Northern Therapeutics Inc.

The SAPPHIRE Trial

Study of Angiogenic cell therapy for Progressive Pulmonary Hypertension: Intervention with Repeat dosing of eNOS-enhanced EPCs

A late phase clinical trial to establish the efficacy and safety of repeat dosing of autologous endothelial progenitor cells (EPCs) transfected with human endothelial NO-synthase (eNOS) in patients with refractory pulmonary arterial hypertension (PAH).

CLINICAL TRIAL PROTOCOL

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Confidentiality Statement

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Protocol Signature Page

Product Name: mc-eNOS-transfected EPCs

Indication: Pulmonary Arterial Hypertension

Northern Therapeutics, Inc. Protocol Number: CT-PAH 002

Health Canada Control Number: 197105, 202459, 219964, 229154, 235968, 252959, 262475

Development Phase: Late Phase

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The SAPPHIRE Trial: Study of Angiogenic Cell Therapy for Progressive Pulmonary Hypertension: Intervention with Repeat dosing of eNOS-enhanced EPCs
A multicentre, late phase clinical trial to establish the efficacy and safety of repeat dosing of autologous endothelial progenitor cells (EPCs) transfected with human endothelial NO-synthase (eNOS) in patients with Pulmonary Arterial Hypertension (PAH) on top of conventional treatments.

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Site Qualified Investigator Approval:

I have read this clinical trial protocol and the investigator's brochure and agree to conduct the trial in accordance with the protocol and Standard Operating Procedures (SOPs) in the Study File Binder. I also agree to comply with applicable laws and regulations and ICH guidelines for good clinical practice. I will obtain approval from this institution's Research Ethics Board prior to initiating the trial, allow access to source documents and agree to inspection by auditors from the Sponsor, Ottawa Hospital Research Institute and regulatory authorities. I will assure that the investigational products supplied by the Sponsor will be used only as described in the above named protocol; if any other use is desired written permission must be obtained from the Sponsor. I agree to oversee the conduct of all study personnel assigned to this trial at this investigative site as noted on the study's Delegation Log.

Print Name _____ Signature _____ Date _____

Protocol Synopsis

Title of Study: Study of Angiogenic Cell Therapy for Progressive Pulmonary Hypertension: Intervention with Repeat dosing of eNOS enhanced EPCs (The SAPPHIRE Trial)

Clinical Phase: Late Phase

Study Design: Multi-centre, randomized, double blind, placebo-controlled, 3-arm protocol.

Number of Patients: 45 randomized

Primary Objective: The SAPPHIRE clinical trial seeks to establish the efficacy and safety of repeated monthly dosing of autologous EPCs transfected with human eNOS (eNOS) in patients with symptomatic severe PAH on available PAH-targeted medical therapy

Primary Endpoint: The primary endpoint is the 6 minute walk distance (6MWD) at 6 months.

Therapeutic Dosing Regimen: We will study the cumulative effect of repeated dosing of eNOS-transfected autologous EPCs, up to a maximum total cumulative cell dose of 160 million cells.

Study participants will be randomized into one of three arms:

Arm 1. Four monthly IV injections of placebo (Plasma-Lyte A saline solution) in the first 6 months (Course 1) followed by four monthly IV injections of 20M autologous eNOS-transfected EPCs in the second 6 months (Course 2; total cumulative dose of 80M cells).

Arm 2. Four monthly IV injections of 20M autologous eNOS-transfected EPCs with in the first 6 months (Course 1) followed by four monthly IV injections of placebo (Plasma-Lyte A) in the second 6 months (Course 2; total cumulative dose of 80M cells).

Arm 3. Four monthly IV injections of 20M autologous eNOS-transfected EPCs in the first 6 months (Course 1) followed by a repeat of four monthly IV injections of 20M eNOS-transfected autologous EPCs in the second 6 months (Course 2; total cumulative dose of 160M cells).

Study Description: Randomization to one of the 3 arms will take place following the screening assessments and satisfactory apheresis collection. Subsequent treatment/follow-up assessments will take place over a 12-month period. Once the 12-month trial data collection is completed, the trial will convert to a long-term follow-up registry with the goal of collecting safety data over a 10-year period.

1. Study Background and Rationale

Pulmonary Arterial Hypertension (PAH) is a progressive and incurable disease which has a prognosis that is as poor as for many malignancies. PAH is associated with profound perturbations in endothelial function, characterized by an imbalance in the production of endothelium-derived vasoconstrictor and vasodilator factors. In particular, decreased nitric oxide (NO) bioavailability results in inappropriate vasoconstriction, increased muscularization and reduced thromboprotection in the pulmonary circulation. Although there have been a number of advances in the therapy of PAH in the last several decades, all current treatments are essentially palliative and most patients ultimately progress, either receiving lung transplantation as a final resort or succumb to right heart failure. Thus, there is an unmet need for new therapies of PAH that can restore the pulmonary vascular structure and function, and improve pulmonary hemodynamics in advanced PAH.

PAH is characterized by marked increases in pulmonary vascular resistance (PVR). In the early stages of the disease this is thought to be due to arteriolar vasoconstriction and progressive increases in muscularization, particularly of the distal arteriolar bed. However, in more advanced disease, PAH is characterized by widespread loss of pulmonary microvasculature which results either from complex remodeling leading to obliteration or degeneration of pre-capillary arterioles. While the mechanism of lung arteriolar loss is uncertain, there is general consensus that it is triggered by endothelial cell apoptosis, which possibly leads directly to microvascular regression or secondarily to the emergence of growth-dysregulated vascular cells which contribute to luminal occlusion.

We have developed a novel method for achieving selective gene transfer to the pre-capillary arteriolar bed, which is the critical site of pathology in PAH. This method involves the ex vivo transfection of autologous early outgrowth endothelial progenitor cells (EPCs), harvested by apheresis from the patient's own circulating blood, and the introduction of these genetically engineered cells back into the pulmonary circulation through an intravenous injection. In pre-clinical studies, we have demonstrated that after a brief period of culture, EPCs develop a strong angiogenic phenotype and are efficiently filtered by the lung, lodging at the level of the pre-capillary arteriole, where these cells can contribute to local lung microvascular repair to restore the continuity of the pulmonary microcirculation.

Gene therapy offers the potential to address genetic abnormalities underlying PAH, and to produce a durable improvement or even reversal of the dysfunctional and damaged vessels in the pulmonary vasculature. Pre-clinical studies in experimental models of PAH have shown that cell-based gene transfer of human endothelial nitric oxide synthase (eNOS) is effective in preventing PAH induced by monocrotaline (MCT), which produces a rapidly progressive and universally fatal form of this disease in rats. As well, we have shown that eNOS gene therapy can reverse established PAH in this model, and that the combination of eNOS gene transfer with EPCs (i.e., cell and gene therapy) provided the greatest benefit in terms of reversing established PAH in animal models. These data are detailed in the Investigator's Brochure.

A phase 1 dose-ranging safety trial, Pulmonary Hypertension and Cell Therapy (PHACeT; ClinicalTrials.gov Identifier: NCT00469027), was performed using autologous EPC transiently transfected by electroporation with plasmid DNA containing the full coding sequence of human eNOS. This study enrolled 7 patients with a diagnosis of idiopathic PAH; 3 received a total of 7 million cells (Panel 1), 3 received 23 million cells (Panel 2) and one patient received 50 million

cells (Panel 3). Cells were delivered by central venous injection through the pacing port of a Swan-Ganz catheter with continuous monitoring of pulmonary arterial pressures in an intensive care environment. For added safety, the total cell dose delivered was divided into 3 aliquots and delivered over 3 days such that the initial "test" dose in Panel 1 was 1 million cells on day 1, followed by 3 million on days 2 and 3 (total of 7 million cells). Participants in Panel 2 received 3, 10 and 10 million cells (total of 23 million cells), and those in Panel 3 received 10, 20 and 20 million cells (total of 50 million cells), on days 1, 2 and 3 respectively. The primary safety analysis was change in pulmonary hemodynamic parameters before and 30 minutes after cell delivery. No deterioration in pulmonary hemodynamics was seen during cell delivery. Administration of 20 million cells as a single injection repeated on two consecutive days in Panel 3 and 23 million cells as a cumulative cell dose delivered over 3 days in Panel 2 were well tolerated. Moreover, there was a statistical trend towards improved total pulmonary resistance over the 3 day delivery period; although no sustained reduction in pulmonary arterial pressure or resistance was seen at 3 months. Of note, there were significant improvements in 6 minute walk distance (6MWD), which peaked at 1 month (~60 meters, $p<0.01$), persisting to 6 months (~40 meters, $p<0.05$). Quality of life measures (SF-36) and functional class were improved over the course of the study as well. Only one patient died during the study period.

The SAPPHIRE trial includes two important design changes to increase the potential efficacy and improve the safety of eNOS gene-enhanced cell therapy for PAH. The first is to repeat cell therapy on a monthly basis, to increase the cumulative cell "dose" that will be delivered over the 12 month study period. Thus, participants will receive 4 or 8 monthly injections of 20 million cells (depending on the study arm), for a maximum of 80 or 160 million cells, respectively. The second is to use minicircle DNA, rather than conventional bacterial plasmid DNA, for eNOS transfection. The advantages of minicircle transfection include greater efficiency of transfection and expression of the gene product as well as to minimize exposure of cells to bacterial DNA sequences. Bacterial DNA contains cPG motifs which can induce an innate immune response leading to rapid clearance of the foreign DNA from the cell. In contrast, the minicircle technology removes most of the bacterial backbone of the plasmid and generates small rings of DNA that contains only the promoter and the human eNOS sequence. These advantages will increase the magnitude and duration of the eNOS transgene expression, while also reducing the chance of host immune response.

2. Trial Objectives and Purpose

SAPPHIRE seeks to establish the short and longer-term efficacy and safety of intravenous delivery of autologous culture-derived, eNOS minicircle transfected EPCs in participants with severe PAH.

2.1. Primary Objective

To determine whether a course of four doses of eNOS gene-enhanced autologous EPCs, delivered monthly, is effective in improving 6 minute walk distance (6MWD) at 6 months in participants with severe PAH who are also receiving PAH-targeted treatments (Arms 2 and 3 vs. Arm 1; see Figure 1).

2.2. Secondary Objectives

1. To compare the sustained effect of delivery of 4 vs. 8 doses of the cell therapy product on 6MWD at 12 months (i.e. Arm 2 vs. Arm 3)
2. To assess durability of any improvement in 6MWD at 3 vs. 9 months post last dose of cell therapy product (Arm 2 only)

3. To determine the incremental effect of delivery of 8 vs. 4 doses of the cell therapy product on 6MWD at 6 and 12 months (Arm 3 only)
4. To assess the cumulative effect of 4 doses of the cell therapy product on pulmonary vascular resistance at 6 months (Arms 2 and 3 vs. Arm 1)
5. To compare the sustained effect of delivery of 4 vs. 8 doses of the cell therapy product on pulmonary vascular resistance at 12 months (i.e. Arm 2 vs. Arm 3)
6. To assess durability of any improvement in pulmonary vascular resistance at 3 vs. 9 months post last dose of cell therapy product (Arm 2 only)
7. To determine the incremental effect of delivery of 8 vs. 4 doses of the cell therapy product on pulmonary vascular resistance at 6 and 12 months (Arm 3 only)
8. To assess effect of cell therapy on Quality of Life measured by the SF-36 at 6 months (Arms 2 and 3 vs. Arm 1)
9. To compare the sustained effect of delivery of 4 vs. 8 doses of the cell therapy product on Quality of Life measured by the SF-36 at 12 months (i.e. Arm 2 vs. Arm 3)
10. To assess the effect of cell therapy on a combined endpoint of death, or clinical worsening of pulmonary arterial hypertension at 6 and 12 months
11. To assess effect of cell therapy on measures of RV function by echocardiography at 6 and 12 months
12. To assess effect of cell therapy on RV function by Magnetic Resonance Imaging (MRI) at sites where this is available in patients with no contraindications for MRI (i.e. prostacyclin infusion pump, claustrophobia) at 6 and 12 months

3. Study Design, Treatment & Duration

SAPPHIRE will use autologous progenitor cell-based gene delivery to enhance lung microvascular repair and regeneration in severe symptomatic PAH. A total of 45 participants will be randomized in this multi-centre, late phase, randomized, double-blind, placebo-controlled, 3-arm protocol. Up to nine centres across Canada will participate. The trial is sponsored by Northern Therapeutics and has been registered at www.ClinicalTrials.gov (NCT03001414). Study recruitment is expected to be completed by the end of 2024.

The primary endpoint is the 6 minute walk distance (6MWD) at 6 months. The primary effect measure is difference in 6MWD at 6 months between patients receiving a course of 4 monthly doses of eNOS-EPCs compared to placebo. As well, a number of secondary endpoint measures will be performed to provide additional insights into the safety and efficacy of this treatment.

Study participants will be randomized into one of three arms (**Figure 1**):

Arm 1. Four monthly IV injections of the placebo saline solution (Plasma-Lyte A) in the first 6 months (Course 1) followed by four monthly IV injections of 20 million autologous eNOS-transfected EPCs in the second 6 months (Course 2; total cumulative dose of 80 million cells).

Arm 2. Four monthly IV injections of 20 million autologous eNOS-transfected EPCs in the first 6 months (Course 1) followed by four monthly IV injections of placebo (Plasma-Lyte A) in the second 6 months (Course 2; total cumulative dose of 80 million cells).

Arm 3. Four monthly IV injections of 20 million autologous eNOS-transfected EPCs in the first 6 months (Course 1) followed by a repeat of four monthly IV injections of 20 million eNOS-

transfected autologous EPCs in the second 6 months (Course 2; total cumulative dose of 160 million cells).

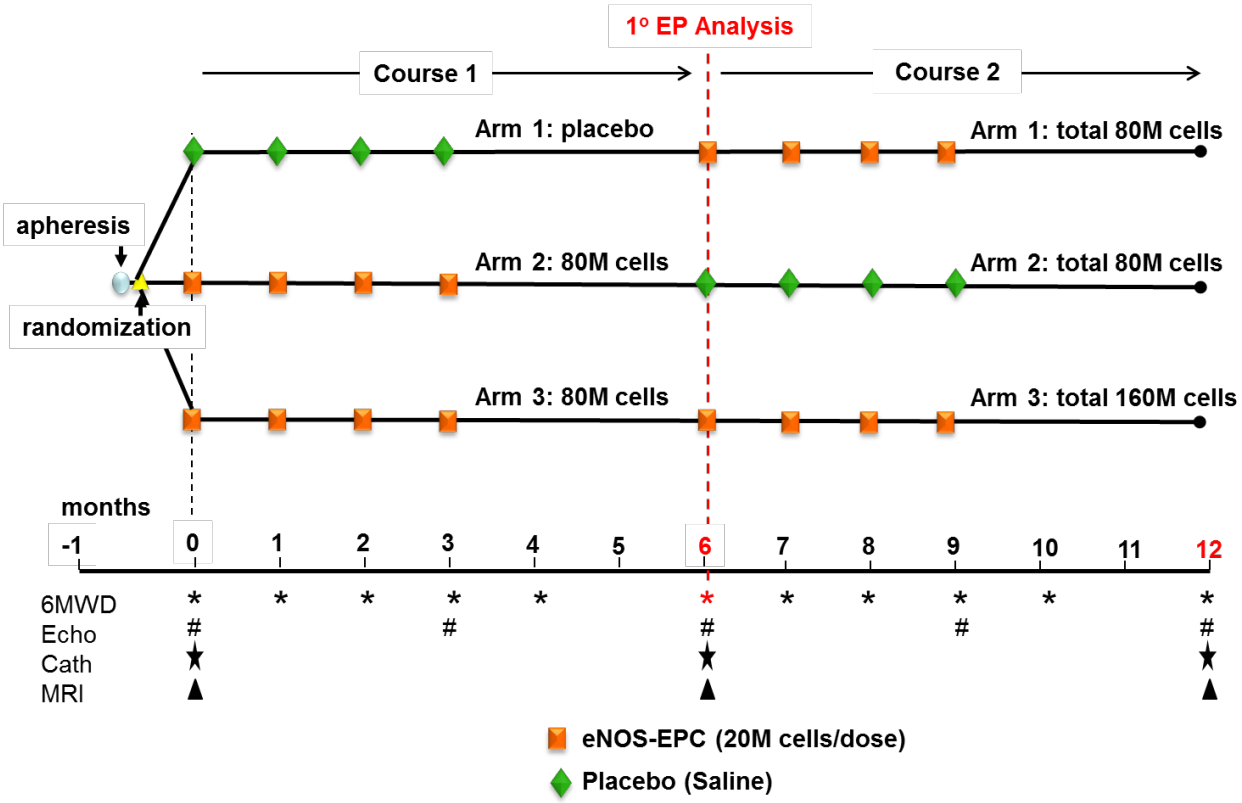


Figure 1: The SAPPHIRE 3-Arm trial design.

Rationale for the 3-arm trial design: The SAPPHIRE trial is designed to maximize the generation of relevant results to inform further development or application of the cell therapy approach. The rationale for the main elements of the study is summarized below.

- **Course 1:** The first 6 months of the study has a standard design with 2:1 randomization to study product or placebo:
  - Arm 1: Participants will receive 4 monthly placebo injections.
  - Arms 2 and 3: Participants will receive 4 monthly injections of the cell therapy product.

  The primary effect measure is at the 6 month time point.

  However, the study continues beyond 6 months in order to address a number of important secondary endpoints. ***Importantly, the blind will continue over the entire 12 month period of the study***.

- **Course 2**: In the second 6 month period, the study treatments will be altered to address specific questions about the timing and durability of this cell therapy:

- Arm 1: Participants previously receiving placebo will now receive a course of 4 cell therapy treatments allowing all participants in this trial to have the opportunity to be given the active study product.
- Arm 2: Participants previously receiving EPCs will now receive the placebo study product to allow an assessment of the durability of any potential benefit of the cell therapy provided in Course 1 over the remaining 6 months of the study.
- Arm 3: Participants previously receiving EPCs will now receive a second course of cell therapy treatments, for a total of 8 doses of eNOS-transfected EPCs. This Arm will determine whether 8 repeated doses of the cell therapy product provides a greater incremental benefit than 4 doses.

After the 12-month treatment and follow-up period is complete, the study will be converted to a long-term follow-up registry extending to 10 years.

A detailed schematic of the study flow is found **Appendix A.**

4. Screening and Eligibility

Referrals for screening will be made by physicians who follow patients for clinical management of PAH at participating SAPPHIRE trial sites, all of which are specialized pulmonary hypertension clinics. Health records will be reviewed in compliance with the Federal Personal Information Protection and Electronic Documents Act (PIPEDA), provincial privacy laws, and local hospital privacy policies.

The research consent process will be initiated at the research site for potential participants who appear to be eligible based on available clinical information. For potential participants who wish to proceed with the trial and who provide written consent, research investigations will then commence. Screening investigations will be completed as outlined in the schedule of assessments in **Appendix B**.

4.1. Inclusion Criteria:

Patients participating in the study must meet ALL of the following inclusion criteria:

- Age ≥ 18 years, ≤ 80 years
- Established diagnosis of PAH due to the following:
  - Idiopathic or heritable PAH;
  - Scleroderma associated PAH (limited or diffuse);
  - Drugs (anorexigens) or toxins;
  - Congenital heart defects (atrial septal defects, ventricular septal defects, and patent ductus arteriosus) repaired ≥ 1 years
- WHO functional class II, III, or IV on appropriate stable therapy for PAH for at least 3 months prior to the screening period and up until randomization, apart from modification of anticoagulant or diuretic dosages, or small adjustments in prostaglandin dose that are considered by the Investigator to be consistent with stable parenteral therapy.
- Able to walk unassisted (oxygen use allowed). Aids for carrying oxygen (such as a wheel chair or walker) are permitted provided they are not also required as mobility aids.

- An average 6-Minute Walk Distance (6MWD) of ≥ 125 meters and ≤ 440 meters on two consecutive tests during the Screening period
- Previous diagnostic right heart cardiac catheterization (RHC) at the time of PAH diagnosis with findings consistent with PAH: specifically, mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg (at rest); pulmonary vascular resistance (PVR) ≥ 3 WU; pulmonary capillary wedge pressure (PCWP) (or left ventricular end diastolic pressure) ≤12 mmHg if PVR ≥ 3 to < 5 WU, or pulmonary capillary wedge pressure (PCWP) (or left ventricular end diastolic pressure) ≤ 15 mmHg if PVR ≥ 5 WU. If repeat testing has occurred since initial diagnosis, the most recent results should be used.
- Echocardiography performed within 12 months prior to the Screening Period confirming a left atrial volume index (LAVI) of ≤ 34 ml/m^2 and the absence of any clinically significant left heart disease including evidence of more than mild left-sided valvular heart disease, systolic or diastolic left ventricular dysfunction
- Ventilation and perfusion (VQ) nuclear scan performed as part of the initial workup to establish the diagnosis of PAH showing absence (i.e. low probability) of pulmonary embolism. If repeat testing has occurred since initial diagnosis, the most recent results should be used. In the absence of a VQ scan to establish eligibility, a CT angiogram that has been reviewed by a radiologist with expertise in the work up for pulmonary endarterectomy and deemed negative for chronic thromboembolic disease may be used instead.
- Pulmonary function tests conducted within 2 years prior to the Screening Period to confirm: total lung capacity (TLC) ≥ 65% the predicted value; and forced expiratory volume at one second (FEV1) of ≥ 65% the predicted value
- Must have a resting arterial oxygen saturation (SaO2) ≥88% with or without supplemental oxygen as measured by pulse oximetry at the Screening Visit
- Must not be enrolled in an exercise training program for pulmonary rehabilitation within 3 months prior to the Screening Visit and must agree not to enroll in an exercise training program for pulmonary rehabilitation during the Screening Period and the first 6 months of the study. Participants enrolled in an exercise program for pulmonary rehabilitation 3 months prior to screening may enter the study if they agree to maintain their current level of rehabilitation for the first 6 months of the study
- Women of child-bearing potential (defined as less than 1 year post-menopausal and not surgically sterile) must be practicing abstinence or using two highly effective methods of contraception (defined as a method of birth control that results in a low failure rate, i.e., less than 1% per year, such as approved hormonal contraceptives, barrier methods [such as a condom or diaphragm] used with a spermicide, or an intrauterine device). Subjects must have a negative ß-hCG pregnancy test during the Screening period and negative urine pregnancy test results at all other study visits
- Must be willing and able to comply with study requirements and restrictions.

4.2. Exclusion Criteria*:*

Patients will not be included in the study if ANY of the following exclusion criteria are present:

- Pregnant or lactating
- PAH related to any condition not covered under inclusion criteria, including but not limited to pulmonary venous hypertension, pulmonary veno-occlusive disease, pulmonary capillary hemangiomatosis, or chronic thromboembolic pulmonary hypertension

- Evidence of more than mild interstitial lung disease on Chest CT within the last 5 years (last 3 years for patients with scleroderma associated PAH)
- Treatment with an investigational drug, device or therapy within 3 months prior to the screening period or is scheduled to receive an investigational drug, device or therapy during the course of the study
- Any musculoskeletal disease or any other disease that would significantly limit ambulation
- Unrepaired or recently repaired (< 1 year) congenital systemic-to-pulmonary shunt other than patent foramen ovale
- Patients having three or more of the following four AMBITION study[1] HFpEF risk factors will be excluded:
    - BMI ≥ 30 kg/m^2,
    - History of essential hypertension,
    - Diabetes mellitus (any type)
    - Historical evidence of significant coronary artery disease (CAD) by ANY ONE of the following:
        - History of MI
        - History of PCI
        - Prior coronary angiography evidence of CAD (>50% stenosis in ≥1 vessel)
        - Previous positive Stress Test
        - Previous CABG
        - Stable angina
- Creatinine clearance <30 ml/min (using the Cockroft-Gault formula) or requires hemodialysis
- Inability to undergo the apheresis procedure due to poor venous access or laboratory tests that are not within acceptable ranges (not including INR for patients on Coumadin)
- Childs-Pugh class C liver cirrhosis
- Previous atrial septostomy
- Any other clinically significant illness or abnormal laboratory values (measured during the screening period) that, in the opinion of the Investigator, might put the subject at risk of harm during the study or might adversely affect the interpretation of the study data
- Anticipated survival less than 1 year due to concomitant disease
- History of cancer in the past 5 years (except for low grade and fully resolved non-melanoma skin cancer)
- Results during screening consistent with current infection with HIV, Hepatitis B(HBV) or C(HCV), human T-cell lymphotropic virus (HTLV- I/II) or syphilis
- Systemic arterial systolic blood pressure < 85 mm Hg
- Known allergy to gentamicin or amphotericin
- Patients who have participated in any gene therapy study or an angiogenic growth factor protein study
- Patients unable to provide informed consent and comply with the visit schedule.

[1] Galiè N, Barberà JA, Frost AE, et al. Initial use of ambrisentan plus tadalafil in pulmonary arterial hypertension. N Engl J Med 2015;373:834-44. DOI: 10.1056/NEJMoa1413687

4.3. Screening Evaluations:

After informed consent has been obtained and documented, participants will be screened for study-specific inclusion/exclusion criteria through screening visit assessments and procedures, which will occur within a 2 week period, no more than 3 months prior to the apheresis procedure and randomization. A detailed schedule of study assessments is found in **Appendix B**.

Each participant enrolled in the trial will be assigned a unique study number that will be used in all study-related documentation pertaining to the individual. The number will be a combination of the site number provided by the coordinating centre and a sequential enrolment number. In the event of a screen failure, a 2nd screening visit may be performed; however, a new study number must be assigned to the participant.

Screening evaluations will include the following:

- Recording of patient demographics, medical history, concomitant medications
- Physical examination by the principal investigator or a sub-investigator
- Vital signs (blood pressure, heart rate, respirations) and measurement of height and body weight
- Assessment and recording of WHO functional class *(*see **Appendix C**)
- Chest x-ray
- Resting 12 lead ECG
- O_2 saturation measurement by peripheral device
- Laboratory tests: sodium, potassium, chloride, TCO_2, blood sugar, BUN, Creatinine, Calcium, Magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, PT, PTT, INR, HIV-1/2, HBV, HCV, HTLV-I/II, *Treponema pallidum* (TP) Antibodies, ß-hCG for females with childbearing potential
- Urine collection for routine and microscopic analysis
- 6-minute walk distance (6MWD) test and Borg dyspnea index (two 6MWD tests to be performed within 24 hours, not less than 1 hour apart, with ≤ 15% variation) (see **Appendix D and E**)

Consenting participants who meet all eligibility criteria will undergo an apheresis procedure which will be performed at the participating site according to the SAPPHIRE Apheresis protocol (**Appendix F)**. Participants will then be randomized to one of three study groups (Figure 1) following a satisfactory cell collection procedure and receipt of the sample by the Cell Manufacturing Facility (CMF). In the case of an unsuccessful apheresis, the participant would be given the option of repeating the apheresis procedure which should be scheduled within the 3-month screening period to avoid having to repeat screening procedures.

4.4 Apheresis Procedure:

Participants who qualify for SAPPHIRE, and who meet all of the requirements to undergo apheresis as per the Apheresis protocol in **Appendix F** (and further detailed in the Mononuclear Cell Collection SOP found in the Study File Binder), will have the procedure performed as an out-patient within 3 months of completion of screening assessments.

The procedure involves using a disposable sterile closed blood collection system which is attached to a Spectra Optia® apheresis machine. Leukapheresis is the separation of leukocytes from whole

blood, and returning the remaining blood back to the donor. Leukocytes in plasma will be collected into a blood collection bag. Upon completion of the donation, the collection bag is labelled, packaged, and transported to the CMF. In addition, a data logger will be used to obtain a continuous record of the internal temperature of the container during transportation. A medical courier service will be used to transport the container to the CMF. Full details describing the requirements for transportation of the autologous collection bags are provided in the "**Transportation of Autologous Donation SOP**".
An aliquot of the apheresis product will be stored in the biorepository at the Ottawa Hospital Research Institute (OHRI) for quality testing. In addition, a portion of any unused apheresis product may be stored for future research to further optimize cell manufacturing processes in order to improve the efficacy of the cell therapy product. Samples will be stored for no longer than 15 years, and no genetic testing will be performed.

5. Randomization

Eligible participants will be randomized only following the completion of a satisfactory apheresis collection and receipt of the sample by the CMF. CMF personnel who have been trained by the sponsor to perform randomizations will access the SAPPHIRE web-based randomization system available from the Ottawa Methods Centre (OMC) located at OHRI. Personnel requiring access to this system will receive individual confidential login accounts from Data Management Services at the OMC.

After accessing the SAPPHIRE randomization system, the web-page will prompt the user to confirm participant eligibility by completing an electronic randomization case report form. Once completed, the web-based program will generate a randomization number on the screen. The requesting CMF user will print the screenshot containing the randomization number and will file it in the CMF participant binder. A copy will then be scanned and emailed to the study site for placement in the participant's study file.

Randomization to treatment allocation will be performed using a randomly permuted block design of fixed sizes of 3, stratified based on the average of the two screening 6MWD test results (≤ 300 m vs. > 300m) and type of PAH (scleroderma associated PAH vs. all other eligible PAH types). Since our sample size for this late phase trial is relatively small, stratification is used to promote balance across the arms with respect to the baseline measure of the primary outcome, as well as type of PAH, an important prognostic factor for response to therapy. In particular, scleroderma associated PAH tends to progress more rapidly and show less favourable response to PAH-specific therapies. The allocation sequence will be generated using a computer-generated algorithm operated by the unblinded statistician for the trial located at the OMC. The study sponsor/CRO will monitor all randomizations throughout the conduct of the trial. The OMC will also generate a blinded email notification to the study site, study sponsor, and the CMF as confirmation of successful randomization. The CMF will subsequently receive a notification with the unblinded treatment allocation in order to proceed with manufacturing of the participant's study product.

6. Study Product Manufacturing

The following sections will briefly describe the methods for gene and cell manufacture and the procedures for *ex vivo* gene transfer.

6.1. Human eNOS minicircle DNA manufacture:

The human eNOS gene was derived from human umbilical vein endothelial cells, and its sequence and activity was verified as previously described (see *Investigator Brochure*). It has been subcloned into the minicircle vector (System Biosciences), with the final eNOS minicircle product manufactured by Aldevron in accordance with cGMP source manufacturing procedures.

6.2. EPC isolation and culture:

The apheresis product will be enriched for monocytes by a process similar to Ficoll gradient centrifugation using the closed-system Sepax cell separation device (Biosafe SA) and cultured in a GMP compliant cell manufacturing facility located at the OHRI. The cells are cultured in media containing human serum (tested to be free of specific infectious agents) and recombinant human growth factors and cytokines. Full details of cell culture are provided in the cell manufacturing Standard Operating Procedures (SOPs) provided to Health Canada in the chemistry and manufacturing information in Module 3 of the Clinical Trial Application.

6.3. Ex vivo gene transfer:

Nonviral gene transfer will be performed by mixing the eNOS minicircle DNA with JetPEI® (a chemically defined linear polyethylenimine derivative manufactured under GMP conditions, Polyplus) and applying the conjugates to the cells for several hours as described in detail in the cell manufacturing SOPs provided to Health Canada in Module 3 of the CTA.

6.4. Instructions for safe handling:

The Cell Manufacturing Facility will prepare the transfected autologous cell therapy or placebo product for each participant dose-delivery according to the randomization allocation for each participant, consisting of either 20 million eNOS-transfected cells suspended in a physiological buffer (Plasma-Lyte A, Baxter) containing 25g/L Human Albumin (Alburex 25) USP for injection, or a similar volume of Plasma-Lyte A containing 25g/L Human Albumin for the placebo product. Each 8 ml dose will be placed into a sterile 10 ml Luer-Lock syringe which is then labelled, packaged, and transported in accordance with Transportation of Dangerous Goods (TDG) regulations to the investigative site along with a temperature data logger to obtain a continuous record of the internal temperature of the container during transportation. A medical courier service will be used to transport the container to the study site.

The eNOS-transfected EPCs product has been shown to be viable for at least 48 hours under conditions relevant to those for transportation to the participating sites. All best efforts will be made to ensure that the study product will be administered to the participant within 24 hours of sealing the syringe. In no case will the study product be delivered to a participant after 48 hours have elapsed.

6.5. Dosing Regimen:

The SAPPHIRE trial will study the cumulative effect of repeated delivery of a “unit” dose of 20 million eNOS-transfected autologous EPCs. The phase 1 dose-ranging PHACeT-1 study established that a dose of 20 million eNOS-transfected autologous EPCs could be delivered at one time as a single cell infusion into the pulmonary circulation of patients with PAH without acute adverse hemodynamic consequences. Participants will receive a maximum of 8 doses delivered monthly for a total cumulative dose of 160 million cells. We have shown that the cumulative administration of up to 200 million cells during a one-time delivery procedure was well tolerated in large animal (porcine) preclinical model of severe pulmonary hypertension (see the

Investigator's Brochure). Using cardiac output to adjust for differences in size between rodents and humans, we estimate that a total dose of ~75 million cells would be roughly equivalent to 1.5 million cells, which was the effective dose established in the rat PH model, as described in detail in the *Investigator's Brochure*. Cardiac output is more relevant for normalization than body weight since cell distribution is limited, at least initially, to the lung microvasculature, and lung microvascular area is directly proportional to cardiac output.

In the SAPPHIRE trial the delivery of study product will occur in two "courses": in the first 6 months (Course 1), participants will receive a "unit" dose of 20 million cells in Arms 2 and 3, or an injection of an identical volume of physiological buffer (Plasma-Lyte A) containing 25g/L Human Albumin USP for the placebo in Arm 1, repeated on a monthly basis for 4 doses (total dose of 80 million cells in Arms 2 and 3). In the second 6 months (Course 2), the delivery of cell therapy product will be repeated in Arm 3 for a cumulative cell dose of 160 million over the 12 month study period; while participants in Arms 1 and 2 will receive either 4 doses of the cell product or placebo, respectively; for a cumulative cell dose of 80 million cells over the study period. A one month interval was chosen since this time period corresponded to the maximal improvement in 6MWD seen in the phase 1 PHACeT trial.

In the event that the CMF is unable to produce a sufficient number of cells to provide a total dose of 20 million cells for any treatment visit, then the total number of available cells will be administered to the participant, and the total cell dose will be recorded so that the total cell dose that was delivered can be calculated for each patient over the course of 4 or 8 cell treatments.

7. Study Visits

7.1. Initial Treatment Visit: Month 0

Under normal circumstances, administration of the study product will be performed a maximum of 11 days following apheresis. This includes both expected shipping and processing time for the apheresis product.

Pre-treatment procedures (performed following randomization and prior to study product delivery):

- Physical exam by the principal investigator or co-investigator
- Concomitant medication recording
- Vital signs (blood pressure, heart rate, respirations)
- Assessment and recording of WHO functional class *(*see Appendix C)
- O_2 saturation (peripheral device)
- Laboratory tests: sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, PT, PTT, INR (if warfarin sodium has been held for RHC procedure), brain natriuretic peptide (BNP), C-reactive protein (CRP)
- Additional blood samples will be taken for measurement of circulating biomarkers to be stored for no more than 15 years in the central biorepository at OHRI (no genetic testing will be performed)
- Urine collection for routine and microscopic analysis
- Urine pregnancy test for females with childbearing potential (to be done prior to administration of study product on the same day)

- **6MWD test (Baseline study)** and Borg dyspnea index (see Appendix D and E)
- Resting 12 lead ECG
- Pulmonary function testing (PFTs) [TLC, FVC, FEV_1/FVC, DLCO]
- 2D Echocardiogram with Doppler and saline contrast (as per Echocardiography Protocol)
- Quality of Life Questionnaire – SF-36 (see Appendix G)
- Out-patient right heart catheterization for hemodynamic assessment (BP, HR, RAP, RVP, PAP, mPAP, PAWP, PVR, SVR, CO, CI, SvO2)
  - Patients who are currently receiving anticoagulant therapy (warfarin sodium, NOAC) will be managed according to the standard procedure at participating sites
  - Cardiac output will be determined using the thermodilution and/or Fick methods
- Cardiac MRI at sites where this is available (only for participants who are not on prostacyclin pumps and have no other exclusions for MRI such as claustrophobia) according to the Cardiac MRI Protocol.

Administration of study product:

- Insertion of a peripheral IV cannula (20 gauge or larger)
- Blood sample will be taken prior to study product delivery to assess levels of inflammatory markers, including IL-2, IL-6 and TNF, by multiplex cytokine array or other appropriate assay
- A total of 20 million cells (8 ml; concentration of 2.5 million cells per ml), or an equivalent volume of saline placebo, will be injected by hand injection at a rate of no more than 2 ml/minute in an outpatient clinic environment that is equipped for repeated assessments of BP, HR and oximetry as per the Study Product Delivery Protocol (**Appendix H**).

Post-treatment procedures:

- Monitoring of BP, HR, and oximetry every 15 minutes for at least one hour
- Adverse event monitoring and assessment
- Discharge from clinic no earlier than one hour post treatment if judged to be clinically stable at the discretion of the investigator.

7.2. Follow-up/Treatment Visits: Months 1, 2, and 3 Post First Delivery of Study Product (±4 days; can occur over 2 days)

Pre-treatment Procedures:

- Physical examination by the principal investigator or sub-investigator
- Assessment of WHO functional class (*see Appendix C*)
- Assessment of adverse events and serious adverse events
- Vital signs
- Concomitant medication recording
- 12 lead ECG
- O_2 saturation (peripheral device)
- Laboratory tests: sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, CBC with differential, ESR, BNP, CRP
- Urine pregnancy test for females with childbearing potential (to be done prior to administration of study product on the same day)
- 2D Echocardiogram with Doppler and saline contrast (at 3 month visit only)

- 6MWD test and Borg dyspnea index (*see Appendix D and E*)
- Quality of Life Questionnaire – SF-36 (at Month 3 visit only - *see Appendix G*).

Administration of study product:

- As per section 7.1 (According to Study Product Delivery Protocol – *Appendix H*)

Post-treatment procedures:

- As per section 7.1 (According to Study Product Delivery Protocol – *Appendix H*)

7.3. Follow up Visit: Month 4 Post First Delivery of Study Product (±4 days; can occur over 2 days)

- Physical examination by the principal investigator or sub-investigator
- Assessment of WHO functional class (*see Appendix C*)
- Assessment of adverse events and serious adverse events
- Vital signs
- Concomitant medication recording
- 12 lead ECG
- O_2 saturation (peripheral device)
- Laboratory tests: electrolytes, sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, CBC with differential, ESR, BNP and CRP
- Blood sample will be taken to assess levels of inflammatory markers, including IL-2, IL-6 and TNF, by multiplex cytokine array or other appropriate assay
- Urine pregnancy test for females with childbearing potential
- 6MWD test and Borg dyspnea index (*see Appendix D and E*).

7.4. Follow up/Treatment Visit: Month 6 Post First Delivery of Study Product (±5 days; can occur over 4 days)

Pre-treatment Procedures:

- Physical examination by the principal investigator or sub-investigator
- Assessment of WHO functional class (*see Appendix C*)
- Assessment of adverse events and serious adverse events
- Vital signs
- Concomitant medication recording
- 12 lead ECG
- O_2 saturation (peripheral device)
- Laboratory tests: sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, CBC with differential, ESR, PT, PTT, INR (if warfarin sodium has been held for RHC procedure), BNP level, and CRP
- Additional blood samples for measurement of circulating biomarkers will be taken to be stored for no more than 15 years in the central biorepository at OHRI (no genetic testing will be performed)
- Urine pregnancy test for females with childbearing potential (to be done prior to administration of study product on the same day)
- Urine collection for routine and microscopic analysis

- 2D Echocardiogram with Doppler and saline contrast (as per echocardiography protocol)
- Cardiac MRI at sites where this is available (only for participants who are not on prostacyclin pumps and have no other exclusions for MRI such as claustrophobia) according to Cardiac MRI protocol
- PFTs [TLC, FVC, FEV_1/FVC, DLCO]
- Chest x-ray
- **6MWD test (for primary EP analysis)** and Borg dyspnea index (*see Appendix D and E*)
- Quality of Life Questionnaire – SF-36 (*see Appendix G*)
- Out-patient right heart catheterization for hemodynamic assessment (BP, HR, RAP, RVP, PAP, mPAP, PAWP, PVR, SVR, CO, CI, SvO2)
  - Patients who are currently receiving anticoagulant therapy (warfarin sodium, NOAC) will be managed according to the standard procedure at participating sites
  - Cardiac output will be determined using the thermodilution (in triplicate) and/or Fick methods.

Administration of study product:

- As per section 7.1 (According to Study Product Delivery Protocol – *Appendix H*)

Post-treatment procedures:

- As per section 7.1 (According to Study Product Delivery Protocol – *Appendix H*)

7.5. Follow up/Treatment Visits: Months 7, 8 and 9 Post First Delivery of Study Product (±4 days; Can occur over 2 days)

Pre-treatment Procedures:

- Physical examination by the principal investigator or sub-investigator
- Assessment of WHO functional class (*see Appendix C*)
- Assessment of adverse events and serious adverse events
- Vital signs
- Concomitant medication recording
- 12 lead ECG
- O_2 saturation (peripheral device)
- Laboratory tests: sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, CBC with differential, ESR, BNP, CRP
- Urine pregnancy test for females with childbearing potential (to be done prior to administration of study product on the same day)
- 2D Echocardiogram with Doppler and saline contrast (at 9 month visit only – as per echocardiography protocol)
- 6MWD test and Borg dyspnea index (*see Appendix D and E*)
- Quality of Life Questionnaire – SF-36 (at Month 9 visit only - *see Appendix G).*

Administration of study product:

- As per section 7.1 (According to Study Product Delivery Protocol – *Appendix H*)

Post-treatment procedures:

- As per section 7.1 (According to Study Product Delivery Protocol – *Appendix H*)

7.6. Follow up Visit: Month 10 Post First Delivery of Study Product (±4 days; Can occur over 2 days)

- Physical examination by the principal investigator or sub-investigator
- Assessment of WHO functional class *(see Appendix C*)
- Assessment of adverse events and serious adverse events
- Vital signs
- Concomitant medication recording
- 12 lead ECG
- O_2 saturation (peripheral device)
- Laboratory tests: sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, CBC with differential, ESR, BNP and CRP
- Blood sample will be taken to assess levels of inflammatory markers, including IL-2, IL-6 and TNF, by multiplex cytokine array or other appropriate assay
- Urine pregnancy test for females with childbearing potential
- 6MWD test and Borg dyspnea index (*see Appendix D and E*).

7.7. Follow up Visit: Month 12 Post First Delivery of Study Product (±5 days; Can occur over 4 days)

- Physical examination by the principal investigator or sub-investigator
- Assessment of WHO functional class (*see Appendix C*)
- Assessment of adverse events and serious adverse events
- Vital signs
- Concomitant medication recording
- 12 lead ECG
- O_2 saturation (peripheral device)
- Laboratory tests: electrolytes, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, CBC with differential, ESR, PT, PTT, INR (if warfarin sodium has been held for RHC procedure), BNP level, and CRP
- Blood sample will be taken to assess levels of inflammatory markers, including IL-2, IL-6 and TNF, by multiplex cytokine array or other appropriate assay
- Additional blood samples for measurement of circulating biomarkers will be taken to be stored for no more than 15 years in the central biorepository at OHRI (no genetic testing will be performed)
- Urine pregnancy test for females with childbearing potential
- Urine collection for routine and microscopic analysis
- 2D Echocardiogram with Doppler and saline contrast as per echocardiography protocol
- Cardiac MRI at sites where this is available (only for participants who are not on prostacyclin pumps and have no other exclusions for MRI such as claustrophobia) according to Cardiac MRI protocol

- PFTs [TLC, FVC, FEV_1/FVC, DLCO]
- Chest x-ray
- 6MWD test and Borg dyspnea index (*see Appendix D and E*)
- Quality of Life Questionnaire – SF-36 (*see Appendix G*)
- Out-patient right heart catheterization for hemodynamic assessment (BP, HR, RR, RAP, RVP, PAP, mPAP, PCWP, PVR, SVR, CO, CI)
  - Patients who are currently receiving anticoagulant therapy (warfarin sodium, NOAC) will be managed according to the standard procedure at participating sites
  - Cardiac output will be determined using the thermodilution and/or Fick methods.

7.8. Concomitant Medication:

All PAH-specific medications will be permitted during the study period.

7.9. Rescue Medication and Risk Management:

All treatment visits will take place in an outpatient hospital setting with access to full resuscitation measures, including a "crash cart" and "code blue" team, and study product injections will be performed by a study investigator.

Potential immediate risks of this cell therapy include adverse events related to possible pulmonary or systemic embolization, and allergic or other immune reactions (see Section 11.9 – Analysis of Safety). To ensure optimal safety following administration of the study product, vital signs and O_2 saturation will be closely monitored for a minimum of 1 hour post study product delivery, with approval required by a study investigator prior to discharge from the clinic. While the possibility of immune reactions has been minimized by using an autologous cell product, standard clinical therapies including antihistamines and corticosteroids will be available for treatment of symptoms should the need arise.

7.10. Rescheduling Missed Study Treatment Visits:

In the event that a study participant is unable to attend a scheduled treatment visit, or the study product does not arrive at the study site on time for delivery, every effort will be made to reschedule the treatment visit so that a new study product can be manufactured and administered within 2 weeks of the cancelled visit.

In cases where the study product administration cannot be performed within 2 weeks of the originally scheduled visit date, the dose for the study visit will be considered "missed" and the next study visit will take place according to the protocol visit schedule.

8. Post Treatment Phase Conversion to a Long-Term Follow-up Registry

Changing the study to a long-term follow-up registry following completion of the 12-month treatment phase of the trial will permit participants to enroll into other trials following data collection at the 12-month visit post first administration of study product. The registry will consist of a yearly telephone contact (± 14 days) to capture serious adverse event data over a 10 year period.

9. Blinding/Unblinding

9.1. Emergency Unblinding:

Since there is no antidote to cell therapy, the need for emergency unblinding during the 12 month treatment/follow-up period will be unlikely; however, unblinding may be undertaken if it is thought to be essential for effective treatment and management decisions for the study participant. The site Investigator will be responsible for contacting the trial medical consultant, Dr. Duncan J. Stewart, directly to discuss the rationale for unblinding. After consultation with Dr. Stewart (or delegate), if there is a need to unblind, Dr. Stewart will then contact the Manager of Data Management Services at the Ottawa Methods Centre (OMC) to ascertain the actual treatment assignment. The OMC is available 24 hours/day, 7 days/week. Dr. Stewart will then relay the treatment assignment to the site Investigator. The participant will then no longer receive any further planned administrations of study product but will be followed for the duration of their study period as an "off-protocol" study participant. Study personnel will record and report the unblinding as a protocol violation. Protocol violations related to unblinding will be monitored by the Study Executive and DSMB. Local personnel in the cell manufacturing facility (CMF) will also have a record of the participant's treatment allocation.

9.2. Treatment Blinding:

Treatment allocation will remain blinded to all study participants, investigators, clinical research staff, persons performing study assessments/procedures, and data analysts from the time of randomization until database lock. Only the following study personnel will have access to unblinded treatment allocation: the unblinded study statistician and CMF personnel responsible for study product manufacturing. The study statistician will be the author of the Master Randomization List and will act as the primary contact to the CMF for any questions pertaining to study treatment allocation. The total processing period in the CMF will be equal (7±2 days) for all study products, including placebo, in order to maintain blinding of the investigator and clinical research staff. The OMC study statistician will securely maintain the confidentiality of the Master Randomization List from all other parties involved in the trial prior to database lock.

9.3. Study Stopping Criteria:

Enrolment and administration of the study product at all sites will be immediately placed on hold with the occurrence of any one of the following: i) death, ii) major cardiovascular event, or iii) a life-threatening response (i.e. immune reaction) that is deemed "*definitely related*" to the *study product* by the trial Data Safety and Monitoring Board (DSMB). The Chair of the DSMB will be notified promptly and be provided with copies of de-identified source information for review and will make recommendations for protocol amendments or revisions to the Investigator's Brochure and/or the risk section of the informed consent form as required. The sponsor will inform Health Canada immediately of the "*definitely-related*" *unexpected and serious event* that met the predefined stopping criterion. Site investigators will be provided with the case information for reporting to their local REB. The Steering Committee may consider resuming the trial after consideration of the DSMB recommendations, and, if applicable, any submission and approval of any suggested protocol/consent amendments by Health Canada and the local REBs.

9.4. Premature Trial Discontinuation:

Premature trial discontinuation may occur as a result of an event that triggers the protocol-defined study stopping criteria. Termination or suspension of the trial may also result from a recommendation made by the Data and Safety Monitoring Board. The Steering Committee will

review any DSMB recommendations and will rule on any consequential actions to be implemented by the sponsor. If the sponsor is required to terminate or suspend the trial, the sponsor will notify the principal investigators in writing. The investigators, in turn will promptly inform their research staff, the trial participants and their local Research Ethics Board of this development. The sponsor will follow-up with the investigative sites by sending the principal investigators written instructions for participant and trial close-out procedures. The investigators in turn, should assure appropriate follow-up for the study participants. The sponsor will notify the Biologics and Genetic Therapies Directorate of Health Canada no later than 15 calendar days after the date of trial discontinuation.

The sponsor may terminate a site's participation due lack of enrollment, concerns of data integrity, protocol noncompliance or investigator conflict of interest. If the investigator terminates or suspends the trial without prior agreement of the sponsor, the investigator will inform the institution where applicable, and the investigator/institution must promptly inform the sponsor and the REB and should provide both with a detailed written explanation of the termination or suspension.

If the Research Ethics Board (REB) terminates or suspends approval of a trial, the principal investigator will inform the institution where applicable and the investigator/institution must promptly notify the sponsor and provide the sponsor with a detailed written explanation of the termination or suspension.

9.5. Premature Participant Withdrawal:

Premature withdrawal of study participants may be based on any of the following reasons:

- Sponsor Recommendations (i.e. Safety-specific protocol requirements)
- Investigator Decision (i.e. Participant non-compliance or if participant cannot safely perform the procedures required in the protocol)
- Participant Decision (No longer wishes to take part in the trial)
- Death of the study participant
- Pregnancy during the 12 month study treatment/follow-up period (refer to Section 9.7 for guidance)

Following the receipt of the study product, a participant may withdraw consent for study follow-up at any time for any reason. In addition, a participant may be considered withdrawn for failure to return for study visits, or become lost to follow up for any other reason.

If a participant withdraws consent for further study follow-up procedures and assessments, every effort should be made to continue to follow the participant for their safety and the collection of safety data. This would ideally include completion of a treatment phase premature withdrawal visit (see section 9.8) and the long-term follow-up registry annual phone calls.

The PI or research staff must determine whether the participant wishes to:

- Withdraw from study follow-up procedures and assessments, but permit telephone follow-up or review of clinic/hospital records for data collection
- Withdraw from study follow-up procedures and assessments and decline further contact, however permit their accumulated data to be included in the study analysis
- Withdraw from study follow-up procedures and assessments and decline further contact and access to personal health information and indicate data collected to date cannot be used

in the final analysis. Source documents cannot be destroyed until completion of the long-term storage period (25 years).

The primary reason for premature withdrawal and the participant's decision to decline study follow-up must be recorded in the participant's source documents and on the Treatment Phase Exit eCRF.

9.6. Lost To Follow-up:

In the case of a participant becoming lost to follow up, the investigator should demonstrate "due diligence" by performing the following recommended steps:

- Contact participant three times and clearly document those efforts. Contacts may be done either by telephone contacts, email or mail correspondence. Documentation of any telephone contacts should also include dates and times
- If unsuccessful after three times, a registered letter should be sent to the participant's address requesting the participant to sign for the letter to attempt to confirm survival status
- If no response, clearly document efforts and complete all source documents and eCRFs as applicable
- Confirm status as lost to follow-up.

9.7. Pregnancy:

In the event that a participant becomes pregnant during the 12-month treatment phase of the trial, the following steps should be taken:

- The participant will not receive any further study product injections
- The site Investigator will contact the study medical consultant, Dr. Duncan Stewart (or delegate), within 24 hours of their knowledge of the pregnancy to request unblinding of the participant's treatment allocation
- If the unblinding reveals that the participant has received at least one cell dose, she will continue to be followed for the duration of her pregnancy and the treatment phase according the protocol visit schedule and procedures, with the exception of imaging tests and right heart catheterizations. In addition, the long-term follow-up registry annual calls should be completed.
- Following birth of the baby, health information at birth should be collected and filed with source documents and entered in the Pregnancy Follow-up eCRF. In the event of any medical concerns at birth, an adverse event form or serious adverse event form should be completed as appropriate
- If the unblinding reveals that the participant has not received at least one cell dose, the participant will be withdrawn from the trial with a study exit visit completed (section 9.8).

9.8. Treatment Phase Premature Withdrawal Visit:

In the event of early withdrawal from the 12-month treatment phase of the trial, a Premature Withdrawal visit will be completed with the following timing:

1. 30 ± 2 days after the last study product injection (for participants who exit the treatment phase between Months 0-4 or 6-10)
2. 5 ± 2 days following withdrawal from the treatment phase (for participants who exit the treatment phase between Months 4-6 or 10-12)

The following assessments will be performed:

- Physical exam by the principal investigator or co-investigator
- Concomitant medication recording
- Vital signs (blood pressure, heart rate, respirations)
- Assessment and recording of WHO functional class *(*see Appendix C)
- O_2 saturation (peripheral device)
- Laboratory tests: sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, PT, PTT, INR, brain natriuretic peptide (BNP), C-reactive protein (CRP)
- Urine collection for routine and microscopic analysis
- Urine pregnancy test for females with childbearing potential
- 6MWD test and Borg dyspnea index (see Appendix D and E)
- Resting 12 lead ECG
- Assessment of adverse events and serious adverse events

10. Adverse Event Reporting

10.1. Adverse Event (AE):

An adverse event is any untoward medical occurrence in a research participant administered an investigational product and which does not necessarily have a causal relationship with the study product. An AE can therefore be any unfavourable and unintended sign (including an abnormal lab finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product.

The following definitions are to be used for guidance for determining severity of adverse events:

- Mild – An awareness of symptoms but easily tolerated
- Moderate – Symptoms interfere with normal daily activities
- Severe – Symptoms are incapacitating with inability to perform daily activities

10.2. Adverse Drug Reaction (ADR):

All noxious and unintended responses to an investigational product related to any dose should be considered adverse drug reactions. The phrase “responses to an investigational product” means that a causal relationship between the investigational product and an adverse event is at least a reasonable possibility (i.e. the relationship cannot be ruled out).

10.3. Serious Adverse Event (SAE) or Reaction:

An SAE is any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in congenital anomaly/birth defect
- Based on medical judgement, is an important medical event that may require medical intervention to prevent one of the outcomes listed above.

Note: The term "life-threatening" in the definition of "serious" refers to an event in when the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

10.4. Unexpected Adverse Drug Reaction:

UADR is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (i.e. the Investigator's Brochure for an unapproved investigational product). Reports which add significant information on specificity or severity of a known, already documented serious ADR constitute unexpected events. For example, an event more specific or more severe than described in the Investigator's Brochure would be considered "unexpected".

10.5. Expected Adverse Events in This Patient Population:

The following is a list of expected adverse events in the PAH population which should be used for guidance when determining expectedness of adverse events for the trial:

- Right heart failure
  - Peripheral edema
  - Pleural or pericardial effusions
  - SOB/dyspnea
  - Liver dysfunction
- Chest pain, angina
- Syncope
- Hypotension
- Supraventricular arrhythmias, atrial fibrillation
- Side effects of concomitant PAH therapies
  - Prostanoids and prostaglandin receptor agonists: hypotension, headache, flushing, nausea, vomiting, diarrhea, jaw pain, thrombocytopenia, musculoskeletal pain
  - ERAs: peripheral edema, elevated LFTs, anemia, headache, nasal congestion
  - PDE5 inhibitors and sGC stimulators: Headache, flushing, hypotension, priapism, GERD, visual changes
  - High dose Lasix: Hearing loss
  - Anticoagulants: Bleeding, bruising
- Hypoxemia, intrapulmonary shunting
- Right to Left shunting with rising right sided pressures
- Renal dysfunction
- Pulmonary Edema (in the context of a very severe reduction in cardiac output)

10.6. Expected Adverse Events during Apheresis

Guidance for determining expectedness of adverse events that occur during apheresis can be found in the Apheresis Risks section of the Informed Consent Form.

10.7. Determination of Adverse Event Relationship to Study Product:

For study participants having received at least one dose of the study product, guidance for determining the relationship and expectedness of adverse events can be found in Section 5 of the Investigator's Brochure.

10.8. Recording and Reporting Adverse Events:

At each contact with the patient, the investigator will seek information on adverse events by specific questioning, and as indicated, by examination. All adverse events occurring during the study period will be recorded. Information on non-serious adverse events should be recorded in the case report form with indication of the possibility of relationship to the cell delivery procedure or the delivered cells. Causality of all adverse events must be determined by the Principal Investigator or a sub-investigator. **A serious adverse event form must be completed for all serious events and sent to Allphase Clinical Research Inc. by email to SapphireSAEReports@allphaseclinical.com within 24 hours of the investigator's knowledge of the event.**

Serious adverse events that occur during the long-term follow-up registry do not require expedited reporting to the sponsor; however, the SAE form must be completed in the electronic data capture system with related source documents uploaded.

At the time of the initial SAE report, the following must be recorded on the SAE Report Form: participant unique study number, randomization number, description of the event, date of onset of event, any treatment given for the event, whether investigative treatment was discontinued prematurely, the reason why the event was classified as serious, and the investigator's assessment of the association between the event and the study product/procedure. The Principal Investigator must also provide prompt reporting of any follow-up information on each serious adverse event. This should include copies of the completed supplemental serious adverse event forms and any other diagnostic information that would assist in the understanding of the event.

In addition, the Principal Investigator is required to report all adverse events to the REB in accordance with local reporting requirements. The following AEs do not normally require reporting to the REB:

- Serious adverse events that are considered expected
- Serious adverse events that are considered not related to the investigational product or research procedures, whether the event is expected or not
- Non-serious adverse events, whether expected or not.

Northern Therapeutics, Inc. is responsible for reporting *Unanticipated Problems (serious and unexpected events which are deemed related or possibly related)* to Health Canada. Events that occur during the SAPPHIRE clinical trial must be reported within **15 days** after becoming aware of the information if the event is neither fatal nor life threatening. However, if an event is fatal or life threatening, the information should be reported within **7 days** after becoming aware of the information. A complete report which includes an assessment of the importance and implication of any findings should be submitted within **8 days** of the initial report.

Events that occur after participants have been transitioned to the SAPPHIRE registry do not require expedited reporting by the study sponsor; however, following completion of the registry, results of the long-term safety data analysis will be published and submitted to Health Canada.

11. Analytical Plan

11.1. Sample Size:

A total sample size of 39 randomized participants (13 per arm) in an analysis of covariance using a planned comparison between the average of the two treatment arms versus control achieves 80% power to detect a minimally important mean difference of **50 meters** in the primary outcome (6MWD) using an F-test with a two-sided 5% significance level. The SAS procedure GLMPOWER was used to conduct the calculation (SAS Institute Inc. 2008. SAS/STAT® 9.2. User's Guide. Cary, NC: SAS Institute Inc). The common standard deviation is assumed to be 85 and the correlation between baseline and 6 months is 0.80. These estimates are similar to those observed in our PHACeT trial where the standard deviation was 80 meters and the correlation between baseline and 6 month was 0.86. To account for 10% potential attrition, we will randomize 45 subjects (15 allocated to each arm).

11.2. General Statistical Approach:

The primary analyses will be by intent-to-treat but we will also conduct "per protocol" and "as treated" analyses. General linear models will be used to allow inclusion of all data in the analysis without the need to use ad hoc imputation procedures such as last observation carried forward. This maximum-likelihood based approach is fully efficient (assuming that covariates are completely observed).

11.3. Descriptive Statistics:

Descriptive statistics will be used to compare demographic characteristics and baseline prognostic factors of participants allocated to the three study arms. Measures of central tendency and dispersion will be calculated for all baseline variables in each study arm. Clinically important differences will be identified and adjusted for in all subsequent adjusted analyses. The normality of the distributions for primary and secondary outcome measures on a continuous scale will be assessed by visual inspection of histograms and normal probability plots. Normalizing transformations will be applied where necessary.

11.4. Analysis of Primary Outcome (6MWD at 6 months):

The primary analysis of our primary outcome, 6MWD assessed at baseline, one, two, three, four and six months, will use a general linear model with time (coded as a categorical variable) and group by time interaction as fixed covariates. As recommended for efficient analyses of longitudinal randomized controlled trials, baseline differences between the arms will be constrained through exclusion of the main effect for group[2]. In addition to providing the unadjusted effect, we will adjust our estimate for the stratification factors (6MWD ≤300m vs. > 300m and scleroderma APAH vs. all other eligible PAH categories), as well as age, gender and baseline medication use (PDEVi and sGC stimulators vs. all other PAH-specific medications). The model will be estimated using Restricted Maximum Likelihood (REML), and degrees of freedom will be computed using the Kenward-Roger approach[3]. Suitable covariance structures to account for correlation among repeated measures over time will be identified using likelihood ratio tests and information criteria (AIC and BIC). For the primary endpoint, the adjusted least square mean difference between the average of the two active treatment arms (Arms 2 and 3) versus placebo

[2] Fitzmaurice GM, Laird NM, Ware JH. Applied Longitudinal Analysis, Second Edition Hoboken, NJ: Wiley, 2011
[3] Kenward MG, Roger JH. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics. 1997;53(3):983–97.

(Arm 1) at 6 months, together with 95% confidence interval, will be obtained and assessed at the two-sided 5% level of significance. Additional exploratory pairwise comparisons of least square mean differences at one, two, three, and four months will be conducted.

11.5. Analysis of 6MWD at 12 months:

To compare the effect of sustained delivery of 4 vs. 8 doses of the cell therapy product on 6MWD at 12 months (secondary objective 1), the regression model will include repeated measures up to 12 months and be used to estimate the adjusted least square mean difference between Arm 2 and Arm 3 at 12 months, together with 95% confidence interval. Additional pairwise comparisons of least square mean differences at 7, 8, 9 and 10 months will be conducted.

11.6. Analysis of the Durability of Primary Outcome (6MWD at 6 months):

To assess the durability of any improvement in 6MWD at 3 months vs. 9 months post last dose of cell therapy product (secondary objective 2), the repeated measures model up to month 12 will be used to estimate the least square mean difference from 6 months to 12 months within Arm 2 together with its 95% confidence interval, and used to test the hypothesis of no deterioration.

11.7. Analysis of the Incremental Effect of Delivery of 4 vs. 8 doses (6MWD from 6 to 12 months):

To estimate the incremental effect of delivery of 4 vs. 8 doses on the 6MWD (secondary objective 3), the repeated measures model up to month 12 will be used to estimate the least square mean difference from 6 months to 12 months within Arm 3 together with its 95% confidence interval.

11.8. Analysis of Additional Secondary Outcomes:

Secondary objectives with respect to pulmonary hemodynamics (i.e., PAP, cardiac output, RAP PAWP), quality of life, and other continuous secondary outcomes will be analyzed using an approach similar to that used for analysis of our primary outcome of 6MWD. The combined endpoint of death or clinical worsening of pulmonary arterial hypertension up to 12 months will be described using Kaplan-Meier plots and compared across the 3 study arms using log-rank tests. Chi square test statistics and relative risks with 95% confidence intervals will be used to determine the effect of four doses of treatment on the combined endpoint at 6 months (Arm 1 vs. Arms 2 and 3). The combined endpoint at 12 months in Arm 3 will be described using 95% confidence interval and compared to historical event rates.

Clinical worsening of pulmonary arterial hypertension will be defined by any of the following:

1. All-cause mortality
2. Hospitalization due to worsening cardiopulmonary status attributable to progression of disease
3. Decrease in 6MWD by ≥ 15%
4. Worsening of WHO functional class.

11.9. Analysis of Safety:

Safety endpoints related to the tolerability and safety of injection of the study product in patients with severe PAH will be divided into early and late endpoints.

Early (within 1 hour of each study product administration):

- Clinical evidence of allergic or other immune reaction that in the opinion of the investigator is possibly related to the cell product injection
- Evidence of pulmonary or systemic embolization
- Symptomatic hypotension
- A clinically significant drop in O_2 saturation.

Late (within 1 month [visits 0-2 and 6-8] or 3 months [visits 3 and 9] of study product delivery):

- Evidence of significant immune activation (i.e. increases in ESR, CRP)
- Evidence of new and more than mild right to left shunting (i.e. greater than grade 2 shunting) by semi-quantitative analysis on agitated saline contrast echocardiography; or new evidence of rapid appearance of contrast in the left atrium.

A safety analysis is planned after the first twelve participants complete primary endpoint data collection at the 6-month follow-up visit. Safety data will be sorted by treatment arms (blinded) to determine the distribution of potential safety concerns and be provided to the DSMB for review. The unblinded treatment allocation will be available from the statistician should the DSMB determine that a safety concern exists that necessitates unblinding.

11.10. Sensitivity Analyses:

To assess for any potential bias due to attrition, we will examine differences between patients with complete data and those lost to follow-up based on all available covariates at baseline. A sensitivity analysis using multiple imputation under assumptions of "missing not at random" will be used to examine potential bias due to informative drop-out[4].

12. Quality

12.1. Data Safety Monitoring Board (DSMB):

Members of an external Data and Safety Monitoring Board have been appointed to monitor the safety of participants during the study. The members are not affiliated with Northern Therapeutics, Inc. and will not participate in the enrollment or treatment of participants in the study and will declare any conflicts of interest. The DSMB will be responsible for assuring that study participants will not be exposed to unnecessary or unreasonable risks and that the study will be conducted according to the highest scientific and ethical standards. Detailed descriptions of the DSMB members' roles and responsibilities are included in the study's DSMB Terms of Reference.

12.2. Trial Steering Committee:

All active study site Principal Investigators will serve as members of the Steering Committee.

Additional Members:

- Dr. David Courtman is a basic scientist and will be directing the cell manufacturing process at the Ottawa Hospital Research Institute (OHRI) cell manufacturing facility in the Sinclair Centre of Regenerative Medicine.

[4] RJA Little. 1995. Modeling the drop-out mechanism in repeated-measures studies. Journal of the American Statistical Association 90(431): 1112-1121.

- Dean Fergusson is the Director of the Clinical Epidemiology Research Program of OHRI, and Professor of Medicine at the University of Ottawa and will advise on matters pertaining to the conduct and oversight of the trial.
- Dr. Antonio Giulivi is the Vice Chair of the Research Department of Pathology Lab Medicine and Section Head of Transfusion Medicine at The Ottawa Hospital with expertise in apheresis procedures, and will serve as a consultant for the MNC Collections for the trial.
- Monica Taljaard is a Biostatistician and Senior Scientist at the Ottawa Hospital Research Institute (OHRI) and Associate Professor in the School of Epidemiology, Public Health and Preventative Medicine at the University of Ottawa. She will provide statistical support from the Ottawa Methods Centre at OHRI.

12.3. Monitoring:

Study monitoring is a sponsor responsibility that ensures that the rights and well-being of human subjects are protected, the reported trial data are accurate, complete, and verifiable from source documents, and that the conduct of the trial complies with the protocol, GCP, and regulatory requirements.

Monitoring for the trial will be performed by the sponsor/CRO according to the detailed monitoring plan for the trial. This will consist of a combination of on-site and remote monitoring to be conducted at regularly scheduled intervals.

12.4. Data Management:

Data will be collected at each participating site and entered into electronic CRFs (eCRFs) following scheduled assessment periods. The site coordinator will review the information entered into the eCRFs for completeness of information prior to submitting the forms to the OHRI Methods Centre data management services (DMS) group, the centralized data center where the data will be stored in an electronic database on a secure server with nightly backup. When queries arise, sites will be contacted to provide appropriate corrections. As part of quality assurance methods, all queries and corrections will be performed using electronic media to provide appropriate audit trails on the data elements that are changed. The Ottawa Methods Centre manager will oversee all activities at the data center, to prepare the interim and final datasets. The datasets will be provided to a Methods Centre statistician who will prepare reports and models for the analysis of the trial.

12.5. Record Retention:

To comply with Health Canada regulations, participating investigators at all sites will retain trial related records for 15 years. Records must be kept to enable linkage of participants' identity to CRF data (master log). This includes sufficient information from hospital and clinic records such as all original signed informed consent forms, source records, and detailed records of cell manufacturing. The records should be retained by the Site Investigator according to local requirements, International Conference on Harmonization – Good Clinical Practice (ICH-GCP) and as specified in the Clinical Study Agreement. After 15 years, all paper study records will be destroyed by shredding or incineration, and all electronic study records will be securely deleted.

12.6. Privacy

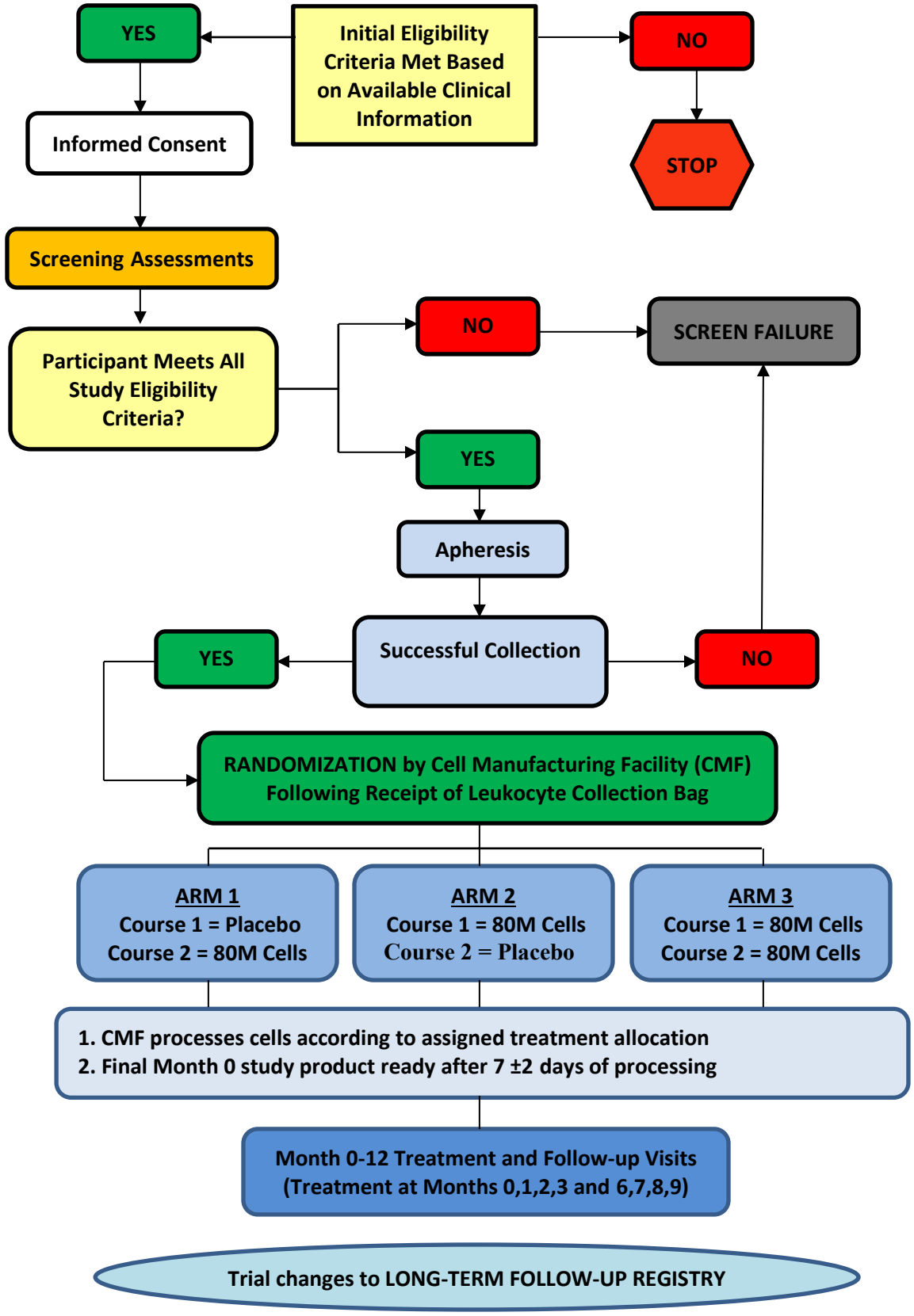
Health records will be reviewed in compliance with applicable provincial privacy laws as well as local hospital privacy policies. All personal health information will be treated in a confidential manner with respect to its collection, use, and disclosure.

Participant names or potentially identifying personal health information will not be provided to the sponsor or CRO. Only the unique participant number or randomization number will be entered in the eCRF, and any source documents provided to the sponsor (or CRO) must first be de-identified.

The consent form will describe to the participant who will have access to their information and for what purposes (e.g., Health Canada, Research Ethics Board (REB), research sponsors and personnel monitoring/auditing the research on their behalf).

Source documents containing participant identifying information must be retained securely at participating sites; however, these documents should ideally be kept separate from all study documents containing the unique participant numbers and randomization number. A master list that links participant identities to their unique participant numbers will be maintained at all study sites and stored separately from all other study records.

Appendix A – SAPPHIRE Study Flow Diagram



Appendix B – Schedule of Assessments

Time point / Assessment	Screening phase	Treatment Phase								Registry
		Month 0	Months 1, 2, 3 (±4 days)	Month 4 (±4 days)	Month 6 (±5 days)	Months 7, 8, 9 (±4 days)	Month 10 (±4 days)	Month 12 (±5 days)	Premature Withdrawal *(see Note[11])*	Yearly Telephone Call x 10 (±15 days)
Informed consent	X									
Eligibility Assessment	X									
Demographics, medical history	X									
Concomitant Medication Recording	X	X	X	X	X	X	X	X	X	
Physical exam by investigator	X	X	X	X	X	X	X	X	X	
WHO class	X	X	X	X	X	X	X	X	X	
Vitals signs (*See Note[1]*)	X	X	X	X	X	X	X	X	X	
Body weight & height	X									
O_2 saturation	X	X	X	X	X	X	X	X	X	
Clinical Blood Tests (*See Note[2]*)	X(a)	X(b)	X(c)	X(c)	X(b)	X(c)	X(c)	X(b)	X(b)	
Inflammatory biomarkers (*See Note[3]*)		X	X	X	X	X	X	X		
Biomarkers for storage (*See Note[4]*)		X			X			X		
Urine pregnancy test (*See Note[5]*)	X[1]	X[2]	X[2]	X	X[2]	X[2]	X	X	X	
Urinalysis	X	X			X			X	X	
12-Lead ECG	X	X	X	X	X	X	X	X	X	
6MWD Test (*See Note[6]*)	X(*)	X	X	X	X	X	X	X	X	
Borg Dyspnea Index (*See Note[7]*)	X	X	X	X	X	X	X	X	X	
Apheresis (*See Note[8]*)	X									
QOL Questionnaire (*See Note[9]*)		X	X[3]		X	X[4]		X		
Chest x-ray	X				X			X		
2D Echocardiogram with Doppler and saline contrast (See *Note[10]*)		X	X[5]		X	X[6]		X		
Cardiac MRI		X			X			X		
Hemodynamic Measurements		X			X			X		
PFTs		X			X			X		
Study Product Administration		X	X		X	X				
A/E & SAE Assessment		X	X	X	X	X	X	X	X	X

NOTE[1]: **Vital Signs:** Blood pressure, heart rate, respirations (all measured seated after 10 minutes of rest)

NOTE[2]: **Clinical Blood Tests:**

X(a) sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, PT, PTT, INR, HIV-1/2, HBV, HCV, HTLV-I/II, *Treponema pallidum* (TP) Antibodies, ß-hCG for females with childbearing potential

X(b) sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, PT, PTT, INR, brain natriuretic peptide (BNP), C-reactive protein (CRP)

X(c) sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, BNP, CRP

NOTE[3]: **Inflammatory Biomarkers:** For treatment visits (Month 0-3, 6-9), samples must be drawn immediately prior to study product delivery.

NOTE[4]: **Biomarkers for Storage:** Samples will be stored in the OHRI biorespository for up to 15 years.

NOTE[5]: **Urine Pregnancy Test:** **X^1** Must be performed in the morning prior to apheresis on the same day
X^2 Must be performed prior to product administration on the same day

NOTE[6]: **6MWD Test:** **X(*)** During screening, two 6MWD tests to be performed within 24 hours with at least 1 hour of rest between the tests.
X One 6MWD test to be performed at all other visits

NOTE[7]: **Borg Dyspnea Index:** Measured at the beginning and end of the 6MWD test

NOTE[8]: **Apheresis:** To be performed within 3 months of completion of screening assessments.

NOTE[9]: **QOL Questionnaire:** **X^3** Administered only at 3 month visit
X^4 Administered only at 9 month visit

NOTE[10]: **2D Echocardiogram:** **X^5** Performed only at 3 month visit
X^6 Performed only at 9 month visit

NOTE[11]: **Premature Withdrawal Visit:** Performed for participants withdrawn from the trial during the treatment phase:

1. 30 ± 2 days after the last study product injection (for participants who exit the treatment phase between Months 0-4 or 6-10)
2. 5 ± 2 days following withdrawal from the treatment phase (for participants who exit the treatment phase between Months 4-6 or 10-12)

Appendix C – Who Functional Class Assessment of Pulmonary Hypertension

WHO Functional Assessment (WHO Symposium 1998) *

Class I – Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnea or fatigue, chest pain or near syncope.

Class II – Patients with pulmonary hypertension resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dyspnea or fatigue, chest pain or near syncope.

Class III – Patients with pulmonary hypertension resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes undue dyspnea or fatigue, chest pain or near syncope.

Class IV – Patients with pulmonary hypertension with inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnea and/or fatigue may even be present at rest. Discomfort is increased by any physical activity.

* Modified after the New York Heart Association Functional Classification

Appendix D – 6 Minute Walk Distance (6MWD) Test Protocol

The 6MWD test is designed to evaluate exercise capacity while performing an everyday activity. It is often used for measuring the response to medical interventions in patients with moderate to severe heart or lung disease and has the advantage of not requiring complicated exercise equipment or specialized technicians to administer the test.
The specific details that describe the technical aspects of the test, the required equipment, the safety aspects, the participant instructions, and the test procedure are found in the SAPPHIRE **6 Minute Walk Distance Test Protocol** found in the Study File Binder.

General Considerations:

1. The 6MWD test is a non-encouraged test.

2. Repeat tests throughout the trial should be performed at about the same time of day in order to minimize intraday variability.

3. The location of the 6MWD test must be of adequate length and width and rarely traveled by other individuals. The same walking course location should be used for each participant at the study site.

4. Testing should be performed in a location where a rapid, appropriate response to an emergency is possible.

5. Participants who require supplemental oxygen should be tested using the same method of delivery (i.e. liquid oxygen or oxygen tank) and flow rate for all 6MWD tests performed throughout the trial. If the flow rate must be increased during subsequent visits due to worsening gas exchange, this should be noted on the worksheet.

The Screening 6MWD Test:

1. Two 6MWD tests will be performed within 24 hours with at least one hour of rest between them.

2. In order for a participant to be eligible for further study participation, there must be ≤ 15% variation between the two tests, and the averaged result must be ≥ 125 meters and ≤ 440 meters.

3. The % difference between the tests will be calculated by taking the absolute value of the difference between the two distances and dividing it by the larger of the two walk distances.

Testing for Other Study Visits:

For all other study visits, a single 6MWD test will be performed. The Month 0 6MWD distance test will be used as the baseline value for subsequent comparisons during the study.

Appendix E – Borg Dyspnea Index

The Borg Scale will be used by the person administering the 6MWD test to obtain a rating of dyspnea from the participant at the following time points:

1. Immediately prior to beginning the 6MWD test.
2. Immediately following the 6MWD test. Before asking the participant to rate their dyspnea at the end of the 6MWD test, remind the participant of the number they selected at the beginning of the test.

Use the following dialogue to obtain the dyspnea rating:

"This is a scale that asks you to rate the difficulty of your breathing. It starts at number 0 where your breathing is causing you no difficulty at all and progresses through to number 10 where your breathing difficulty is maximal. How much difficulty is your breathing causing you right now?"

Perceived Breathlessness (Borg scale)

0	**NOTHING AT ALL**
0.5	**VERY VERY SLIGHT (just noticeable)**
1	**VERY SLIGHT**
2	**SLIGHT**
3	**MODERATE**
4	**SOMEWHAT SEVERE**
5	**SEVERE**
6	
7	**VERY SEVERE**
8	
9	**VERY VERY SEVERE (almost maximum)**
10	**MAXIMUM**

Appendix F – Apheresis Protocol

Participants who meet all eligibility criteria following completion of the screening evaluations will be scheduled to undergo an out-patient apheresis procedure at the study site.

This apheresis protocol describes general aspects of the apheresis procedure. Specific procedures to be followed for the collection of MNCs are detailed in the SAPPHIRE **Mononuclear Cell Collection SOP**.

Responsibilities:

The overall responsibility for ensuring adherence to all aspects of mononuclear cell collection (MNC) for this protocol is that of the Principal Investigator. The Principal Investigator will delegate the responsibility of performing the apheresis procedure to Registered Nurses (RNs) who are specially trained in apheresis techniques and must also ensure that the apheresis RNs receive appropriate training on the clinical protocol. The apheresis RNs will be responsible for performing the MNC collection in accordance with the protocol and SOP, data collection and entry onto study-specific worksheets, monitoring of the study participant throughout the procedure, and reporting any adverse events experienced by study participants during MNC collection.

The Principal Investigator may delegate medical supervision for MNC collections performed for this protocol at the study site to another physician who has expertise with apheresis procedures.

All delegation of duties for the apheresis procedure must be documented on the Clinical Staff List maintained by each study site.

General Considerations:

1. MNC collections for the study are performed with orders signed by the PI or co-investigator.

2. In order to proceed with the apheresis, the participant's blood test results (CBC drawn within 24 hours of the procedure) must be within the acceptable ranges for the apheresis unit at the study site, and females of child bearing potential must have a urine pregnancy test performed the morning of the procedure with a negative result.

3. Participants will undergo apheresis using the Spectra Optia® Apheresis System.

4. Following the procedure, transportation of the autologous collection bags will be performed in accordance with the **Transportation of Autologous Donation SOP**.

Appendix G – SF 36 Survey

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. *Thank you for completing this survey!*

For each of the following questions, please mark an ☒ in the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. <u>Compared to one year ago</u>, how would you rate your health in general <u>now</u>?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a <u>Vigorous activities</u>, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b <u>Moderate activities</u>, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
c Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d Climbing <u>several</u> flights of stairs	☐ 1	☐ 2	☐ 3
e Climbing <u>one</u> flight of stairs	☐ 1	☐ 2	☐ 3
f Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g Walking <u>more than a kilometre</u>	☐ 1	☐ 2	☐ 3
h Walking <u>several hundred metres</u>	☐ 1	☐ 2	☐ 3
i Walking <u>one hundred metres</u>	☐ 1	☐ 2	☐ 3
j Bathing or dressing yourself	☐ 1	☐ 2	☐ 3

4. During the <u>past 4 weeks</u>, how much of the time have you had any of the following problems with your work or other regular daily activities <u>as a result of your physical health</u>?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the <u>amount of time</u> you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b <u>Accomplished less</u> than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Were limited in the <u>kind</u> of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d Had <u>difficulty</u> performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. During the <u>past 4 weeks</u>, how much of the time have you had any of the following problems with your work or other regular daily activities <u>as a result of any emotional problems</u> (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the <u>amount of time</u> you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b <u>Accomplished less</u> than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Did work or other activities <u>less carefully than usual</u>	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

6. During the <u>past 4 weeks</u>, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

7. How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>?

None	Very mild	Mild	Moderate	Severe	Very severe
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

8. During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

9. These questions are about how you feel and how things have been with you <u>during the past 4 weeks</u>. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the <u>past 4 weeks</u>…

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Did you feel full of life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Have you been very nervous?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Have you felt so down in the dumps that nothing could cheer you up?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f Have you felt downhearted and depressed?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
g Did you feel worn out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
h Have you been happy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
i Did you feel tired?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

10. During the <u>past 4 weeks</u>, how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

11. How TRUE or FALSE is <u>each</u> of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a. I seem to get sick a little easier than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. I am as healthy as anybody I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Thank you for completing these questions!

Appendix H – Study Product Delivery Protocol

Delivery of the study product to participants will take place at the following visits: Months 0-3 and Months 6-9.

This protocol describes the general requirements for the study product delivery. Specific procedures to be followed are detailed in the SAPPHIRE **Study Product Delivery SOP**.

General Requirements:

1. Delivery of the study product (with associated procedures and post-delivery monitoring) must be the final study procedure/assessment performed during the study visit window.

2. Delivery of the study product must take place in an outpatient setting with access to full resuscitation measures, including a "crash cart" and "code blue" team.

3. Antihistamines and corticosteroids must be available for treatment of immune reactions should the need arise.

4. The study product will be injected by hand at a rate of no more than 2ml/minute only by the Principal Investigator or a delegated co-investigator.

5. The study product will be delivered through a peripheral IV cannula (20 gauge or larger).

6. Participants will be monitored for a minimum of 1 hour post product delivery. HR, BP, and oximetry will be measured every 15 minutes during this time period.

7. Discharge from the clinic must be approved by the Principal Investigator or delegated co-investigator once the participant is judged to be clinically stable.