Title: A single center, prospective study to compare the quality and quantity of the cellular content of M-PRP harvested after peripheral mobilization of progenitor cells using filgrastim versus pegfilgrastim.

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Table of Contents

Abbreviations and Definitions

AE Adverse Event

ACDA Anti-Coagulant Citrate Dextrose A

BMA Bone Marrow Aspirate

cBMA Concentrated Bone Marrow Aspirate

CBC Comprehensive Metabolic Panel

Cc Cubic Centimeter

CRF Case report Form

EDC Electronic Data Capture

GCP Good Clinical Practice

G-CSF Granulocyte Colony-Stimulating Factor

HEENT Head, Eyes, Ears, Nose, and Throat examination

HIPAA Health Insurance Portability and Accountability Act

H_0 Null Hypothesis

H_1 Alternative Hypothesis

HPCs Hematopoietic Stem Cells

INR International Normalized Ratio

IRB Institutional Review Board

Kg Kilograms

M-PRP Mobilized Platelet-Rich Plasma

MPCs Mesenchymal Progenitor Cells

Mg Milligrams

mL milliliters

mm Millimeters

PI Principal Investigator

PB Peripheral blood

PEG Polyethylene glycol

PPP Platelet-Poor Plasma

PRP Platelet-Rich Plasma

PT Prothrombin Time

PTT Partial Thromboplastin Time

QA Quality Assurance

SAE Severe Adverse Event

SOC Standard of Care

SOP Standard Operating Procedure

WBC White Blood Count

1 Background / Scientific Rationale

1.1 Introduction

Musculoskeletal injuries are a significant public health concern globally, contributing a large burden of disability and suffering.[1] Progenitor/stem cell therapies have shown great potential for addressing acute, traumatic and chronic orthopedic injury. [2] Orthopedic surgeons have started to augment surgical procedures and treat degenerative conditions such as osteoarthritis, with injections of bone marrow aspirate (BMA) and platelet rich plasma (PRP). [3-7] Several clinical application studies have concluded that the success of the intervention is dependent upon the number of progenitor cells harvested and utilized. While bone marrow aspiration is frequently utilized for orthopedic applications, the number of cells harvested is variable and dependent on several factors including individual, aspiration technique and location of harvest. The most efficient method involves rapid, small-volume, and multiple aspirations from multiple locations on the iliac crest.[8, 9] In addition, bone marrow aspiration is an invasive procedure requiring more clinical resources and logistics demands. [10] As a result, orthopedic studies have begun to investigate different methods and locations for progenitor cells for orthopedic purposes including injury effusion fluids and arthroscopic by products [11], adipose tissues [12], synovial fluid [13, 14], subacromial bursal cells [15], and mobilized peripheral blood cells. [7, 16, 17]

Bone marrow (BM) contains a variety of stem/progenitor cells, including hematopoietic progenitor cells (HPCs), endothelial progenitor cells (EPCs), and mesenchymal progenitor cells (MPCs). [18] CD34 is a common membrane marker for HPCs and studies have shown very low level of circulating CD34+ cells (10-100 CD34+ cells/ml) in PB under physiological steady-state conditions [19-21]. A mobilization regime is needed to effectively shift progenitor cells from BM niche to PB. Various interleukins, the chemokines, or the hematopoietic growth factors have shown to increase mobilization of progenitor cells with various efficiencies and kinetics. [22, 23] In this study, filgrastim and pegfilgrastim will be used as HPCs mobilizing agents.

1.1.1. Background on Filgrastim and Pegfilgrastim and the Use of Biosimilars

Filgrastim is a human granulocyte colony-stimulating factor (G-CSF) protein with a molecular weight of approximately 19 kilodaltons (kD) and consists of 175 amino acids. Filgrastim is obtained from the bacterial fermentation of a strain of *E. coli.* transformed with a genetically engineered plasmid containing the human G-CSF gene. Pegfilgrastim Fulphila® is a long-acting covalent conjugate of recombinant methionyl human filgrastim, and monomethoxypolyethylene glycol (PEG). To produce pegfilgrastim, a 20 kD PEG molecule is covalently bound to the N-terminal methionyl residue of filgrastim. The average molecular weight of pegfilgrastim is approximately 39 kD. Attachment of PEG increases the size of filgrastim as a result it becomes too large for renal clearance. Consequently, neutrophil-

mediated clearance predominates in elimination of the drug. A single injection of pegfilgrastim produces increased serum concentrations to 42 hours, compared with between 3.5 and 3.8 hours for multiday administration of filgrastim [24, 25].

Granix® (tbo-filgrastim), an FDA-approved biosimilar of filgrastim, will be used for the filgrastim mobilization (see appendix A). Fulphila® (pegfilgrastim-jmdb), an FDA-approved biosimilar of pegfilgrastim, will be used for the pegfilgrastim mobilization (see appendix B). Biosimilars are biological products that have no clinically meaningful differences from a reference product [26]. Biosimilars are approved by the FDA after rigorous evaluation and testing by the applicant [26]. The above biosimilars were selected by the study investigator and a consulting board-certified oncologist based on the similarity of indication, risk/benefit ratio, previous clinical testing, and general availability.

1.1.2 Indication, Dosage and Safety of Filgrastim and Pegfilgrastim

Filgrastim and Pegfilgrastim are indicated to increase survival in patients acutely exposed to myelosuppressive doses of radiation and to decrease the incidence of infection in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia.

Previous work has determined that the optimal dosage of filgrastim for cell mobilization is 10 mcg/kg/day for four or more consecutive days which is inconvenient for the patients **[27].** According to the FDA label, patients undergoing autologous peripheral blood progenitor cell collection and therapy 10 mcg/kg/day subcutaneous injection of filgrastim is administered for at least 4 days before first leukapheresis procedure. For pegfilgrastim according to the FDA label, 6 mg of pegfilgrastim is administered subcutaneously once per chemotherapy cycle to patients with cancer receiving myelosuppressive chemotherapy. The above suggested dosage will be used in the study. Pegfilgrastim may be preferred over filgrastim, due to its convenient once-per-cycle subcutaneous administration as compared to daily administration of filgrastim for at least 4 days.

Several clinical studies have shown filgrastim and pegfilgrastim to be safe and effective for mobilizing peripheral blood progenitor cells in patients and healthy donor. [28-31] Both the pharmaceutical agents increase the production of progenitor cells in the bone marrow leading to increased release and circulation of progenitor cells in the peripheral blood **[24, 32, 33]**. The long-term safety of filgrastim mobilization has been reported with bone pain as a common adverse effect and no association with neoplastic risks identified. **[27, 34-37]** In one study involving 126 cancer patients, 44% reported mild-to-moderate muscle and bone pain and 7% reported headaches.[33] The Spanish National Donor Registry reports that out of 736 donors, 90% reported bone pain and 33% had headaches.[34] In preclinical and clinical studies, pegfilgrastim has been shown to have comparable efficacy and safety profile to filgrastim with pegfilgrastim only requiring one injection to achieve the same effect as multiple day injections of filgrastim. **[24, 38-42]** Pegfilgrastim is comparable to filgrastim for mobilization of

peripheral blood progenitor cells as shown by CD34+ (marker for hemopoietic stem cells) cells. **[43, 44]** A single dose of pegfilgrastim was able to mobilize a sufficient number of $CD34^+$ in a multiple myeloma patient not responsive to two previous attempts with high or standard dose chemotherapy followed by filgrastim.**[45]**

1.2 Rationale for the study

Mainly the previous study of mobilization of progenitor cells to PB is focused on hematologic oncologic clinical practice of hematopoietic stem cell transplant. Pharmaceutical mobilization by filgrastim, followed by peripheral harvest of cells with apheresis is used more commonly than bone marrow aspirate for the hematologic oncologic clinical practice of hematopoietic stem cell transplant **[46]**. These previous studies have evaluated the potential of filgrastim and pegfilgrastim to improve apheresis harvest. However, combining pegfilgrastim mobilization with peripheral blood harvest and concentration with a PRP device has not been fully evaluated. The PI recently completed a study on developing M-PRP product created by peripheral blood mobilization of progenitor cells with filgrastim and processing with Arthrex Angel system and comparing it with concentrated bone marrow aspirate (cBMA). The most significant finding of this study was that the M-PRP product contained a higher concentration of platelets and monocytes when compared to cBMA. As expected, M-PRP had significantly greater WBC, monocytes, and HPCs compared to PRP alone. This study found overall a similar cellular product with an easier harvest with M-PRP when compared to cBMA **[47]**.

The multiday administration of filgrastim is inconvenient for the patients; therefore, the PI in this study aims to utilize pegfilgrastim as a mobilizing agent for creating M-PRP product in one subcutaneous administration. In this study the M-PRP product created by either pegfilgrastim or filgrastim will be evaluated *in vitro* or bench top. The PI hypothesizes that both of the pharmaceutical mobilization agents would result in similar M-PRP product. It is proposed that the progenitor cells isolated in M-PRP would be of similar property to cultured bone marrow derived cells (when cultured in hypoxia), with established multi-potentiality and differentiation potential to the mesodermal lineage. Previous studies have shown that circulating cells in PB are multipotent and possess differentiation potential. [16, 48, 49] Due to convenience, pegfilgrastim is a more practical mobilizing agent; thus, could be preferred in creating potent point of care M-PRP product for application in orthopedic medicine.

In this study, in conjunction with the mobilizing agent, the M-PRP processing requires a concentrating PRP system, Arthrex Angel system. The Arthrex Angel system uses centrifugation and optics to more precisely separate cell types using buffy coat method. All cells have a density range and non-uniformly after centrifugation. Arthrex Angel system has the unique ability to isolate specific cells using inherent properties of cells that absorb differing wavelengths of light. The settings on the system can be controlled to adjust the proportion of cells versus plasma. Increasing the setting from 7% to 15%, the Angel system isolates more cells from a deeper portion of the buffy coat, which results in capturing more HPCs per volume.

Also, the volume of the cPRP can be increased with autologous PPP, which consists of growth factors and other beneficial proteins. The Arthrex Angel system combined with peripheral mobilization could be used as point of care method for creating effective M-PRP product. (Device pamphlet, Appendix H)

2 Protocol Design

The proposed study is a prospective, randomized controlled, single-center laboratory study involving 10 healthy volunteers. Once the potential participant has cleared the screening, consented to the study procedures, completed the medical interview, and laboratory blood testing, the subject will undergo two serial mobilization events. The scheduling of the mobilization events will be varied across the 10 participants to counter sequencing effects of the mobilization events. 5 heathy donors will be administered standard filgrastim mobilization regimen of 10 mcg/kg per day for 4 days will be followed by a standard pegfilgrastim mobilization regimen consisting of one 6 mg injection separated by 8 weeks for 5 of the participants. The other 5 healthy donors will receive the reverse order of the pharmaceutical agent, first pegfilgrastim followed by filgrastim.

On the first day of the study, a first blood draw of 130 mL will be performed which will be used to create standard PRP for laboratory testing and subjects will begin a filgrastim or pegfilgrastim dosage series. After the specified time (4 days for filgrastim and 7 days for pegfilgrastim), a second 130 mL of blood will be harvested and processed with the Arthrex Angel system to create M-PRP for laboratory testing. The standard PRP and M-PRP cellular content will be studied and quantified *in vitro* with cell counting, cell culturing and protein analysis. 8 weeks after the second blood harvest, the subjects will return for a third 130 mL of blood draw, followed by administration of a second mobilizing agent (pegfilgrastim or filgrastim). After the specified time (4 days for filgrastim and 7 days for pegfilgrastim), the patients will return for a fourth blood draw of 130mL. The sample will be processed with the Arthrex Angel system to create M-PRP for laboratory testing. The cellular content of the M-PRP product will be studied and quantified *in vitro* with cell counting, cell culturing and protein analysis. Thereafter, the cellular content of M-PRP product will be compared between filgrastim and pegfilgrastim mobilization agents.

3 Objectives

3.1 Primary Objective

The objective of this study is to compare and quantify the cellular content (*in vitro)* of the mobilized M-PRP products harvested after a standard four-day filgrastim mobilization regimen versus one-day pegfilgrastim regimen when combined with Arthrex Angel System. This study would be useful to further develop optimized point of care orthopedic product in future.

3.2 Primary Effectiveness Endpoint

The Automated hematological analysis will be performed on all M-PRP products to quantify total red blood cell (RBC), white blood cell (WBC), monocyte, platelet, and hematopoietic progenitor

cell (HPC) concentration. The cell count of the M-PRP products will be performed and cells will be cultured and characterized under different differentiation conditions. The protein analysis of the M-PRP will be carried out via ELISA and/or flow cytometry.

4. Hypotheses

4.1Null Hypothesis:

H_0-Particpants will have not a similar cellular content (*in vitro)* of the mobilized M-PRP products harvested after a standard when comparing a 4-day filgrastim mobilizing treatment to a one-day pegfilgrastim mobilizing treatment.

4.2 Alternative Hypothesis:

H_1- Participants will have a similar cellular content (*in vitro)* of the mobilized M-PRP products harvested after a standard when comparing a 4-day filgrastim mobilizing treatment to a one-day pegfilgrastim mobilizing treatment.

5 Treatment Plan

5.1 Participant Eligibility

The proposed study is a controlled laboratory study involving 10 healthy male volunteers between the ages of 19 and 39 years with weight 50-100 kg. The age was limited because the cellular components of PRP have been found to be dependent on these variables. [50, 51] The weight was restricted due to the logistics of pre-filled syringes and the cost of Filgrastim. Female volunteers will be excluded to avoid the potential of any fetal risks from administration of filgrastim during pregnancy. The general exclusion criteria were based on associated risk factors with mobilizing agents. Subjects will be selected using the following inclusion and exclusion criteria:

5.2 Inclusion Criteria:

1. Healthy Male 19-39 of age and weight 50-100 kg

2. Subject consents to coming 5 serial days for filgrastim treatment and additional blood draw and then 8 weeks later two addition visits for pegfilgrastim treatment and 7 days later blood draws. The above order of administration will be provided to 5 participants and the additional 5 will receive pegfilgrastim treatment first followed by filgrastim to counter sequencing effects.

5.3 Exclusion criteria:

1. Female

2. Weight < 50kg or > 100kg

3. Previous allergic reaction to filgrastim, PEG, lidocaine, latex, acrylic or any other injectable numbing agent

4. History of Diabetes

5. Abdominal tenderness to palpation

6. Unclear lung fields on physical exam

7. Splenomegaly

8. Significant cardiovascular, renal, hepatic, or pulmonary disease

9. WBC over 20,000/mcL upon initial screening CBC

10. Blood disorders, autoimmune disorders, disorders requiring immunosuppression, cancer, an ongoing infectious disease, or sickle cell or other blood disorders.

6 Participant Enrollment

10 healthy male participants will be recruited through the Andrews Institute physician practices via word of mouth and utilizing a recruitment flyer (see appendix F) at the Andrews Institute campus. Potential participants will be prescreened for inclusion and exclusion criteria through standard of care medical evaluations. Participants meeting the inclusion criteria will have the study explained to them by one of the members of the investigating team, and they will be given an opportunity to participate if they are interested. Once a potential participant has agreed to be involved in the study, they will go through the described informed consent process.

6.1 Screening Process:

Once interested participants are identified and prescreened, a screening visit will be scheduled. During the initial visit, a screening form will be completed and reviewed. If an individual answers "yes" to any of the initial screening exclusion questions, they will be informed that they do not qualify, and they will be informed that they can keep their screening form. If all answers are "no" then the form will be placed in the study documents.

Once the screening requirements are met, the informed consent will be provided to ensure the volunteer understands the details of the study including the benefits and risk factors. The subject will be provided sufficient time to consent and sign the informed consent form (ICF). The principal investigator (PI) will be available to answer any questions or provide clarifications during the informed consent process. After the volunteer has consented to participate in the study, a medical interview, and a blood test will be administered (CBC, CMP) will be conducted. The medical interview will include a complete physical examination and review of the blood test to ensure that no exclusion conditions exist. Physical examination will include height/weight, head/neck, cardiovascular, lung, and abdominal examinations. Standard vitals will be taken.

6.2 Informed Consent 21 CFR 50

In adherence to the 21 CFR 50, Protection of Human Subjects Guidelines, the informed consent process will be performed by one of the study investigators or staff, in the research office that has received training on the informed consent process. No aspects of the study will be conducted prior to obtaining informed consent from each participant. The purpose and methods of the study along with the expected effects will be reviewed with each potential participant. Each participant will be provided a copy of the consent and sufficient time will be given for the opportunity to read and ask any questions about the study. After signing of the informed consent document, participants will be given a copy for their records.

The designee will review with each participant that they are free to refuse to the study or to withdraw from it at any time.

6.3 Consent Withdrawal:

During the informed consent procedure, participants will be informed that if at any point during the study, consent may be withdrawn. To withdraw consent, participants can request in writing to withdraw HIPAA authorization and the research site will not use or provide any health information to researchers. At this time, the link between the participant's health information will be severed with the research team. This process for consent withdrawal will be reviewed with each participant and identified barriers will be addressed at the time of informed consent.

6.4 Benefits:

There is no direct benefit to participants participating in this study. This study may help direct clinical practice in alternative method to extract stem cells that can be used to augment surgical procedures.

6.5 Compensation:

Compensation will be provided to participants on a pre-set schedule. A $50.00 dollar stipend will be provided to participants on the first day of receiving the mobilization agents. Each subsequent scheduled study treatment visit will award the participant a $75.00 stipend until the total of $500.00 is met.

7 Study Procedures

7.1 Screening Visit:

Upon obtaining consent from the participant, the Research Team will complete the screening process along with the screening form. The subject will be provided the filgrastim and pegfilgrastim label handout (Appendix A & B) to continue to best practices. The Research Team will obtain history of the participant to check inclusion and exclusion criteria. If not excluded, the study physician will complete another physical examination including height/weight, head/neck, cardiovascular, lung, and abdominal examinations. Standard vitals will be taken. The blood draw will be completed (CBC, CMP) and sent to the lab. Once labs are reviewed and are within the

acceptable limits, WBC <20k and coagulation studies are normal, the subject may be scheduled for the initial mobilizing regimen which may take place on the same day as screening.

7.2 Filgrastim Treatment Plan:

During the collection/treatment visit, the physician will complete another physical examination including height/weight, head/neck, cardiovascular, lung, and abdominal examinations. Standard vitals will be taken. A standard venipuncture will be performed on the left or right upper extremity. Before the treatment 130.0 ml of blood will be drawn and processed with the Arthrex Angel® System (Arthrex, Inc, Naples, FL USA). The PRP will be analyzed for cellular components total cell count, cell growth and protein (chemokine/cytokine) analysis. .

After the blood draw, the volunteers will undergo 4 consecutive days of mobilization with filgrastim. The 10 mcg/kg dose of filgrastim will be administered to the volunteers subcutaneously into the thigh. Dosages will be rounded to 300 mcg, 600 mcg, 780 mcg, 840 mcg. Appendix A, a filgrastim information handout will be given after filgrastim administration. After 4 doses of the filgrastim, 130.0 mL of blood will be drawn and will be processed with the Arthrex Angel system. The M-PRP samples will be analyzed for cellular components total cell count, cell growth and protein (chemokine/cytokine) analysis. The safety of the volunteer will be followed up via a phone call at 7 day and 28 days after mobilized blood collection.

There will be 8 weeks interval between the two treatments.

7.3 Pegfilgrastim Treatment Plan

During the pegfilgrastim visit, the physician will complete another physical examination including height/weight, head/neck, cardiovascular, lung, and abdominal examinations. Standard vitals will be taken. A standard venipuncture will be performed on the left or right upper extremity. Before the treatment 130.0 ml of blood will be drawn and processed with the Arthrex Angel® System (Arthrex, Inc, Naples, FL USA).The PRP samples will be analyzed for cellular components total cell count, cell growth and protein (chemokine/cytokine) analysis. After the blood draw, the volunteers will undergo 1 injection of pegfilgrastim. The 6mg dose will be administered to the volunteers subcutaneously into the thigh. Appendix B, a pegfilgrastim information handout will be given after pegfilgrastim administration.

One week after the pegfilgrastim administration, another 130 mL blood draw will occur by a standard venipuncture on the left or right upper extremity. The blood will be processed with the Arthrex Angel system to generate M-PRP. The M-PRP samples will be analyzed for cellular components total cell count, cell growth and protein (chemokine/cytokine) analysis. The safety of the volunteer will be followed up via a phone call at 7 day and 28 days after mobilized blood collection.

8 Review of Safety

8.1 Adverse Event (AE)

An adverse event is any untoward or unfavorable medical occurrence in the human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in the research, whether considered related to the subject's participation in the research.

8.2 Serious Adverse Event (SAE)

Serious adheres events are any events that:

- Result in death
- Is life threatening, or places the participant at immediate risk of death from the event as it occurred
- Requires or prolongs hospitalization
- Causes persistent or significant disability or incapacity
- Results in congenital anomalies or birth defects
- Is another condition which investigators just to represent significant hazards

8.3 Unanticipated Problem (UP):

Defined by DHHS 45 CFR part 46 as any incident, experience, or outcome that meets the following criteria.

- unexpected, in terms of nature, severity, or frequency, given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the study population.
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);
- suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4 AE & SAE Collection and Reporting

Throughout the study the research team will monitor the occurrence of AE and SAE. Data will be collected if an instance occurs, and the PI will be notified. All AE data, such as onset date, resolution date, outcome and treatments given will be documented in the source documents and will be recorded in the EDC and analyzed for severity to follow reporting protocol if severity level.

Follow-up will occur using the provided safety monitoring form if AE occurs. The follow up will end either when the symptoms resolve or up to 30 days past the end of the study participation.

8.5 Risks and Discomforts:

As with any research involving participants, there is the inherent risk of a breach in patient confidentiality though this will be minimized using participant code numbers and adherence to all HIPAA guidelines. Standard sterile precautions will be utilized for the administration of filgrastim and pegfilgrastim, but with any procedure there is risk of infection, bleeding, and swelling.

Filgrastim is administered subcutaneously via a single prefilled syringe for manual use or for use with the On-body Injector for filgrastim which is co-packaged with a single prefilled syringe.

Anticipated Discomforts of Filgrastim
Aching in the bones and muscles

Possible Serious Adverse Events (SAE) of Filgrastim
Splenic rupture
Serious lung problem called acute respiratory distress syndrome (ARDS).
Serious allergic reaction
Sickle cell crises
Kidney injury (glomerulonephritis).
Capillary leak syndrome.

Additional response to these adverse events can be found in Appendix B.

Anticipated Discomforts of Pegfilgrastim
Aching in the bones and muscles

Possible Serious Adverse Events (SAE) of Pegfilgrastim

Splenic rupture.
Serious lung problem called acute respiratory distress syndrome (ARDS).
Serious allergic reaction.
Glomerulonephritis
Leukocytosis
Thrombocytopenia
Aortitis
Capillary leak syndrome.

Contraindications to the use of Filgrastim and pegfilgrastim include patients with a history of serious allergic reactions to human granulocyte colony-stimulating factors such as Filgrastim or Pegfilgrastim. Most common adverse reactions in patients undergoing peripheral blood progenitor cell mobilization and collection ($\geqslant$ 5% incidence) are bone pain, pyrexia (fever) and headache. Although extremely rare, cases of splenic rupture, acute respiratory distress syndrome, serious allergic reactions, fatal sickle cell crisis, capillary leak syndrome, cutaneous vasculitis, and glomerulonephritis have been reported in patients undergoing treatment with Filgrastim and pegfilgrastim. For this reason, patients will be monitored with a review of symptoms and a physical examination upon each visit, including ascertaining blood pressure and pulse, as well as a lung, heart, and abdomen examination.

The approximate duration of the study will be a 10 week period. During this 10 week period a total of four blood draws will occur with the amount of 130 mLs each draw with a total amount of 520 mL of blood drawn. To review the safety of this practice, a comparison between the acceptable blood donation practice was reviewed. For whole blood donation, one pint (~473.16 mL) of blood is drawn in one sitting with the acceptable inter-response time of 56 days. During the duration of this entire study, a total of 520 mL of blood will be drawn over the 10 week duration, lowering the risk of the blood draws significantly less than the risks with blood draw regimes.

8.6 Safety Monitoring Plan:

To ensure close monitoring and support of the participants due to the use of filgrastim, a safety and data monitoring plan will be implemented. The participant safety monitoring plan will include follow up via a phone call at 7 day and 28 days after mobilized blood collection. At this time, patients will be asked an open-ended question by the research team: “How has your general health been and did you find the study uncomfortable?” Responses will be summarized in Appendix C

"Monitoring Form." Expected side effects include bone pains and muscle aches. Any unexpected severe adverse events will be documented and reported to the PI and the IRB notified.

9 Data Analysis & Management Procedures

All personal information is strictly confidential, and no names will be disclosed except as required by law. All information and data collected during this research will be recorded in spreadsheeting source and within the EDC. Records related to this study will be securely retained in a secure location for a period of 3 years after the completion of the study or longer as required by law. At that time, all records will be properly destroyed.
Laboratory data will include CBC with differentials, characterization of cell content and results from ELISA testing. All data will by organized by an anonymous identifier and will not be linked or identifiable to the study participants.

9.1 Data Collection

Data will be collected using the EDC system. Reports of data will be used by internal site monitors to ensure accuracy of data elements.

9.2 Statistical Analysis

All patient data will be entered into EDC. The investigators will meet at appropriate intervals to evaluate and analyze the data. All compiled data will be de-identified. The numbers of cells will be determined per microliter of total volume collected. Cytokine levels will be determined according to volume collected.

9.3 Statistical Considerations

Calculations of standard metrics including means, ranges, and standard deviations will be performed.

10 Quality Control and Assurance

All protocols will be monitored and analyzed data will be checked for accuracy by the principal investigator and /or a designated AREF research team member. All medical data will be kept in compliance with HIPAA guidelines.

11 Regulatory Requirements

11.1 21 CFR 50- Informed Consent:

In adherence to the 21 CFR 50, Protection of Human Subjects Guidelines, the informed consent process will be performed by one of the study investigators or staff, in the office. No aspects of the study will be conducted prior to obtaining informed consent from each participant. The purpose and methods of the study along with the expected effects will be reviewed with each potential participant. Each participant will be provided a copy of the consent and sufficient time

will be given for the opportunity to read and ask any questions about the study. After signing of the informed consent document, participants will be given a copy for their records.

The designee will review with each participant that they are free to refuse to the study or to withdraw from it at any time.

11.2 Participant Confidentiality:

Signed consent forms for each subject will be deidentified by a coding system with the subject's unique study identification system. Authorization to use each subject's personal health information will be obtained during the informed consent procedure to adhere to the federal Health Insurance Portability and Accountability Act (HIPAA). The consent will specifically grant permission to use health information obtained as part of the presented study.

11.3 Consent Withdrawal:

During the informed consent procedure, participants will be informed that if at any point during the study consent may be withdrawn. To withdraw consent participants can request in writing to withdraw HIPAA authorization and the research site will not use or provide any health information to researchers. At this time, the link between the participant's health information will be severed with the research team. This process for consent withdrawal will be reviewed with each participant and identified barriers will be addressed.

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