Title: Efficacy of bone marrow aspirate concentrate compared with platelet rich plasma for the treatment of symptomatic knee osteoarthritis: A randomized, controlled clinical trial

Principal Investigator: Joshua Hackel, MD

Co-Investigators: Adam Anz, MD

James R. Andrews, MD

Charles E. Leddon, PhD

Nicole Rendos, PhD

Funding Mechanism: Emcyte Corporation

Institution(s): Andrews Research and Education Foundation (AREF)
1020 Gulf Breeze Pkwy.
Gulf Breeze, FL 32561
(850) 916-8790

Andrews Institute
1040 Gulf Breeze Pkwy.
Gulf Breeze, FL 32561
(850) 916- 8790

1 Background / Scientific Rationale

In the United States it is estimated that 47 million people have been diagnosed with arthritis. 4.3 million have been diagnosed with isolated osteoarthritis of the knee and knee arthritis accounts for 20% of disability claims[1]. Prevalence of the disease continues to climb and is expected to double by 2030. In addition 4 out of 5 patients with osteoarthritis have movement limitations[1]. Currently there are no curative treatments for osteoarthritis. Surgical options include arthroscopy, osteotomies, and total joint replacement, but not all patients with knee osteoarthritis are candidates for surgical treatments, and others choose non-operative treatments instead of undergoing a procedure. Non-surgical treatment options include medications such as non-steroidal anti-inflammatory's (NSAIDs), injectables such as corticosteroids and visco-supplementation, or physical treatments such as rehabilitative therapy, unloading braces,

and activity modifications (i.e. canes or walkers). Additionally weight loss can help in the battle to reduce pain in cases of knee osteoarthritis.

The clinical efficacy of hyaluronic acid or corticosteroid injections has been reported, but effects are short lived. This has led to additional injection options such as autologous derived blood products which have been documented to alleviate symptoms related to knee osteoarthritis, with recent comparative studies suggesting a greater, long-lasting effect with these blood derived products like platelet rich plasma (PRP).[2-8] Additionally some studies have suggested an increased positive effect with a combination of medications with PRP.[9-12]

While PRP shows promise in helping restore function to these patients, there are still concerns with PRP's long term outcomes. Another option that has become more popular for physicians treating this debilitation condition is bone marrow aspirate concentrate (BMA), which use's undifferentiated cells found in the bone marrow to promote healing and tissue regeneration.[13] These cells have the ability to replicate into a multiple different tissue types. With BMA, the marrow is concentrated provide better healing of the damaged tissue and aid in growth and repair. The full benefits of BMA are still unknown, but studies have shown the treatment can reduce swelling, relieve pain, and improve healing in articular cartilage and bone grafts.[14]

Autologous BMA has shown promising clinical potential as a therapeutic agent in regenerative medicine, including the treatment of osteoarthritis and cartilage defects,[13-14] and the clinical efficacy platelet rich plasma has been documented to alleviate symptoms related to knee osteoarthritis.[2-8] However, randomized, prospective comparison of the two techniques has not been reported in the literature and long term follow-up for both treatments is limited, and especially limited in the use of BMA for osteoarthritis treatment.

2 Objectives

The main objective of this study is to compare the effectiveness of bone marrow aspirate concentrate (BMC) to platelet rich plasma (PRP) injections for the treatment of knee osteoarthritis in regards to pain and function at multiple time points up to 24 months post injection.

3 Subject Eligibility

Patients between the ages of 18 and 80, with evidence of knee osteoarthritis will be screened for eligibility in the study. All subjects must present with pain or swelling of the knee of at least 4 months in duration and a Kellgren-Lawrence score between 1

and 3 upon x-ray evaluation. Potential subjects must be willing and able to provide informed consent and be willing and able to return for scheduled follow-up visits.

Exclusion criteria include: Patients with major mechanical axis deviation of more than 50% into either compartment (varus or valgus) or that have had a corticosteroid injection within 3 months or a hyaluronic acid injection within 6 months will not be eligible. Patients will be excluded if they have a history of the following medical conditions - diabetes, autoimmune disorders, disorders requiring immunosuppression, rheumatoid arthritis, hemophilic arthropathy, infectious arthritis, Charcot's knee, Paget's disease of the femur or tibia, or history of cancer. In addition anyone with an ongoing infectious disease or significant cardiovascular, renal or hepatic disease will be excluded.

4 Subject Enrollment

120 patients will be recruited through physician practices and with print, flyer or web based advertisements (AREF and EmCYTE websites). Those patients who contact the clinical trials department through the physician's office or by referral from an advertisement or otherwise will be prescreened for adherence to the inclusion and exclusion criteria via phone or in person.

Once the potential participant has been prescreened for inclusion and exclusion criteria, and agreed to be screened for participation in the study they will be instructed on study enrollment protocols which will include a visit with one of the physician investigators for evaluation, x-ray's and confirmation of adherence to the inclusion criteria. Subjects will also be instructed that they must not take any prescription or over the counter non-steroidal anti-inflammatory drugs (NSAIDS) for 3 weeks prior to the evaluation appointment. Following the physician evaluation and confirmation subjects will again have all study requirements explained to them and go through the described informed consent process. The subject will then be randomly placed in one of the two study groups and be scheduled to receive either a PRP injection or BMC injection in their affected knee.

5 Study Design and Procedures

Upon enrollment and completion of the informed consent process, patients will be randomized into the 2 study groups of 60 each, based on a list compiled by a random number generator prior to initiation of the study. Both Groups will complete the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and the Subjective International Knee Documentation Committee Subjective Score (IKDC) questionnaires prior to any treatment. Due to the invasive nature of obtaining marrow aspirate blinding of the subjects and clinicians will not be performed. Group 1 [PRP]: Will receive a single (1) intra-articular injection of PRP. Group 2 [BMC]: Will receive a single (1) intra-articular injection of BMC after obtaining informed consent. Both types of injections and all associated procedures will be performed in the clinic or lab setting with local anesthesia. Following the injection patients will be asked to complete a short questionnaire documenting the BMC or PRP collection, preparation and injection experience. Ten patients will be randomly selected from both (20 total subjects) the PRP and

BMC groups to have analysis performed on the BMC and PRP as described below. This analysis will not be performed on patients not randomly selected. In patients randomly selected after the BMC or PRP have been prepared for injection, a small sample (1 mL) of the BMC or PRP will be separated and sent to an independent lab (Biosciences Research Associates, Inc) for analysis. The PRP will be analyzed with a CBC and the BMC will be analyzed for TNC, human CD34+ hematopoietic stem/progenitor cell assay, CFU-F, plasma free hemoglobin, cell viability percent, and hemolysis percent. Evaluation, a 4 view x-ray series (long leg, lateral, sunrise, and bilateral Rosenberg), and injections will be provided at no charge to the patients. Patients will then be given standard follow-up care instructions that include no use of NSAIDS for at least 7 days and partial weight bearing on the limb for 2-3 days, followed by the initiation of a standard physical therapy program at one week following injection.

After the injection subjects will be required to complete the WOMAC and IKDC questionnaires as well as report any medications they are using via email or phone (paper method will be available on request) at 1, 3, 6, 9, 12, 18, and 24 months following the injection.

PRP protocols

All subjects who will receive a PRP injection will be treated under the normal standards of care for such a treatment. These procedures include drawing of blood from the patient using a standard aseptic technique (step by step instructions found in appendix A - Technique Manual) into a syringe with anticoagulant (see appendix B – MSDS sodium citrate USP). From the syringe of patients randomly selected for analysis 1 mL of blood will be separated and sent to a lab for analysis. The remaining blood in the case of patients randomly selected or all of the blood in those not randomly selected will then be placed into a single concentrating device and placed in a centrifuge for 1.5 minutes at 3800 RPM which will separate the red blood cells from the plasma and platelets (see appendix C – centrifuge Op Manual). The plasma and platelets will then be separated off with a syringe and re-spun for 5 minutes at 3800 RPM in the centrifuge to separate the platelets from the plasma. The platelets will then be separated off for injection into the patient's knee (step by step instructions found in appendix A - Technique Manual). The PRP preparation procedure results in 7 mL of product in patients randomly selected for analysis 1 mL will be separated and sent to a lab for analysis leaving 6 mL for injection. Both the 1 mL of blood and the 1 mL of PRP of patients randomly selected for analysis will be sent to an independent lab (Biosciences Research Associates, Inc) to undergo analysis for CBC. The treating physician will then inject the PRP into the affected knee joint following standard aseptic technique per the physician's normal standard of care. The above mentioned processes will be performed using the EmCyte GenesisCS Component Concentrating System Pure PRP® II-60 mL 2015kit (product number (GS60-PURE-II) (EmCyte Corporation, Fort Myers, Florida) which has been approved by the FDA for use in PRP treatments (see Appendix D – FDA letter). This entire process will be completed in a single patient visit. Any unused portion of a sample in the physician's office will be disposed of through standard biohazard waste disposal systems as required by law.

BMA Protocols

Subjects who receive a BMA injection will again be treated under the normal standards of care for such a treatment. These procedures include a bone marrow harvest from the posterior iliac

crest using a prone and posterior approach. The trocar will be rinsed with 15 mL of heparin (see MSDS heparin). A total of 50 mL of bone marrow will be drawn into a syringe containing 10 mL of anticoagulant (see appendix B – MSDS sodium citrateUSP). The filter will be rinsed with 3 mL of heparin. The bone marrow will then be filtered. From the syringe with filtered bone marrow, for patients randomly selected for analysis, 1 mL of filtered BMA will be separated and sent to a lab for analysis. The remaining bone marrow will be separated into a single concentrating device, counterbalanced and placed in a centrifuge for 2.5 minutes at 3800 RPM for separation of the bone marrow concentrate. The plasma and cell concentrate will then be separated off with a syringe and stopcock, then transferred into the concentrating accessory, counterbalanced and re-spun for 7 minutes at 3800 RPM in the centrifuge to separate the cell concentrate from the plasma. After the plasma is drawn off and swirled to resuspend, the BMC will be drawn into a syringe for injection into the patient's knee (step by step instructions found in appendix A - Technique Manual). The BMC production procedure results in 7 mL of product for patients randomly selected for analysis 1 mL will be separated and sent to a lab for analysis. Both the 1 mL of BMA and the 1 mL of BMC of patients randomly selected for analysis will be sent to an independent lab (Biosciences Research Associates, Inc) to undergo analysis for TNC,human CD34+ hematopoietic stem/progenitor cell assay and CFU-F, plasma free hemoglobin, cell viability percent, and hemolysis percent.. This sample will be packaged in the company's standard sample packaging and shipped per instructions (see appendix F – BSR Quality Evaluation Program Specimen Preparation Kit) for delivery to the lab to undergo verification testing. The treating physician will then inject the BMC into the affected knee joint following standard aseptic technique per the physician's normal standard of care. The above mentioned processes will be performed using the EmCyte GenesisCS Pure BMC®-60 ml 2015kit (product number BC60-PURE) (EmCyte Corporation, Fort Myers, Florida) which has been approved by the FDA for use BMC treatments (see Appendix E – FDA Letter). This entire process will be completed in a single patient visit. Any unused portion of a sample in the physician's office or in the lab will be disposed of through standard biohazard waste disposal systems as required by law.

6 Expected Risks and Benefits

Risks and Discomforts: Potential risks include those expected with any injection including syncope, dizziness, headache, nausea, tachycardia, infection (septic arthritis, phlebitis, and osteomyelitis), bleeding, or pain. Additionally the patient may experience knee stiffness or inflammation associated with the procedures. For BMA harvest, there is the potential for pain, bleeding or infection at the harvest site, as well as the possibility of retroperitoneal hematoma. The filter and the trocar from the bone marrow aspiration kit will be rinsed with heparin. Risks with the use of heparin include hypersensitivity, hemorrhaging, heparin-induced thrombocytopenia (HIT), heparin-induced thrombocytopenia and thrombosis (HITT), and delayed onset HIT and HITT. As with any research involving patients there is the inherent risk of a breach in patient confidentiality though this will be minimized through the use of subject code numbers and adherence to all HIPAA guidelines.

Benefits: Direct benefits of the study include a physician visit, x-rays and an injection of BMA and/or PRP at no cost, as well as potential pain relief, improvement of knee function, and improvement of quality of life, which all could lead to the avoidance of surgical intervention. It is also believed that the information obtained in this study will help advance treatment of osteoarthritis through the use of regenerative medicine.

7 Data Management Procedures

All personal information is strictly confidential and no names will be disclosed except as required by law. All research information will be managed and accessible by only the named investigators and their medical staff. Data sheets will have names and identifying information replaced with a subject code to protect the confidentiality of the subject. All information provided by the subject during this project will be recorded on the appropriate forms and stored in a locked room in the AREF research facility. In addition all subject data forms will be scanned and stored, along with summary information forms, spreadsheets, and photos, in a secure password protected folder on a laptop in which only study investigators will have access to, and will be permanently deleted following any and all publications or presentations have been completed related to this research. All records related to this research will be retained in a secure location for a period of 3 years following the completion of all study related activities, at which time they will be properly destroyed.

8 Data Analysis

All data will be entered into an excel spreadsheet for analysis that will include obtaining standard measures such as means and standard deviation. Data will be analyzed as scores on the two questionnaires as well as across subsets of the individual questionnaires for function, quality of life and pain scores. Additional determinations based on medication usage and failure rates will be examined as well.

9 Statistical Considerations

A power analysis was performed to determine sample size based upon recent clinical trials comparing PRP to HA for the treatment of osteoarthritis. Analysis utilized the average of standard deviation to detect average of group mean effect at 80% power. This determined that 25 patients per group would be needed to reach statistical significance with the WOMAC and that 50 patients would be needed per group for the IKDC. Considering a potential dropout rate of 20%, 60 patients per group was chosen as the sample size.

Data will be analyzed for differences between groups using a repeated measures ANOVA design, using a significant p-value of < 0.05 for rejecting the null hypothesis. Additional subjective analysis will be performed on data involving the patient experience with regards to the injection.

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

Informed Consent:

The informed consent process will be performed by a study coordinator from the Andrews Research and Education Foundation. All subjects will have the study described to them and will be given as much time as they would like to read an approved, stamped version of the informed consent document. After signing the informed consent document, they will be given a copy for their records.

Subject Confidentiality:

Subject confidentiality information is listed above in #7 (Data Management Procedures). All medical data will be recorded and stored in compliance with HIPAA guidelines.

12 References

1. Hootman JM, Helmick CG. Projections of US prevalence of arthritis and associated activity limitations. Arthritis Rheum 2006; 54: 226-9.

2. Spakova T, Rosocha J, Lacko M, Harvanova D, Gharaibeh A. Treatment of Knee Joint Osteoarthritis with Autologous Platelet-Rich Plasma in Comparison with Hyaluronic Acid. American Journal of Physical Medicine & Rehabilitation. May 2012;91(5):411-417.

3. Kon E, Mandelbaum B, Buda R, et al. Platelet-Rich Plasma Intra-Articular Injection Versus Hyaluronic Acid Viscosupplementation as Treatments for Cartilage Pathology: From Early Degeneration to Osteoarthritis. Arthroscopy-the Journal of Arthroscopic and Related Surgery. Nov 2011;27(11):1490-1501.

4. Cerza F, Carni S, Carcangiu A, et al. Comparison Between Hyaluronic Acid and Platelet- Rich Plasma, Intra-articular Infiltration in the Treatment of Gonarthrosis. American Journal of Sports Medicine. Dec 2012;40(12):2822-2827.

5. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. Am J Sports Med. 2013;41(2):356-364.

6. Li M, Zhang C, Ai Z, Yuan T, Feng Y, Jia W. Therapeutic effectiveness of intra- knee- articular injection of platelet-rich plasma on knee articular cartilage degeneration. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi (Chinese Journal of Reparative and Reconstructive Surgery). Oct 2011;25(10):1192-1196.

7. Filardo G, Kon E, Di Martino A, et al. Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial. Bmc Musculoskeletal Disorders. Nov 2012;13.

8. Vaquerizo V, Plasenicia M, Arribas I, et al. Comparison of intra-articular injections of plasma rich in growth factors (PRGF-Endoret) versus Durolane

hyaluronic acid in the treatment of patients with symptomatic osteoarthritis: a randomized controlled trial. Arthroscopy 2013;29(10):1635-1643.

9. Convery P, Milligan K, Quinn P, et al. Low-dose intra-articular ketorolac for pain relief following arthroscopy of the knee joint. Anaesthesia. 1998 Nov 53(11):1125-1129.

10. NG H, Nordstrom U, Axelsson K, et al. Efficacy of intra-articular bupivacaine, ropivacaine, or a combination of ropivacaine, morphine, and ketolorac on posteroperative pain relief after ambulatory arthroscopic knee surgery: a randomized double-blinded study. Regional Anesthesia and Pain Medicine. 2006 Jan;31(1):26-33.

11. Gupta A, Axelsson K, Allvin R, et al. Postoperative pain following knee arthroscopy: the effects of intra-articular ketorolac and/or morphine. Regional Anesthesia and Pain Medicine. 1999 May;24(3)225-230.

12. Beitzel K, McCarthy MB, Cote MP, et al. The effect of ketorolac tromethamine, methylprednisolone, and platelet-rich plasma on human chondrocyte and tenocyte viability. Arthroscopy. 2013 Jul;29(7):1164-74

13. Hauser RA, Orlosfsky A. Regenerative injection therapy with whole bone marrow aspirate for degenerative joint disease: a case series. Clin Med Insights Arthritis Musculoskelet Disord. 2013 Sep 4;6:65-72.

14. Saw KY, Hussin P, Loke SC, Azam M, Chen HC, Tay YG, Low S, Wallin KL, Ragavanaidu K: Articular cartilage regeneration with autologous marrow aspirate and hyaluronic acid: an experimental study in a goat model. Arthroscopy 2009;25(12)1391-1400.