant is terminated or withdraws from the study following the receipt of the second treatment, the participant will again be encouraged to return for all study visits for safety monitoring but will not be replaced. A maximum of one participant may be replaced, regardless of timing of withdrawal.

Table 2 contains these follow-up and replacement decisions for each scenario.

Table 2: Participant Follow-up and Replacement Decisions

Scenario		Original Participant Follow-up	Included in ITT?	Replacement Decision
1	Participant terminates/withdraws after randomization prior to cellular/sham therapy on Day 0 (includes inadequate sample following bone marrow aspiration)	No further assessments performed; participant only followed to close-out any AEs.	No	Additional patient will be accrued as a replacement
2	Participant receives 1st treatment but withdraws during Period 1 / refuses further treatment	Participant encouraged to follow visit schedule at least through Month 6 or longer (for safety monitoring)	Yes	Additional participant will be accrued as a replacement (up to 1 time)
3	Participant has inadequate sample following bone marrow aspiration (period 2)	Participant cannot receive 2nd treatment; is necessarily unmasked	Yes	Additional participant will be accrued as a replacement (up to 1 time)
4	Participant receives 2nd treatment but withdraws during Period 2	Participant encouraged to follow visit schedule through Month 12 (for safety monitoring)	Yes	No replacement

4.0 GENERAL ANALYSIS POPULATIONS, DEFINITIONS AND CONVENTIONS

4.1 Analysis Populations

4.1.1 Screened Population

The screened population consists of candidates that meet basic study eligibility criteria who signed the written informed consent (IC) at the initiation of the screening process and were enrolled into segment A.

4.1.2 Intent-to-Treat Population

The (modified) intent-to-treat population consists of all participants who were randomized and received the first injection (cellular or sham). For analyses, these participants will be analyzed in the arm to which they were randomized.

4.1.3 Safety Population

The safety population includes all participants who completed written informed consent.

4.1.4 Per Protocol Population

No per protocol population is planned for this study.

4.2 General Definitions

4.2.1 Study Periods

The time of a participant's participation in the study will be divided into three periods. The Screening Period refers to the time between signing of informed consent to the day prior to the day of the first treatment. Study Period 1 refers to the time between the day of the first treatment (Treatment 1) up to but not including the day of the second treatment (Treatment 2). Study Period 2 refers to the time between the day of the second treatment up to and including the 12-month visit, recognizing that for those who initially receive cellular therapy, Study Period 2 occurs post cellular therapy and therefore is not a true sham period.

4.2.2 Baseline

For all visits occurring during Study Period 1, baseline will be defined based on the measurements taken prior to Treatment 1 (during the screening/baseline visits). For all visits occurring during the Study Period 2, baseline will be defined based on the measurements taken during the Month 6 visit immediately prior to Treatment 2. It is noted that for participants in the sham/deferred treatment group, these two sets of baseline measurements should be similar due to the fact that no active treatment is given during the Study Period 1. We will review the values of these two sets of baseline measures to examine the extent to which this is true.

4.2.3 Adverse Event

An adverse event (AE) is any untoward medical occurrence, whether or not considered treatment- or procedure-related, which occurs during the conduct of the study. Medical conditions or diseases present before a participant has study treatment on Day 0 are only considered adverse events if they worsen after the participant has signed ICF (temporal association).

A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the study treatment caused the adverse event. A reasonable possibility implies that there is evidence that the study treatment caused the event.

An adverse reaction is any adverse event caused by the study treatment.

4.2.4 Serious Adverse Event

A Serious Adverse Event (SAE) is defined as any adverse event that suggests a significant hazard, contraindication, side effect, or precaution. This includes, but may not be limited to any of the following events:

1. Death
2. Life-threatening (Life-threatening means that the study participant was, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred.)
3. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
4. Inpatient hospitalization or prolongation of existing hospitalization
5. Congenital abnormality or birth defect
6. Important medical event that may not result in one of the above outcomes, but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event.
7. Vision-threatening (Refer to Section 8.3, Vision threatening Adverse Events)

Hospitalization or surgery for diagnostic or elective procedures for a preexisting condition is not considered an SAE. A pre-existing condition can be an SAE if the frequency, intensity, or character of the condition worsens after signing of informed consent. If any questions arise regarding what may qualify as an adverse event or SAE, the Medical Monitor at the DCC will be consulted.

Unexpected Event or Reaction: An event or reaction is considered “unexpected” when its nature (specificity) or severity is not consistent with applicable product information, such as safety information provided in the package insert, the investigational plan, or the investigator’s brochure.

4.2.5 Post-treatment AEs

Post-treatment AEs are events that occur on or after the day of the first treatment (cellular or sham).

4.3 Tables, Figures and Listings Conventions

Safety analyses will be summarized for the safety population. All other analyses described in this document will be done for the ITT population and summarized by randomized treatment group and overall. For all summaries of ITT population, participants will be analyzed according to the treatment arm to which they were randomized regardless of the subsequent sequence of events regarding study drug exposure.

Continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, percentiles (median, maximum, and minimum). Categorical variables will be summarized in terms of frequencies and/or percentages.

Any deviations from the above general conventions will be noted in the subsequent sub-sections.

5.0 STUDY POPULATION

Eligible participants include adult men and women having persistent vision loss due to CRVO with or without concurrent retinal artery occlusion in the study eye and meet all of the inclusion criteria and none of the exclusion criteria as outlined in the study protocol.

As this is a phase I/II study and the number of participants is small, all available data from participants who meet the protocol's eligibility criteria and who were not involved in any significant protocol violations will be used. The intent-to-treat population consists of all randomized participants who receive the first treatment.

5.1 Eligibility Criteria for Selection of Study Population

5.1.1 Study Eye Inclusion/Exclusion Criteria

Inclusion criteria for the study eye are:

1. Clinical diagnosis of central retinal vein occlusion (CRVO) confirmed by review of medical records and screening assessment.
2. BCVA obtained during the screening period is in the range of 20/40+ to 20/400- (ETDRS letter score in the range of 18 to 73, inclusive).
3. Duration of vision loss from CRVO $\geq$ 6 months to $\leq$ 42 months.

Exclusion criteria for the study eye are:

1. Previous eye treatment with intravitreal or periocular steroids, laser or intraocular surgery within 6 months prior to enrollment (i.e., date ICF signed) or treatment is expected to be given during the study period.
2. For eyes requiring treatment to prevent recurrent macular edema, on-going intravitreal anti-VEGF treatment is expected to be given at an interval < every 8 weeks during the study period or anti-VEGF therapy was started less than 24 weeks prior to informed consent.
3. History or concurrent ocular herpes infection.
4. Active non-herpetic eye infection diagnosed within 8 weeks from enrollment (i.e., date ICF signed).
5. Glaucoma requiring treatment with more than 2 medications, laser or intraocular surgery.
6. Active uveitis or history of recurrent uveitis or uveitis involving the posterior segment.
7. Presence of cataract that is impairing vision.
8. Presence of lens or lens implant subluxation.
9. History of ocular trauma that is currently impairing vision.
10. Any concurrent optic nerve or retinal disease that is visually significant or likely to progress to visual significance during the 1-year study follow-up. The excluded eyes include eyes with AREDS category 2 to 3 age-related macular degeneration (AMD) with foveal involvement of drusen or RPE changes, and any AREDS category 4 AMD eyes. For eyes with ERM, the excluded eyes include eyes with OCT evidence of foveal deformation. For optic nerve disease, eyes with any associated visual field deficit or history of associated CNVM are excluded. For glaucoma eyes, eyes requiring glaucoma laser trabeculectomy or glaucoma surgery to maintain IOP are excluded.
11. Active retinal or iris neovascularization.
12. Macular edema requiring on-going therapy or where such treatment is expected during the study period, with the exception of anti-VEGF treatment given at an interval of 8 weeks or greater.

13. Significant media opacity precluding view of the fundus for examination, photography or optical coherence tomography (OCT) including cataract and vitreous haze.
14. High myopia (≥ 9 diopters)
15. Amblyopia
16. Other cause contributing to vision loss at screening.
17. History of any of the following procedures: corneal transplant, glaucoma surgery, or intraocular silicone oil.

5.1.2 Participant-Level Inclusion/Exclusion Criteria

Participants must meet the following inclusion criteria:

1. Age ≥ 18 years of age.
2. Female participants of child-bearing potential (see **APPENDIX B** in protocol for definition) must not be pregnant or breastfeeding and have a negative urine pregnancy test within 14 days prior to sham injection and/or CD34+ cell injection.
3. Females of childbearing potential must have had a hysterectomy, be completely abstinent from intercourse or must agree to practice effective contraception for the duration of the study. Acceptable methods of contraception include hormonal contraception, intrauterine device, barrier methods (diaphragm, condom) with spermicide, or surgical sterilization (tubal ligation).
4. Able and willing to sign informed consent.
5. Able to keep follow-up appointments for at least 12 months as determined by the investigator.

Participants with any of the following criteria will be excluded from the study:

1. Concurrent treatment with an investigational drug or device.
2. Concurrent use of systemic immunosuppressive therapy or history of use within 3 months prior to enrollment (i.e., date ICF signed).
3. Concurrent use of anticoagulation therapy except for aspirin without an acceptable safe stopping plan for study treatments.
4. Known history of coagulopathy or other hematologic abnormality that may put participant at risk for bleeding or infection or raise concerns about quality or quantity of CD34+ cells isolated.
5. History of allergy to fluorescein dye.
6. Participant who has had a prior or concomitant malignancy with the exception of the following: 1) adequately treated basal or squamous cell carcinoma of the skin, or 2) any other malignancy from which the patient has remained disease free for more than five years.
7. Current active systemic infection as evidenced by fever greater than 100.4 or any evidence of systemic infection as determined by the study physician.
8. Any diagnosis of active infection or vaccination within 8 weeks prior to receipt of study treatment.
9. Diabetes mellitus with known systemic complications by self-report or physician-determined by medical history or examination.

10. History of prior radiotherapy to head/neck area.
11. Poorly controlled hypertension with systolic > 180 or diastolic > 95.
12. Serious medical or psychiatric condition that, in the opinion of the Investigator, would make study participation hazardous to the participant or compromise study findings or would prevent the participant from completing the study.
13. Any physical characteristic that precludes ability to perform study diagnostic testing.

6.0 ANALYSIS OF PARTICIPANT CHARACTERISTICS

Baseline demographics and characteristics such as gender, age, ethnicity, and race will be summarized by treatment group. A summary of baseline medical history data will present the number and percent of participants with each captured condition organized by body system and treatment group. A summary of baseline ocular history data in both the study and fellow eyes will present the number and percent of participants with each captured ocular condition organized by treatment group.

7.0 OUTCOME ANALYSIS

7.1 Primary Outcomes

The primary objective of the study is to assess the safety and feasibility of the stem cell therapy. The primary safety outcome focuses on the participants' adverse event experience during Treatment Period 1, whereas the secondary safety outcome encompasses the AE experience throughout study duration. Section 7.1.1 describes analyses for the primary and secondary safety outcomes and section 7.1.2 describes the feasibility outcome analyses.

7.1.1 Safety

Any solicited or non-solicited ocular or systemic adverse events (AEs) from all participants will be utilized to summarize safety data for this phase I/II study. Reported AEs will be subdivided into pre-treatment and post-treatment, where post-treatment is defined as those occurring on or after the day of the first treatment (cellular or sham). AEs will be summarized by presenting the number of events, and also by counts and frequencies for the number of participants with AEs, and the severity and relatedness of each AE to study treatment (i.e., CD34+ cells) or study procedures (i.e., intravitreal injection). Summaries will be broken down by the treatment period in which the AE occurred (Treatment Period 1, Treatment Period 2). Summaries of events in the study eye will also be provided. Detailed listings of post-treatment AEs and pre-treatment AEs will be provided.

All AEs will be coded using MedDRA® dictionary version 21.0 or higher. Post-treatment AE incidence rates will be summarized by System Organ Class (SOC) and Preferred Term (PT) as determined by the MedDRA coding, with summaries broken down by the treatment period in which the AE occurred. Summaries of MedDRA coded events in the study eye and fellow eye as well as non-ocular AEs will also be provided. The incidence rate of an AE is calculated as the number of participants who experience the event at least once during the safety window divided by the number of participants at risk (times 100). Incidence rates will be calculated at the PT level, at the SOC level, and for participants with at least one post-treatment AE. If a participant experiences multiple episodes of an event, then the event is only counted once. Fisher's exact test may be performed to compare incidence rates between the two treatment arms.

Post-treatment Serious Adverse Events (SAEs) will be summarized by presenting the number of events, and also by counts and frequencies for the number of participants with post-treatment SAEs, and the relatedness and type of each SAE, broken down by whether the SAE occurred in Treatment Period 1 or Treatment Period 2. A summary of post-treatment MedDRA-coded SAEs

using incidence rates will be provided if enough events have occurred. Incidence rates, as defined above, will be calculated at the PT level, at the SOC level, and for “At Least One Serious Adverse Event.” Detailed listings of post-treatment SAEs and pre-treatment SAEs will be provided.

The primary safety outcome will be assessed at the end of Treatment Period 1 (at approximately month 6). Adverse events that occur during Treatment Period 1 will be broken down by whether the AE occurred following the sham or cellular treatment to assess differences in the adverse event experience between the cellular and sham therapies.

A participant listing of the number of study eye, fellow eye and non-ocular AEs will be provided broken down into 3 time periods (0-6 months following sham therapy, 0-6 months following cellular therapy and 6-12 months following cellular therapy) sorted by number of CD34+ cells injected and treatment arm to examine whether more AEs may be observed in those participants who received more CD34+ cells.

In order to assess whether there are any trends in when adverse events are reported, we will also present adverse events based on the how much time has elapsed from receipt of sham and cellular therapy. Depending on how many events are reported, we may create categories (e.g., events reported between 0- and 1-month post-therapy, events reported between 1 and 6 months, events reported between 6 and 12 months, etc.). In this analysis, data will be collapsed over both treatment arms, and will be summarized for all AEs and study eye AEs.

Summaries and listings of solicited ocular events in the study eye and in the fellow eye will be provided, broken down by treatment arm and visit. In addition, a summary of vision-related events broken down by Treatment Period will be provided in an attempt to combine information about unsolicited and solicited ocular events in a single table. Unsolicited ocular events such as reduced visual acuity and visual impairment and solicited ocular events such as decreased vision and blurred vision will be considered for inclusion.

Events related to bone marrow or sham aspiration will be summarized. In addition, pre-treatment AEs will be provided in a listing.

A summary of post-injection vision and eye examination will be provided, broken down by Treatment Period and will include a summary of intraocular pressure (IOP), any IOP interventions administered, and any changes or symptoms seen following injection.

7.1.2 Feasibility

The number and percent of study participants undergoing successful bone marrow aspiration will be summarized. The mean and range of volume of bone marrow aspirate, number of CD34+ cells isolated and number of CD34+ cells injected intravitreally will be described to determine whether a desired number of stem cells can be isolated from bone marrow aspiration without sedation in an outpatient setting. Any participant who did not tolerate the bone marrow aspiration without sedation and any unsuccessful bone marrow aspiration attempt will be recorded.

Other pre- and post-release analysis parameters such as percent yield of the CD34+ isolation will also be described.

7.1.3 Supportive Analysis for Primary Outcome

The measures collected as supportive analysis measures for the primary outcome include presence of clotting in the bone marrow aspirate, information collected regarding COVID-19 symptoms, testing and exposure, occurrence of pregnancy, and concomitant medications. Clotting data will be presented as a summary for each participant noting their number of CD34+ cells in total bone marrow, the number of syringes used to collect the aspirate and whether there was clotting present in any of the syringes. The COVID-19 questions were added to the data system in August 2020 and each response to each question will be presented in summary format

in counts and percentages. Any pregnancies will be presented in a listing. Each concomitant medication will be summarized by noting the indication(s) for which it was taken, number of participants who reported taking the medication, number of times reported being taken, and the mean number of days taken. Concomitant medications will also be presented in a listing.

7.2 Patient Profiles

In order to assess if safety events affect the efficacy outcomes, patient profiles will also be created. Patient profiles will include a short report for each participant, showing the participant's demographics and baseline information, randomization arm, ocular medications administered during the study, number of cells injected, solicited and non-solicited adverse events, and a few key measures of treatment effect (e.g., visual acuity, OCT, microperimetry). These profiles will exemplify the changes over time to feasibility and whether it correlates with safety events.

7.3 Secondary Efficacy Outcomes

Availability of the secondary outcomes will first be summarized by treatment arm and visit. Due to each secondary outcome having numerous measures associated with it (e.g., the OCT outcome can be measured by looking at macular thickness (central, pericentral and/or peripheral), macular volume, average macular thickness, presence of CME and/or subretinal fluid, integrity of photoreceptor layer, etc.), outcome measures thought to be of most importance were chosen by the Study Chair prior to any knowledge of treatment assignment. Using this pre-selected list of outcome measures, the following strategy will be used to analyze each continuous outcome.

1. Summary table of **descriptive** statistics broken down by treatment arm, Treatment Period and visit for change from baseline in the outcome measure. For VA and microperimetry, the summary table will also include the outcome measure on the original scale.
2. Analysis of **treatment effect** of cellular vs sham therapy during Treatment Period 1 (as described in Section 7.3.1.1) for the outcome measure on the original scale and change from baseline.
3. Analysis of **effect of cellular treatment over time** (as described in Section 7.3.1.2) for the outcome measure on the original scale and change from baseline.

For those outcomes that are categorical, a summary table of counts and frequencies will be provided, with any other planned analyses described within the relevant section.

These analyses will be conducted to gauge any changes in the outcome measures that could be attributed to the cellular therapy and to differentiate these changes from non-treatment factors (i.e., natural course of the condition, learning or placebo effects or testing variations). Graphical measures will be used to summarize and display trends over time. A (modified) intent-to-treat approach will be followed in which all participants who were randomized *and received the first injection* (cellular or sham) will be included in the analysis and analyzed in the arm to which they were randomized.

Based on the observations of the preliminary phase 1 clinical trial, we anticipate any improvement in visual function associated with study cell therapy to occur within 3 months of cell therapy and for any effect to be sustained over time (but not increasing over time).[5] Due to small sample sizes, it is recognized that there is limited power to detect differences (Appendix), but trends may be identified that would encourage future trials of this treatment, provided no safety concerns are found. Significance testing will use 2-sided tests with alpha=0.05. No adjustment for multiple comparisons will be made as these are purely exploratory analyses for hypothesis generation.

7.3.1 Secondary Efficacy Outcome Analysis Methods

7.3.1.1 Treatment Effect of Cellular vs Sham Therapy During Treatment Period 1

Comparisons between the two treatment arms will be performed at 1, 3, and 6 months after the first treatment is administered as a method of exploring the differences between cellular and sham therapies. An independent t-test will be used to analyze the treatment effect for each continuous outcome measure on the original scale and change from baseline.

7.3.1.2 Effect of Cellular Therapy Over Time

The mean change from baseline at 1, 3 and 6 months post-cellular therapy will be assessed in both treatment arms. Comparisons will be made between the two arms to identify any differences in the effect of cellular therapy. If there are no statistically significant differences identified, the treatment arms will be combined, and a within-patient paired t-test will be performed. If a statistically significant difference is found, within-patient paired t-tests will be performed separately in each treatment arm to assess the effect of cellular therapy. Additionally, the effect of cellular therapy will be assessed in the immediate cellular therapy/deferred sham therapy arm at 12 months compared to the screening visit with a within-patient paired t-test.

7.3.2 Secondary Efficacy Outcome Measures

The sections below provide a summary of individual outcome measures, parameters (e.g., mean, mean change), timepoints and analyses that will be performed for each efficacy outcome. Analyses for treatment effect and effect of cellular therapy are described in Sections 7.3.1.1 and 7.3.1.2, respectively. Outcomes will be examined in the study eye unless otherwise noted.

7.3.2.1 Changes in Visual Acuity

Best corrected visual acuity (BCVA) of the study eye will be obtained at all scheduled study visits by a certified visual acuity examiner using ETDRS visual acuity chart and refraction. Table 3 outlines the outcome analyses that will be performed for visual acuity.

Table 3: Visual Acuity Outcomes

Outcome Measure	Parameter	Timepoints	Analyses
ETDRS Visual Acuity Letter Score	Mean, mean change from baseline	All visits	Descriptive
		1, 3 and 6 months following Treatment 1	Treatment effect
		1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time
ETDRS Visual Acuity Improvement and Losses	Categorical change: Decrease by ≥10 letters/within 9 letters/improve by ≥ 10 letters; Decrease by ≥ 15 letters/within 14 letters/improve by ≥15 letters	All visits	Descriptive

7.3.2.2 Changes in Microperimetry

Microperimetry will be used as another measure of macular function and to gauge safety and potential efficacy of the study treatment. Table 4 outlines the outcome analyses that will be performed for microperimetry. Categorical versions of continuous variables may also be explored.

Table 4: Microperimetry Outcomes

Outcome Measure	Parameter	Timepoints	Analyses
% Reduced Sensitivity Average Threshold	Mean, mean change from baseline	All visits	Descriptive
		1, 3 and 6 months following Treatment 1	Treatment effect
		1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time

7.3.2.3 Changes in OCT/OCTA

As a secondary outcome measure and a measure of change in macular morphology, OCT image analysis will be performed. Table 5 outlines the outcome analyses that will be performed for OCT/OCTA. Categorical versions of continuous variables may also be explored.

Table 5: OCT/OCTA Outcomes

Modality	Outcome Measure	Parameter	Timepoints	Analyses
OCT	Macular Thickness by ETDRS zone	Mean change from baseline	All visits	Descriptive
	Macular thickness in ETDRS Zone 1 (Central)	Mean change from baseline	1, 3 and 6 months following Treatment 1	Treatment effect for central macular thickness
			1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time for central macular thickness
	Macular Volume	Mean change from baseline	All visits	Descriptive
			1, 3 and 6 months following Treatment 1	Treatment effect

Table 5: OCT/OCTA Outcomes				
Modality	**Outcome Measure**	**Parameter**	**Timepoints**	**Analyses**
			1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time
	Average Macular Thickness	Mean change from baseline	All visits	Descriptive
	Presence of CME	Count, percentage, change from baseline	All visits	Descriptive
	Presence of Subretinal Fluid	Count, percentage, change from baseline	All visits	Descriptive
	Photoreceptor Layer (IS/OS Junction)	Count, percentage, change from baseline	All visits	Descriptive
	Photoreceptor Layer (ELM)	Count, percentage, change from baseline	All visits	Descriptive
	Presence of hyper-reflective foci in vitreous, intraretinal and subretinal	Count, percentage	All visits	Descriptive
	Number of hyper-reflective foci in vitreous, intraretinal and subretinal	Mean change from baseline	All visits	Descriptive
	Length of longest DRIL in line scans	Mean change from baseline	All visits	Descriptive
	Presence of ERM	Count, percentage, change from baseline	All visits	Descriptive
	Presence of VMT/VMA	Count, percentage, change from baseline	All visits	Descriptive
OCTA	Foveal avascular zone size	Mean change from baseline	All visits	Descriptive
			1, 3 and 6 months following Treatment 1	Treatment effect
			1, 3 and 6 months post-cellular therapy in both arms; 12	Effect of cellular therapy over time

Table 5: OCT/OCTA Outcomes				
Modality	**Outcome Measure**	**Parameter**	**Timepoints**	**Analyses**
			months post-cellular therapy in immediate cell/deferred sham arm	
	Automated Superficial and Deep Vascular Density (VD) by ETDRS zone	Mean change from baseline	All visits	Descriptive
			1, 3 and 6 months following Treatment 1	Treatment effect for central, whole image 3.0mm scan, and whole image 6.0mm scan
			1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time for central, whole image 3.0mm scan, and whole image 6.0mm scan
	VD analysis of superficial plexus and deep plexus	Count, percentage	All visits	Descriptive
	Number of microaneurysms in total ETDRS area	Mean change from baseline	All visits	Descriptive
	Number of telangiectasia in total ETDRS area	Mean change from baseline	All visits	Descriptive
	RNFL Thickness; Whole image capillary density %; Inside disc capillary density %; Retinal peripapillary capillary density %	Mean change from baseline	All visits	Descriptive
			1, 3 and 6 months following Treatment 1	Treatment effect
			1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time

7.3.2.4 Changes in Multifocal ERG

As a secondary outcome measure and an objective measure of macular function, multifocal ERGs will be performed. Table 6 outlines outcome analyses that will be performed for multifocal ERG. Categorical versions of continuous variables may also be explored.

Table 6: Multifocal ERG Outcomes

Outcome Measure	Parameter	Timepoints	Analyses
Number of abnormal amplitude hexagons Mean amplitude S.D. Number of abnormal latency hexagons Mean Latency S.D.	Mean change from baseline	All visits	Descriptive
		1, 3 and 6 months following Treatment 1	Treatment effect
		1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time

7.3.2.5 Changes in Full-Field ERG

As a secondary outcome measure and an objective measure of global retinal function, the change in retinal function in the study eye based on full-field ERG will be assessed. Table 7 outlines outcome analyses that will be performed for full-field ERG. Categorical versions of continuous variables may also be explored.

Table 7: Full-field ERG Outcomes

Outcome Measure	Parameter	Timepoints	Analyses
% Normal amplitude for a-wave and b-wave under scotopic conditions Flicker amplitude % Normal amplitude for ERG+OP a-wave and b-wave under scotopic conditions	Mean change from baseline	All visits	Descriptive
		1, 3 and 6 months following Treatment 1	Treatment effect
		1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time
% Normal latency for a-wave and b-wave under scotopic conditions % Normal amplitude for a-wave and b-wave under photopic conditions % Normal latency for a-wave and b-wave under photopic conditions Flicker latency % Normal latency of ERG + OP a-wave and b-wave under scotopic conditions	Mean change from baseline	All visits	Descriptive

7.3.2.6 Changes in Autofluorescence

As a secondary outcome measure, the change in autofluorescence (AF) in the study eye will be assessed. Table 8 outlines outcome analyses that will be performed for autofluorescence. Categorical versions of continuous variables may also be explored.

Table 8: Autofluorescence Outcomes

Outcome Measure	Parameter	Timepoints	Analyses
Area of definite autofluorescence loss Area of increased autofluorescence	Mean change from baseline	All visits	Descriptive
		1, 3 and 6 months following Treatment 1	Treatment effect
		1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time

7.3.2.7 Changes in Fundus Photography and Fluorescein Angiography

As a secondary outcome measure and to evaluate for changes in retinal perfusion, fundus photography and fluorescein angiography (FA) will be performed throughout the study. Table 9 outlines outcome analyses that will be performed for fundus photography and fluorescein angiography outcomes. For fundus photography, a focused review of the degree of retinal and pre-retinal hemorrhage data will be performed to assess for patterns.

Table 9: Fundus Photography and Fluorescein Angiography Outcomes

Modality	Outcome Measure	Parameter	Timepoints	Analyses
Fundus Photography	Degree of retinal hemorrhage by ETDRS/Networc zones	Count, percentage	All visits	Descriptive
	Degree of pigment changes by ETDRS/Networc zones			
	Degree of retinal fibrosis by ETDRS/Networc zones			
	Degree of NV by ETDRS/Networc zones			
	Degree of pre-retinal hemorrhage by ETDRS/Networc zones			
Fluorescein Angiogram	Foveal avascular zone integrity	Count, percentage	All visits	Descriptive

Table 9: Fundus Photography and Fluorescein Angiography Outcomes				
Modality	**Outcome Measure**	**Parameter**	**Timepoints**	**Analyses**
	Area of non-perfusion within ETDRS Grid Area of non-perfusion within Networc Grid	Mean change from baseline	All visits	Descriptive
			1, 3 and 6 months following Treatment 1	Treatment effect
			1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time
	Grade of capillary non-perfusion	Count, percentage	All visits	Descriptive
	Number of microaneurysms	Mean change from baseline	All visits	Descriptive
			1, 3 and 6 months following Treatment 1	Treatment effect within ETDRS grid and within Networc grid
			1, 3 and 6 months post-cellular therapy in both arms; 12 months post-cellular therapy in immediate cell/deferred sham arm	Effect of cellular therapy over time within ETDRS grid and within Networc grid
	Grade of telangiectatic vascular changes	Count, percentage	All visits	Descriptive

7.3.2.8 Changes in Quality of Life

As a secondary outcome measure and to evaluate for changes in vision-related quality of life, the NEI VFQ-25 will be performed throughout the study. Table 10 outlines outcome analyses that will be performed for quality of life. Categorical versions of continuous variables may also be explored.

Table 10: Quality of Life Outcomes			
Outcome Measure	**Parameter**	**Timepoints**	**Analyses**
Total score Subscale scores	Mean change from baseline	All visits	Descriptive

7.4 Exploratory Outcomes

7.4.1 Advanced Retinal Imaging (Investigational OCT)

Advanced retinal imaging (ARI) will be performed in select participants who have consented and who, in the study eye at screening, have stable fixation on microperimetry and clear media defined as Optovue OCTA signal strength of 6 or greater using the 3mm macular scan. The ARI sub-study involves adaptive optics-OCT imaging and ultra-high resolution OCT/OCTA. A table of the status of ARI acquisition, processing and review will be provided, along with a corresponding listing, to summarize how many participants were eligible for ARI and how many had images acquired, processed, and reviewed. A listing of advanced retinal imaging outcomes measures will be provided. Table 11 outlines the information that will be included in the outcome listing.

Table 11: Advanced Retinal Imaging Outcomes

Modality	Outcome Measure[1]	Timepoints
Adaptive Optics	Integrity of retinal vascular wall, inner and outer retinal layer and IS/OS	Baseline and 1 month following each treatment[2]
	Number of hyperreflective foci along vascular wall, inner and outer retina and vitreous	Baseline and 1 month following each treatment[2]
Ultra-high resolution OCT/OCTA	Total, inner, and outer retinal thickness and DRIL length	Baseline and 1 month following each treatment[2]
	IS/OS integrity	Baseline and 1 month following each treatment[2]
	Number of hyperreflective foci (vitreous, within retina, under retina)	Baseline and 1 month following each treatment[2]
	Superficial, intermediate, and deep plexus vascular density measurements	Baseline and 1 month following each treatment[2]
	Superficial, intermediate, and deep plexus integrity	Baseline and 1 month following each treatment[2]
	Number of microaneurysms, total area of telangiectasia, and total area of retinal neovascularization	Baseline and 1 month following each treatment[2]

[1] Information on the following four locations will be provided: central 3 mm, eccentric 1, eccentric 2, and eccentric 3.

[2] In the immediate cell/deferred sham arm, timepoints include baseline and 1 month following cellular therapy. In the immediate sham/deferred sham arm, timepoints include baseline, 1 month following sham therapy and 1 month following cellular therapy.

7.4.2 Cell Characterization

Table 12 outlines the various cell characterization assays that will be performed on the final cellular product. Descriptive statistics for the various outcome measures will be provided.

Table 12: Cell Characterization Outcomes

Characterization Type	Outcome Measure	Parameter	Time Frame
Pre-release Viability	% of cells with no trypan blue staining	Mean	Day of receipt of stem cells
	% of cells with irregular shape or size and no trypan blue staining	Mean	Day of receipt of stem cells
Post-release viability	Cell viability using 7-AAD	Mean	Day of receipt of stem cells
Post-release phenotype verification using flow cytometry	% of cells CD34+	Mean	Day of receipt of stem cells
	% of cells CD3+	Mean	Day of receipt of stem cells
Flow Cytometry Panel for Cell Surface Markers	% CD34(+) cells	Mean	Day of receipt of stem cells
	% hematopoietic stem cells in CD34+ population: CD133(+)/CD45(+)/ CD34(+)	Mean	Day of receipt of stem cells
	% endothelial progenitor cells in the CD34+ population: CD31(+)/CD45(-) /VEGFR-2(+)/CD34(+)	Mean	Day of receipt of stem cells
	% CD34+ cells with VEGFR-2: VEGFR-2(+)/CD34(+)	Mean	Day of receipt of stem cells
Additional flow cytometry panel analysis for cell surface markers	% CD133(+)	Mean	Day of receipt of stem cells
	% CD31 (+)	Mean	Day of receipt of stem cells
	% CD45 (+)	Mean	Day of receipt of stem cells
	% VEGFR-2 (+)	Mean	Day of receipt of stem cells

7.4.3 Participant Satisfaction

A study survey will collect participants' experiences with various aspects of the study, including their impressions on timing of receipt of stem cell injection vs sham injection. Table 13 outlines the various exploratory outcomes that will be described in tabular format.

Table 13: Participant Satisfaction Outcomes

Outcome Measure	Parameter	Timepoints
Satisfaction with procedures (aspiration and eye injection)	Count, percentage	1 week following each treatment
Pain/discomfort in hip/eye during procedure and 1-week post-procedure	Count, percentage	1 week following each treatment
Perception of which procedure participant received (aspiration and eye injection)	Count, percentage	1 week following each treatment
Perception of improvement in vision	Count, percentage	12 months
Satisfaction with study experience	Count, percentage	12 months
Perception of which procedure participant received first	Count, percentage	12 months
Comfort with various aspects of the study	Count, percentage	12 months

7.5 Methods for Handling Missing Data

As this is an exploratory phase I/II study, all available data will be used for all analyses. If the amount of missing data is >5% and there is reason to believe the data are not missing completely at random (MCAR), an imputation method such as multiple imputation will be considered, recognizing the limitations with these methods due to the small sample size.

8.0 DATA QUALITY

8.1 Data Audits

A summary of data audit results from site interim monitoring visits conducted by DCC monitors will be presented, including total fields audited, total data discrepancies, and error rate.

8.2 Protocol Deviations

Protocol deviations will be summarized by whether they were reported by masked or unmasked staff and will include the number of deviations reported and the frequencies for the types of protocol deviations. A detailed listing of protocol deviations by deviation category will be provided. The listing will include participant ID, date of protocol deviation, date protocol deviation entered in EDC (Electronic Data Capture), deviation type, deviation description, and resolution or corrective action to be taken.

9.0 SOFTWARE TO BE USED FOR ANALYSIS

All analyses will be performed using SAS® Version 9.4 and R Core Team (2023) software.

10.0 UPDATES TO THE STATISTICAL ANALYSIS PLAN

Table 14: SAP Revision History

SAP Version	Date of Approval	Summary of Changes
0.2	13NOV2018	Initial draft version presented to TRUST DSMC
1.0	02APR2024	Finalized Version

11.0 LIST OF PROPOSED TABLES, LISTINGS AND FIGURES

The below listing provides the title of the tables, listings, and figures (TLFs) that will be provided, along with the analysis population used to generate each TLF. See the accompanying "Statistical Analysis Plan Shells" document for the table, listing and figure shells that show the planned layout of the TLFs.

Section	Title	Population
Enrollment, Participant Disposition, and Follow-up	*CONSORT Diagram*	*Prescreened*
	Proposed vs Actual Randomizations	*Screened*
	Figure of Proposed vs Actual Randomizations	*Screened*
	Summary of Screen Failures	*Screened*
	Summary of Participant Disposition by Treatment Arm	*ITT*
	Summary of Attendance at Follow-up Visits by Treatment Arm	*ITT*
	Summary of Early Study Terminations by Treatment Arm	*ITT*
	Summary of Reasons for Missed Visits	*ITT*
Participant Characteristics at Baseline	*Summary of Baseline Demographics by Treatment Arm*	*ITT*
	Summary of Baseline Medical History by Treatment Arm	*ITT*
	Summary of Baseline Ocular History in Study Eye by Treatment Arm	*ITT*
	Summary of Baseline Ocular History in Fellow Eye by Treatment Arm	*ITT*
Patient Profiles	*Patient Profiles*	*ITT*
Primary Outcome – Primary and Secondary Safety	*Summary of Post-treatment Adverse Events for Treatment Period 1 by Treatment*	*ITT*
	Summary of Post-treatment Adverse Events in the Study Eye for Treatment Period 1 by Treatment	*ITT*
	Summary of Post-treatment Adverse Events for Treatment Period 2 by Treatment	*ITT*
	Summary of Post-treatment Adverse Events in the Study Eye for Treatment Period 2 by Treatment	*ITT*
	Listing of Post-treatment Serious Adverse Events by Treatment Arm	*ITT*
	Summary of Post-treatment MedDRA Coded Adverse Events in Study Eye for Treatment Period 1 by Treatment	*ITT*
	Summary of Post-treatment MedDRA Coded Adverse Events in Study Eye for Treatment Period 2 by Treatment	*ITT*

Section	Title	Population
	Summary of Post-treatment MedDRA Coded Adverse Events in Fellow Eye for Treatment Period 1 by Treatment	*ITT*
	Summary of Post-treatment MedDRA Coded Adverse Events in Fellow Eye for Treatment Period 2 by Treatment	*ITT*
	Summary of Post-treatment MedDRA Coded Non-ocular Adverse Events for Treatment Period 1 by Treatment	*ITT*
	Summary of Post-treatment MedDRA Coded Non-ocular Adverse Events for Treatment Period 2 by Treatment	*ITT*
	Summary of Post-treatment MedDRA Coded Serious Adverse Events in Treatment Period 1 by Treatment	*ITT*
	Summary of Post-treatment MedDRA Coded Serious Adverse Events in Treatment Period 2 by Treatment	*ITT*
	Number of Adverse Events by Treatment Received and Number of CD34+ Cells Injected	*ITT*
	Listing of Post-treatment Adverse Events by Treatment Arm and Number of CD34+ Cells Injected	*ITT*
	Adverse Event Time Trends	*ITT*
	Listing of Pre-Treatment Serious Adverse Events by Treatment Arm	*ITT*
	Summary of Solicited Ocular Events in Study Eye by Treatment Arm	*ITT*
	Summary of Solicited Ocular Events in Fellow Eye by Treatment Arm	*ITT*
	Listing of Solicited Ocular Events	*ITT*
	Summary of Vision-related Events in Treatment Period 1	*ITT*
	Summary of Vision-related Events in Treatment Period 2	*ITT*
	Summary of Aspiration Procedure Events	*ITT*
	Listing of Aspiration Procedure Events	*ITT*
	Summary of Vision-related Events in Treatment Period 1	*ITT*
	Summary of Vision-related Events in Treatment Period 2	*ITT*
	Summary of Aspiration Procedure Events	*ITT*
	Summary of Post-injection Vision and Eye Examination by Treatment Period and Treatment	*ITT*
Primary Outcome - Feasibility	*Summary of Pre-release and Post-release Analysis by Treatment Arm*	*ITT*
	Listing of Pre- and Post-release Analysis Parameters by Treatment Arm	*ITT*

Section	Title	Population
	Clotting Data	*ITT*
Supportive Analysis for Primary Outcome	*Summary of COVID-19 Responses*	*ITT*
	Listing of Pregnancies by Treatment Arm	*ITT*
	Summary of Concomitant Ocular Medications	*ITT*
	Listing of Concomitant Ocular Medications	*ITT*
Secondary Outcomes	*Availability of Secondary Outcomes*	*ITT*
	Summary of Visual Acuity Letter Score and Change from Baseline by Treatment Arm	*ITT*
	Summary of Visual Acuity Improvement and Losses by Treatment Arm	*ITT*
	Visual Acuity Letter Score Line Plot by Treatment Arm	*ITT*
	Analysis of Treatment Effect in Visual Acuity	*ITT*
	Analysis of Cellular Treatment Effect Over 1, 3, 6 and 12 Months in Visual Acuity	*ITT*
	Summary of Microperimetry by Treatment Arm	*ITT*
	Microperimetry Line Plots by Treatment Arm	*ITT*
	Analysis of Treatment Effect in Microperimetry Measures	*ITT*
	Analysis of Cellular Treatment Effect Over 1, 3, 6 and 12 Months in Microperimetry Measures	*ITT*
	Summary of Commercial Grade OCT by Treatment Arm	*ITT*
	Commercial Grade OCT Plots	*ITT*
	Analysis of Treatment Effect in Commercial Grade OCT Measures	*ITT*
	Analysis of Cellular Treatment Effect Over 1, 3, 6 and 12 Months in Commercial Grade OCT Measures	*ITT*
	Summary of Commercial Grade OCTA by Treatment Arm	*ITT*
	Quantitative Commercial Grade OCTA Plots	*ITT*
	Qualitative Commercial Grade OCTA Plots	*ITT*
	Analysis of Treatment Effect in Commercial Grade OCTA Measures	*ITT*
	Analysis of Cellular Treatment Effect Over 1, 3, 6 and 12 Months in Commercial Grade OCTA Measures	*ITT*
	Summary of Multifocal ERG by Treatment Arm	*ITT*
	Multifocal ERG Plots	*ITT*

Section	Title	Population
	Analysis of Treatment Effect in Multifocal ERG Measures	*ITT*
	Analysis of Cellular Treatment Effect Over 1, 3, 6 and 12 Months in Multifocal ERG Measures	*ITT*
	Summary of Full-field ERG by Treatment Arm	*ITT*
	Full-field ERG Plots	*ITT*
	Analysis of Treatment Effect in Full-field ERG Measures	*ITT*
	Analysis of Cellular Treatment Effect Over 1, 3, 6 and 12 Months in Full-field ERG Measures	
	Summary of Fundus Autofluorescence by Treatment Arm	*ITT*
	Fundus Autofluorescence Plots	*ITT*
	Analysis of Treatment Effect in Fundus Autofluorescence Measures	*ITT*
	Analysis of Cellular Treatment Effect Over 1, 3, 6 and 12 Months in Fundus Autofluorescence Measures	*ITT*
	Summary of Fundus Photography by Treatment Arm	*ITT*
	Summary of Fluorescein Angiogram by Treatment Arm	*ITT*
	Fluorescein Angiogram Plots	*ITT*
	Analysis of Treatment Effect in Fluorescein Angiogram Measures	*ITT*
	Analysis of Cellular Treatment Effect Over 1, 3, 6 and 12 Months in Fluorescein Angiogram Measures	*ITT*
	Summary of Quality of Life by Treatment Arm	*ITT*
	Quality of Life Plots	*ITT*
Exploratory Outcomes	*Summary of Advanced Retinal Imaging (Investigational OCT) Acquisition, Processing and Review by Treatment Arm*	*ITT*
	Listing of Advanced Retinal Imaging (Investigational OCT) Acquisition, Processing and Review	*ITT*
	Listing of Advanced Retinal Imaging (Investigational OCT) Results	*ITT*
	Summary of Cell Characterization in Color Panel Flow Cytometry by Treatment Arm	*ITT*
	Summary of Post-Procedure Satisfaction Survey Results	*ITT*
	Summary of Post-Procedure Satisfaction Survey Results Aspiration Procedure and Eye Injection	*ITT*
	Listing of Post-Procedure Satisfaction Survey Results	*ITT*
	Summary of Study Satisfaction Survey Results	*ITT*

Section	Title	Population
	Listing of Study Satisfaction Survey Results	*ITT*
Data Quality	*Data Quality Based on Audits*	*N/A*
	Summary of Masked Protocol Deviations by Treatment Arm	*Pre-screened*
	Listing of Masked Protocol Deviations	*Pre-screened*
	Summary of Unmasked Protocol Deviations	*Pre-screened*
	Listing of Unmasked Protocol Deviations	*Pre-screened*

12.0 REFERENCES

1. Kocur I, Resnikoff S. Visual impairment and blindness in Europe and their prevention. *Br J Ophthalmol.* 2002; 86: 716-722.
2. Laouri M, Chen E, Looman M, Gallagher M. The burden of disease of retinal vein occlusion: review of the literature. *Eye.* 2011; 25: 981-988.
3. Rogers S, MoIntosh RL, Cheung N, et al. The prevalence of retinal vein occlusion: pooled data from population studies from the United States, Europe, Asia, and Australia. *Ophthalmology.* 2010; 117: 313-319.
4. Central Vein Occlusion Study Group. Natural history and clinical management of central retinal vein occlusion. The Central Retinal Vein Occlusion Group. *Arch Ophthalmol.* 1997; 115: 486-491.
5. McIntosh RL, Rogers SL, Lim L, et al. Natural history of central retinal vein occlusion: an evidence-based systematic review. *Ophthalmology*. 2010;117:1113–1123.
6. Park SS, Bauer G, Abedi M, et al. Intravitreal autologous bone marrow-derived CD34+ cells for ischemic and degenerative retinal disorders: preliminary phase 1 clinical trial findings. *Invest Ophthalmol Vis Sci.* 2014; 56:81-89.
7. Scott IU, VanVeldhuisen PC, Ip MS, et al, for the SCORE2 Investigator Group. Effect of bevacizumab vs aflibercept on visual acuity among patients with macular edema due to central retinal vein occlusion. The SCORE2 randomized clinical trial. *JAMA*. 2017;317(20):2072-2087.

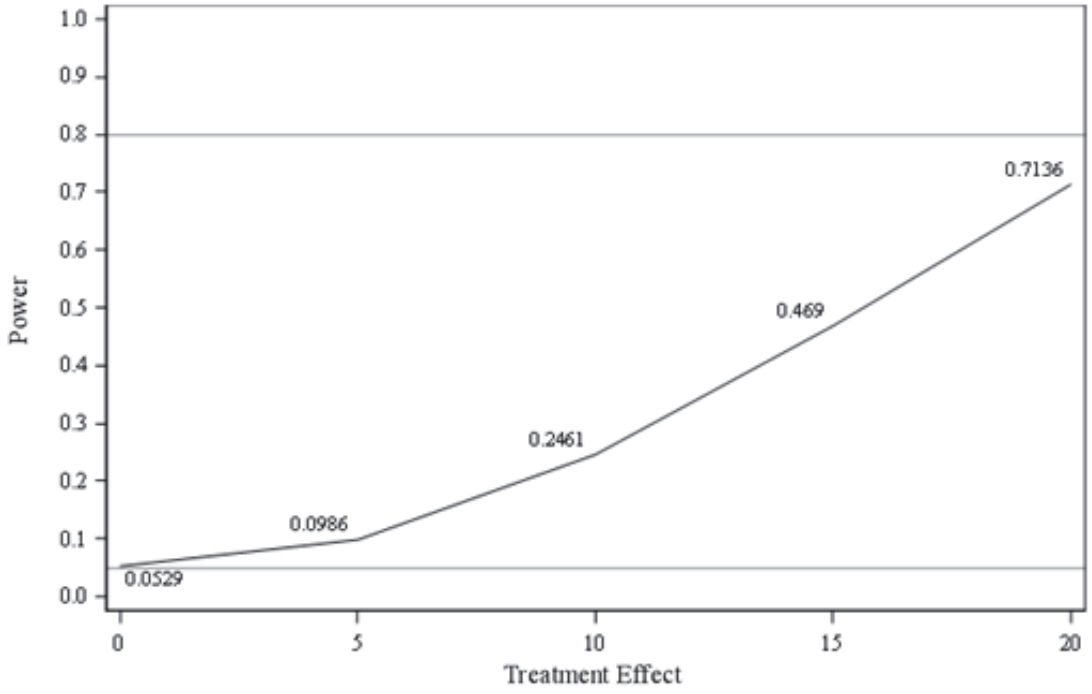
13.0 APPENDIX

13.1 Simulation Results

Simulations were used to produce power curves for the independent t-test. The following plots show the power curves for using the t-test in different scenarios with varying degrees of treatment decay and period effect in the simulated data. Treatment decay is defined as the attenuation of cellular treatment, which might be observed in the immediate cellular therapy/deferred sham therapy group during Study Period 2. Period effect is defined as the effect on treatment of being in Study Period 1 versus being in Study Period 2. Estimates of participant level random effects and measurement error used in these power calculations were derived from visual acuity data in the Study of Comparative Treatments for Retinal Vein Occlusion 2 (SCORE 2)[7].

FIGURE 1 shows the power curve when estimating treatment effect of visual acuity (i.e., difference in number of letters read) holding treatment decay and period effect at 0.

Figure 1: Power Curve for T-test



Standard deviation of the patient level random effect=15.6
Standard deviation of the measurement error=6.2
Treatment decay (Q)=0
Period effect (T)=0

FIGURE 2 and **FIGURE 3** show the power of estimating treatment effect of visual acuity while introducing treatment decay and a period effect, respectively.

Figure 2: Power Curve with Differing Levels of Treatment Decay

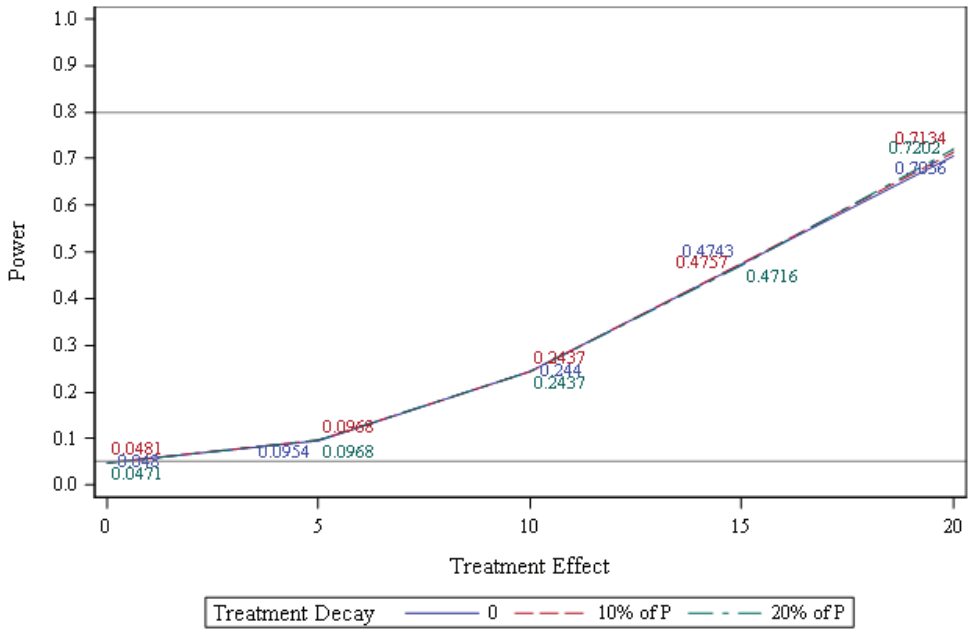
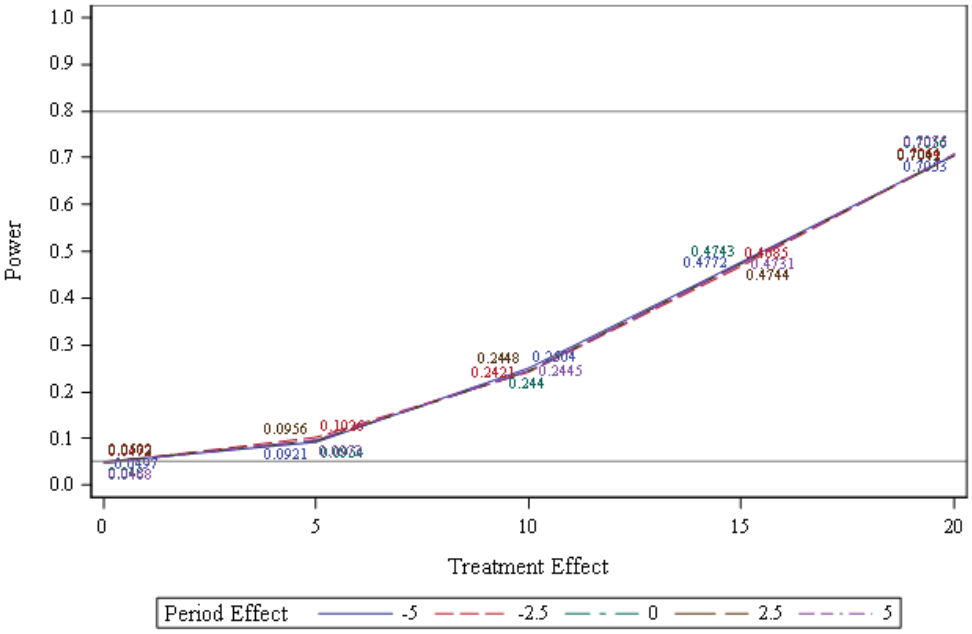


Figure 3: Power Curve with Differing Levels of Period Effect



Figures 2 and 3 show that treatment decay and a period effect do not affect power. The overall conclusion is that power to detect a treatment effect does not approach even 70% until the treatment effect is quite large (difference of 20 letters read).