Investigation of mesenchymal stem cell therapy for the treatment of osteoarthritis of the knee: Effect on pain and quality of life

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1.0 – INTRODUCTION

1.1 – Background

Arthritis has been identified as one of the leading causes of disability in the United States.[1] Osteoarthritis, the most common form of arthritis[2], is most prevalent in the knee[3] and the lifetime risk of symptomatic knee osteoarthritis (KOA) is nearly 45%.[4] Osteoarthritis affects greater than 50% of individuals over the age of 65, and the increasing life expectancy will continue to rapidly increase the cases of KOA and subsequent substantial economic burden to society, payers, and patients.[5] Different non-surgical treatment options exist in the symptomatic management of KOA, yet KOA is a chronic and progressive disease state and the portfolio of non-surgical treatment options available have notable limitations.

Conservative treatment options such as self-management programs, physical exercise, and weight loss are recommended as a first option to address KOA.[6] However, available conservative treatment modalities for KOA merely ameliorate symptoms, and convey variable and often short-term efficacy.[7-8] The pain associated with KOA is most often treated with non-steroidal anti-inflammatory drugs (NSAIDs), which have come under intense scrutiny with respect to their safety in patients with cardiovascular disease as NSAIDs can increase the risk of having a heart attack or stroke.[9] Since nearly one in two Americans with heart disease also have arthritis,[10] there is a necessity to apply non-drug therapies in the treatment of KOA.

Intraarticular therapies including hyaluronic acid (i.e., viscosupplementation) and corticosteroid injections are commonly used in the treatment of KOA.[11-12] Available evidence demonstrates symptom reduction with hyaluronic acid and corticosteroid injections in the short term[13-22]; however, evidence does not consistently support long term improvement nor prevention or reversal of the disease state.[13-16,18,21]

Several surgical techniques are used to address cartilage and joint surface lesions but limitations exists. Autologous chondrocyte transplantation and microfracture techniques, which are used to repair isolated lesions, are not applicable to a more widespread disease state such as KOA.[7,20,23] Additionally, arthroscopic procedures involving lavage or debridement of the joint surfaces are not recommended with a primary diagnosis of KOA.[6]

The accepted option for KOA when previously highlighted options fail is total knee arthroplasty (TKA). Considering the aging demographic in the United States, the demand for primary TKA is expected to increase 673% by 2030, with associated costs increasing exponentially.[8,24] This prognostication may reflect the lack of effective options available to prevent or reverse the progression of KOA. The growing costs[25] added to the risk of significant complications associated with TKA have created a staggering need for a lower risk and lower cost, non-surgical approach.[26]

The field of orthobiologic therapy, specifically mesenchymal stem cell (MSC) therapy, holds promise in the non-surgical treatment of chronic degenerative disease such as osteoarthritis.[7] Currently the most widely used method of obtaining MSCs is by way of bone marrow aspirate concentrate (BMAC). Published human data demonstrates no significant adverse events related to injection of MSCs or other regenerative biologic therapies into osteoarthritic human knee joints.[27-28] Available research suggests MSC injection demonstrates benefits in pain and function at 2 years, and may possibly change the course of the disease, slowing progression or even regenerating cartilage to a small degree.[8,29-31] In addition, the conceptual advantage and early success of using a platelet-rich plasma (PRP) injection as a complement

therapy has been documented.[7,32-35] Despite early promise, further evidence is required in order to determine if MSC injection in patients with KOA can fill a void in effective non-surgical management of KOA with the intention of delaying or preventing the need for surgical management.

To our knowledge this is the first randomized controlled trial to determine the effect of non-surgical, autologous BMAC and PRP intraarticular injections for primary KOA with comparison to a control group receiving a current and common injectable treatment.

1.2 – Specific Aims/Rationale

The purpose of this study is to determine the clinical response to autologous BMAC and PRP injections for KOA with respect to pain, function, and quality of life at up to 1 year following the intervention. This is a prospective randomized controlled trial with the following aims:

> **Specific Aim 1:** To determine if BMAC and PRP treatment of KOA improves Knee injury and Osteoarthritis Outcome Score (KOOS) subscales for patients measuring pain, function, and quality of life at 3 months, 6 months, and 12 months post treatment compared to Gel-One® hyaluronate injections and relative to baseline.
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> A change in 10 points has been suggested[36] and utilized[37] to demonstrate a minimal clinically important difference (i.e., decline or improvement) in each KOOS subscale. In this study, we expect to see a mean improvement of greater than 10 points at 3 months post treatment in the patients receiving BMAC and PRP injections as well as those receiving the Gel-One® hyaluronate injection. However, we expect to see greater improvement in favor of the group receiving the BMAC and PRP treatment at 6 months and 12 months post treatment.
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> **Specific Aim 2a:** To determine if BMAC and PRP treatment of KOA improves pain scores measured by the Numeric Pain Rating Scale (NPRS) at 3 months, 6 months, and 12 months post treatment compared to Gel-One® hyaluronate injections and relative to baseline.
>
> **Specific Aim 2b:** To determine if BMAC and PRP treatment of KOA improves Patient Reported Outcome Measurement Information System (PROMIS) Global Health scores (short form, version 1.1) at 3 months, 6 months, and 12 months post treatment compared to Gel-One® hyaluronate injections and relative to baseline.

2.0 – METHODS

2.1 – Study Population

The study population will consist of patients presenting to McConnell Spine, Sport, and Joint Physicians, which is focused on conservative, non-surgical solutions for orthopedic issues. The clinic is located in a diverse and large geographic area.

All participating patients will be patients of the McConnell Spine, Sport, and Joint Physicians. Patients participating in the study may be patients of the study physician, referred from an outside practice to the

study physician, or referred from other physicians of the McConnell Spine, Sport, and Joint Physicians to the study physician.

The study population will consist of patients who have been diagnosed with KOA (i.e., ICD 10 diagnosis code M17) and have failed initial conservative treatment options. If the patient has bilateral KOA the patient would be excluded unless the patient had only unilateral symptoms. The study will not include patients who have end-stage KOA (i.e., Kellgren-Lawrence grade 4) for which surgical intervention would be more appropriate. Determining a Kellgren-Lawrence grade is a standard of care for diagnosing KOA and is done by reading a standing radiograph of the knee.

The clinic completes an average of approximately 630 office visits per month, 25% of which is related to the care of osteoarthritis. We anticipate that 50% of patients with KOA will satisfy inclusion and exclusion criteria and 80% of eligible patients will be willing to participate in the study. We anticipate enrollment of four patients per month over a maximum enrollment period of 16 months from the start date.

Number of Subjects to be included in the study: 45

We expect that up to 1 of every 3 patients consented will need to be withdrawn prior to the treatment intervention. Withdrawal criteria include use of NSAIDs or corticosteroids within 2 weeks prior to the treatment intervention date, or if a patient's platelet count is found to be <150,000 platelets per microliter of blood or >450,000 platelets per microliter of blood. Withdrawal criteria will be further discussed in section 2.4. We will apply the treatment intervention to only 30 total patients (i.e., n = 15 per treatment group); however, we anticipate consenting up to 45 patients based on possibility of withdrawal from the study. We will discontinue screening and consenting of patients if 30 patients have undergone one of the two treatment interventions.

As attrition following the treatment intervention is only expected to be due to a patient undergoing TKA prior to the 1 year follow-up or due to unforeseen illness, we do not anticipate this having an effect on our population. To further ensure visit compliance, compensation (i.e., a $25 gift card) will be offered with completion of the 3, 6, and 12 month research follow-up visits.

Proposed Study Start Date: October 1, 2016

Proposed Study End Date: April 1, 2019

Inclusion Criteria:

1) Male and female patients 40 to 70 years old
2) Diagnosed with KOA based on the American College of Rheumatology criteria including symptomatic reports and radiographic findings
3) Kellgren-Lawrence grade 1-3 based on a radiograph within 6 months of presentation to the clinic
4) Symptomatic evidence of tibiofemoral osteoarthritis for ≥6 months
5) Average numeric pain rating of 4 – 8 on a scale of zero to 10 (defined as moderate level) over the past week

6) Previous trial of 6 weeks minimum of conservative therapy including physical therapy, weight loss, anti-inflammatory medication, or injection therapy

Exclusion Criteria

1) Grade 4 KOA according to the Kellgren-Lawrence scale
2) History of intraarticular viscosupplementation or steroid injection in the target knee in the past 6 months at the time of the baseline visit or intraarticular injection planned during the trial
3) History of arthroscopic surgery in the target knee in the past 12 months at the time of presentation to the clinic or planned surgery during the trial period (e.g., scheduled for/awaiting arthroscopy or a knee replacement procedure)
4) Bilateral KOA (unless the contralateral knee involvement is limited to radiographic osteoarthritis and not symptomatic)
5) Ipsilateral (same side) or contralateral (opposite side) symptomatic osteoarthritis of hip or ankle
6) Clinically apparent tense effusion or other acute inflammation of the target knee at the time of presentation to the clinic
7) Active infection of either lower extremity such as cellulitis or any skin disease or infection in the area where BMAC is aspirated, blood is drawn, or an injection is given
8) History of diagnosis of any of the following: 1) septic osteoarthritis of any joint, 2) inflammatory arthropathy such as rheumatoid arthritis, gout, pseudogout, lupus, crystalline arthropathy, chondrocalcinosis and other rheumatology diagnoses
9) Cruciate/collateral knee ligament instability, ligament laxity, or meniscal instability of the target knee
10) Significant alignment deformity such as varus/valgus of the target knee in the judgment of the investigator
11) Currently pregnant, nursing, or planning to become pregnant during the trial period
12) Previous or known allergic reaction or hypersensitivity to heparin; sodium citrate; hyaluronan products or specifically Gel-One®; cinnamon; bird products such as feathers, eggs, or poultry; avian proteins
13) Not suitable for BMAC tissue allograft injection per physician (e.g., blood dyscrasia)
14) Unable to be prescribed stable dose of NSAIDs and/or tramadol based on medical history as ad lib use of OTC analgesics will be allowed in both groups after treatment
15) Current cigarette smoker
16) Unable to give informed consent
17) Non-English speaking

2.2 – Hypotheses

Hypothesis 1: We hypothesize that patients that are treated with BMAC and PRP will have improved KOOS subscale scores at 6 months and 12 months post treatment compared to patients treated with Gel-One® hyaluronate injections and relative to baseline. We hypothesize that both patients treated with BMAC and PRP and patients treated with Gel-One® will have improved KOOS subscale scores at 3 months post treatment relative to baseline.

A sample size of 30 total patients undergoing the treatment intervention has been established based on the power calculation ($\alpha = 0.05$, $\beta = 0.20$) of a previous study comparing allogeneic bone marrow MSCs to hyaluronic acid in 30 patients with knee osteoarthritis[30] and a preceding pilot study.[38] The primary outcome tool used in the study by Vega et al. has been demonstrated to have similar effect sizes at 6 and 12 months and similar smallest detectable differences compared to the KOOS scale in two different populations with knee-related deficits.[39,40] A post-hoc power analysis will be completed, if necessary, to evaluate current findings and potentially plan for future studies with this specific study population.

Hypothesis 2a: We hypothesize that patients that are treated with BMAC and PRP will have improved NPRS scores at 3 months, 6 months, and 12 months post treatment compared to patients treated with Gel-One® hyaluronate injections and relative to baseline.

Hypothesis 2b: We hypothesize that patients that are treated with BMAC and PRP will have improved PROMIS Global Health scores at 3 months, 6 months, and 12 months post treatment compared to patients treated with Gel-One® hyaluronate injections and relative to baseline.

2.3 – Study Variables & Outcomes of Interest

Treatment Groups:

- N = 30 patients that will undergo treatment
- Group A: BMAC and PRP injections to target knee (n=15)
- Group B: Gel-One® hyaluronate injection to target knee (n=15)

The following data points will be collected for patients who choose to participate in the study at McConnell Spine, Sport, and Joint Center (see Table 1). Patient data will be collected via the electronic medical record at the study physician's practice, as well as by means of study specific data collection forms (see Appendices). Only the study staff will have access to the paper data collection forms and the Research Electronic Data Capture (REDCap) database created solely for use in this study. Exported REDCap patient data will be de-identified upon study completion, prior to statistical analysis using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Demographics:

- Age, gender, and body mass index (BMI)

Relevant clinical information or past medical history:

- Relevant past medical history related to the involved knee including previous surgical procedures, previous intraarticular injections, other treatments, and course of knee pain
- Kellgren-Lawrence grade of KOA via standing radiograph of the target knee
- Platelet count via complete blood count (Group A only)

Other relevant therapies received following the study treatment:

- Therapies received for the target knee including use of over the counter medications (i.e., Advil, Motrin, or Tylenol), prescription anti-inflammatories or NSAIDs (i.e., Mobic, Naprosyn, Celebrex, or Voltaren), narcotics (i.e., Tramadol, Percocet, Vicodin, or others), or physical therapy

Primary outcome measures:

- Change in KOOS subscale scores which will objectively measure pain, function, and quality of life indicators at 3 months, 6 months and 12 months post treatment (see appendix 4.1 for KOOS scale)

  Mean values for the KOOS subscales have been studied in a population-based sample (i.e., not specific to whether those surveyed actually had knee complaints).[41] The population-based sample of subscale scores (i.e., range of 0-100 with a score of 100 representing the best possible score) in the approximate age group of this study ranged from 61 to 89.5, which also varies by gender.[41] A change in 10 points has been suggested[36] and utilized[37] to demonstrate a minimal clinically important difference (i.e., decline or improvement) in each KOOS subscale.

Secondary outcomes measures:

- Change in pain scores as measured by the NPRS at 3 months, 6 months, and 12 months post treatment (see appendix 4.2 for NPRS scale)

  A two-point change has been established as the minimal clinically important difference as measured by the NPRS.[42] Each patient will have an average pain rating of 4-8 on a 0-10 scale over the past week prior to completion of baseline information to be included in the study.

- Change in the PROMIS Global Health scale (short form, version 1.1) at 3 months, 6 months, and 12 months post treatment (see appendix 4.3 for PROMIS Global Health scale short form, version 1.1)

  The PROMIS Global Health scale (short form, version 1.1) will be useful to describe changes in the study population. The scale has been described as a useful summary of physical and mental health in clinical studies with patient-reported outcomes.[43]

Study Variables Table:

Table 1. Study Variables.

Category	Data points
Demographics	1. Age 2. Gender (0-male, 1-female) 3. BMI
Relevant clinical information or past medical history	1. Previous surgery on target knee (0-no, 1-yes) 2. Previous injection on target knee (0-no, 1-yes) 3. Previous physical therapy for target knee (0-no, 1-yes) 4. Onset/course of target knee pain (0-traumatic, 1- insidious) 5. Kellgren-Lawrence grade (0-grade 1, 1-grade 2, 2-grade 3) 6. Platelet count -Group A only (in thousands per ml of blood)
Other relevant therapies received following the study treatment	1. Pain medications for the target knee (0-no, 1-yes) 2. Over the counter medications for the target knee (0-no, 1-yes as needed, 2-yes daily) 3. Prescription anti-inflammatories or NSAIDs for the target knee (0-no, 1-yes as needed, 2-yes daily)

	4. Narcotics for the target knee (0-no, 1-yes as needed, 2-yes daily) 5. Physical therapy for the target knee (0-no, 1-yes)
Primary outcome measures	1. KOOS pain subscale 2. KOOS symptoms subscale 3. KOOS ADL subscale 4. KOOS sport and recreation subscale 5. KOOS quality of life subscale
Secondary outcome measures	1. NPRS 2. PROMIS Global Health scale score

2.4 – Study Design

Overall Design

This study will be a prospective, 1:1 randomized, controlled trial. The two treatment groups (Group A: BMAC and PRP injections and Group B: Gel-One® hyaluronate injection) will be compared to one another.

A study calendar is included within section 2.4 or can be found in Appendix 4.5.

Patient Identification Procedures

Potential patients can contact the clinic to be screened and will present to the clinic as appropriate. Patients may learn of the clinical trial through routine informational postings in the McConnell Heart Health Center monthly newsletter. Other potential study subjects will be identified by their presentation to the clinic. The physician or study staff will then screen the patient that has presented to the clinic to ensure that the patient satisfies all inclusion and exclusion criteria prior to introducing the trial to the patient.

Consent Process

Patients presenting to the clinic who satisfy inclusion and exclusion criteria will be introduced to the study by the physician or study staff. After the study has been thoroughly explained, the patient has had his/her questions answered, and the patient has agreed to participate in the study, informed consent will be obtained by the study staff.

Patients will be consented at the beginning of their baseline visit. Patients will be given the consent form by either the study physician or a research team member. The patient will be given appropriate time to read through the consent form and have any questions answered. An explanation will be provided to patients along with written documentation regarding their rights as a research patient and how to withdraw authorization for participation in this study. Patients will receive a copy of their consent form for their records. Next, the consented patients will complete the baseline primary and secondary outcome tools, as well as a form asking for relevant past medical history regarding the patient's knee (see Appendix 4.4). Baseline demographic information (i.e., gender, age, body weight, and height) and clinical information (i.e., Kellgren-Lawrence grade) will be obtained from the patient's medical record. The patients will complete the baseline forms with either the study physician or a research team member. Informed consent and baseline procedures will be completed within 14 days of a patient presenting to the clinic and

verbalizing interest in the study. However, informed consent and baseline procedures may be performed on the day of the patient's initial presentation to the clinic.

Randomization

Patients completing informed consent will be randomized in a 1:1 single-blinded fashion. The randomization allocation schedule will be developed by the research team member performing the statistical analyses and will not be shared with the remainder of the research team. Following the completion of informed consent and collection of baseline information for each patient, the research team member responsible for scheduling the treatment procedure will be made aware of group allocation. This research team member will assign a random number to each patient and independently maintain a key to be able to link the patient to the data collected as well as identify the group membership of each patient. This research team member will not be involved in the collection of 3 month, 6 month, or 12 month study variables. The designated research team member who will collect study variables at 3, 6, and 12 months will remain blinded to group allocation throughout the duration of the study. The primary investigator (i.e., the physician providing the treatment) will not be blinded to group allocation as knowledge of group allocation will be essential to deliver two distinctly different treatment procedures and to provide the participant with an explanation of the clinical procedure prior to initiating the treatment. However, the primary investigator will be unaware of the randomization allocation schedule. Randomization will be blocked to prevent selection and accidental bias while ensuring equal allocation to each treatment group.

Study Treatment/Interventions

After randomization, the patient will be scheduled for the treatment intervention within the next 30 days after the completion of informed consent and baseline procedures. All patients will be restricted from use of NSAIDs or oral corticosteroids for 2 weeks prior to the planned treatment procedure.

At the baseline visit, patients randomized to Group A (BMAC and PRP injections to target knee) will have venous blood sampled by the clinic staff in order to determine the patient's platelet count via a complete blood count (CBC) in the lab based on established procedures. Specifically, the venous blood will be drawn in a lavender-top ethylenediamine tetraacetic acid tube. The sample will be mixed by gently inverting 8 times. The sample will be sent to OhioHealth Laboratory Services and be received within 24 hours at ambient temperature.

Following the baseline procedures and prior to treatment intervention, patients will be withdrawn from the study if one of the following occur:

1) a patient uses NSAIDs or corticosteroids within 2 weeks prior to the treatment intervention date, or
2) a patient's platelet count is found to be <150,000 platelets per microliter of blood or >450,000 platelets per microliter of blood.

If a patient's platelet count is <150,000 platelets per microliter of blood or >450,000 platelets per microliter of blood, the patient will be referred appropriately for proper medical follow-up. If a patient is withdrawn prior to the treatment intervention for any of the reasons outlined above, we

will continue enrollment of patients until we have reached a total of 30 patients (i.e., 15 patients per group).

Within 30 days after the completion of informed consent and baseline procedures, the patient will return for appropriate treatment based on group allocation. The treatment procedure for each group is outlined below.

Procedures:

Group A: (BMAC and PRP injections to target knee)

For BMAC preparation, 60 ml of bone marrow will be aspirated from the posterior superior iliac crest by the study physician and handled by the clinic staff utilizing a BC60 PURE BMC® Concentrating System 60mL single use disposable kit (EmCyte Corporation, Fort Meyers, FL, USA). During this procedure, the patient will lie prone. The bone marrow will be aspirated through a single site portal using a T handle Jamshidi™ bone aspiration needle (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Ultrasound will be used to localize the site of aspiration. The limited aspiration will be performed under a local anesthetic. No conscious sedation will be used. The bone marrow will be transferred to the Executive Series Centrifuge II (EmCyte Corporation, Fort Meyers, FL, USA) for centrifugation and resulting bone marrow cell concentration, an approximately 25-minute process. The concentration process will yield approximately 5-6 ml of stromal fluid to be used for injection, under ultrasound guidance, into the subject's target knee by the study physician. Immediately following the injection of the concentrated cells, 4-5 ml of previously separated PRP will be introduced under ultrasound guidance to the subject's target knee (preparation described below) by the study physician.

For PRP preparation, 60 ml of venous blood will be withdrawn from either upper extremity by the appropriate clinical staff. The PRP will be handled using a AB60 Pure, Accellerated Biologics 60mL PURE Concentrating System single use disposable kit (Accelerated Biologics, Tequesta, FL, USA).

Ultrasound guided injection procedures:
Sterile probe preparation will be used in addition to routine sterile techniques using chlorhexadine cleansing solution and aseptic technique. All blood handling, harvesting and interventions will be done using strict aseptic protocol.

Group B: (Gel-One® hyaluronate injection to target knee)

Patients will receive a single injection of Gel-One® (Zimmer, Inc., Warsaw, IN, USA) into the target knee. Injections will be performed by the study physician under real-time dynamic ultrasound guidance. Pre-injection aspirations will be carried out if clinically indicated, however, corticosteroids will not be introduced at any time during the trial.

After identifying anatomical landmarks and marking the injection site, the area will be carefully prepared with a chlorhexidine solution, and allowed to dry. Landmarks will then be re-established. The prepared area will be swabbed with alcohol. Skin will be anesthetized with a

vapo-coolant spray for 3-5 seconds. Immediately after evaporation, a needle will be advanced to the target area and safe placement will be achieved with real time ultrasound guidance. Introduction of the injectate (3 ml syringe of Gel-One® - 1% solution [10 mg/mL], 30mg total hyaluronan) will follow without significant resistance or complaints of pain by the patient. The needle will then be withdrawn, the area cleaned, and a band-aid applied.

Following the treatment based on group allocation, all patients regardless of group allocation will be provided and/or will complete the following:

1. A handout will be provided with instructions to follow and tips of what to expect prior to the 2 week visit follow-up with the study physician (see Appendix 4.6).

2. All patients will complete a visit with the study physician to monitor response to joint injection at 2 weeks (+/- 7 days) and 3 months (+/- 7 days) following the treatment date. At the 3 month visit, all patients will also complete the primary and secondary outcome tools with the study staff.

3. All patients will complete a follow up visit at approximately 6 months (+/- 14 days) following the date of the treatment provided for completion of the primary and secondary outcome tools.

4. All patients will complete a follow up visit at approximately 12 months (+/- 14 days) following the date of the treatment provided for completion of the primary and secondary outcome tools.

Data Collection

Time point	**Measurement**	
Baseline ≤14d from presentation or at presentation	1.Demographic information including gender, age, and BMI 2.Relevant past medical history related to the involved knee including previous surgical procedures, previous intraarticular injections, other treatments, and course of knee pain (i.e., primary osteoarthritis or from previous traumatic injury) 3. Kellgren-Lawrence grade 4. Platelet count -Group A only 5. KOOS subscales 6. NPRS 7. PROMIS Global Health (short form, version 1.1) scale	
RANDOMIZATION – 15 patients per group		
Treatment Delivered ≤30d from Baseline	Group A (n=15) Single episode of BMAC and PRP injections to the target knee	Group B (n=15) Single injection of Gel-One® hyaluronate to the target knee
3 months (+/- 7 days) after treatment	1. KOOS subscales 2. NPRS 3. PROMIS Global Health scale 4. Other relevant therapies received following the study treatment	
6 months (+/- 14 days) after treatment	1. KOOS subscales 2. NPRS 3. PROMIS Global Health scale	

	4. Other relevant therapies received following the study treatment
12 months (+/- 14 days) after treatment	1. KOOS subscales 2. NPRS 3. PROMIS Global Health scale 4. Other relevant therapies received following the study treatment

Risks:

Risks are typical for bone marrow aspiration, blood draw, and intraarticular knee injection and include the following:

Common or likely for blood draw in Group A (participants who will receive BMAC and PRP injections to the target knee):

- Momentary discomfort at the site of the blood draw
- Bruising, redness, swelling, or bleeding at the site of the blood draw
- Feeling of lightheadedness when the blood is drawn

Rare for blood draw in Group A (participants who will receive BMAC and PRP injections to the target knee):

- Infection at the site of the blood draw

Rare for bone marrow aspiration in Group A (participants who will receive BMAC and PRP injections to the target knee):

- Excessive bleeding, particularly in people with low numbers of a certain type of blood cell (platelets)
- Infection, especially in people with weakened immune systems
- Long-lasting discomfort at the site
- Penetration of the internal organs if the aspiration needle is pushed too deeply into and through the hip bone

Common or likely for Group A (BMAC and PRP injections to the target knee):

- Increased temporary pain at the collection and/or injection site
- Swelling, redness, warmth, stiffness, and/or bruising at the collection and/or injection site

Rare for Group A (BMAC and PRP injections to the target knee):

- Bleeding, infection, reaction to local anesthetic, hypersensitivity or allergic reaction to heparin or anticoagulant sodium citrate

Common or likely for Group B (Gel-One® hyaluronate injection to target knee):

- Increased temporary pain at the injection site
- Swelling, redness, warmth, stiffness, and/or bruising at the injection site
- Hip or knee pain

Less common but serious for Group B (Gel-One® hyaluronate injection to target knee):

- Increased swelling to the knee requiring joint aspiration (removal of fluid)

- Increased pain to the knee requiring an intraarticular steroid injection
- Worsening arthritis related to the injection
- Upper respiratory tract infection

Rare for Group B (Gel-One® hyaluronate injection to target knee):

- Bleeding; infection; muscular weakness; spasms; dizziness; rash; itching; back pain; headache; migraine; high blood pressure; burning sensation; gait disturbance; increased blood liver enzyme, alkaline phosphatase, alanine aminotransferase, urea, and increased or decreased white blood cell count
- Reaction to local anesthetic,
- Allergic/non-allergic reaction to Gel-One® accompanied by cold sweat, paleness, or low blood pressure
- Need for arthroscopy (surgical inspection) of the knee related to the injection

Safety Endpoints:

Patients who experience a significant complication from bone marrow harvest, blood draw, or injection therapy including infection, synovitis with effusion, or excessive pain, will be treated with the appropriate medical intervention. If a patient's platelet count is found to be <150,000 platelets per microliter of blood or >450,000 platelets per microliter of blood after the baseline visit, the patient will be withdrawn from the study and referred appropriately for proper medical follow-up.

Safety Reporting Plan:

The study team or study physician will be responsible for gathering information related to adverse events. The PI will review, evaluate, and monitor AE/SAEs.

Non-serious adverse events that are considered to be related or possibly related to the research will be reported annually upon IRB renewal. Unanticipated problems that are not adverse events, protocol violations, or enrollment exceptions will be reported within 5 business days from the time the study team received knowledge of the event.

Some adverse events will be expected including those listed as "common or likely", "less common", or "rare" for each treatment group. Other adverse events that are unexpected and are possibly related to the study treatment will be reported within 5 business days of notification of the event. Adverse events that are found to be occurring at a higher rate than expected will be reported within 5 business days of noting a pattern of occurrence. Adverse events that are considered serious, unexpected, and possibly related to the study treatment (i.e., SAEs) will be reported within 1 business day of the notification of the event. SAEs will be reported to the OhioHealth IRB via e-mail reporting using the Adverse Event Template Form, per institutional and national guidelines. SAEs will be tracked for 90 days following notification via chart review.

Recording of Adverse Events:

At each contact with the subject following the treatment intervention, the study team will seek information on adverse events by patient completion of an Adverse Event Collection Form (see Appendix 4.7) and, as appropriate, by examination. Information on all adverse events will be

tracked throughout study participation using the collection form. All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results will be recorded.

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the probable cause.

When an adverse event has been identified, the study team will take appropriate action necessary to protect the study participant and then complete the necessary paperwork and/or log. The primary investigator will evaluate the event and determine the necessary follow-up and reporting required.

Surveys/Questionnaires

As described previously, the patients' knee pain, function, and quality of life measures will be assessed via standardized survey tools (i.e., KOOS subscales, NPRS, and PROMIS Global Health scale) at baseline, 3 months, 6 months, and 12 months. The KOOS subscales (i.e., pain, symptoms, activities of daily living, function in sports and recreation, and knee related quality of life) combine for 42 questions in a 5 point rating scale format. The NPRS is a semantic differential scale ranging from zero (i.e., no pain) to ten (i.e., worst imaginable pain). The PROMIS Global Health scale (short form, version 1.1) is a five point rating scale consisting of 10 questions. The patient will independently complete each clinical scale once comprehension is assured. The research team member administering the clinical scales will be available to answer patient questions regarding completing the scale. This research team member will be unaware of group allocation.

Study Calendar

	Study Calendar							
	Presentation	**Baseline** ≤14d from presentation or at presentation	**periodWithdrawal**	**Treatment** ≤30d from Baseline	**2-Weeks after treatment** +/- 7d	**3-Months after treatment** +/- 7d	**6-Months after treatment** +/- 14d	**12-Months after treatment** +/- 14d
Screen for eligibility	x							
Informed consent		x						
Demographics		x						
Past Medical History		x						
KOOS subscales		x				x	x	x
NPRS		x				x	x	x
PROMIS		x				x	x	x
Other relevant						x	x	x

therapies								
Randomization		x						
CBC (Group A)		x						
Withdrawal[a]			x					
Treatment				x				
Visits	x	x		x	x	x	x	x
SAE Evaluation					x	x	x	x

[a]NSAIDs or oral corticosteroids during the 2 weeks leading up to the treatment visit or platelet count <150,000 per microliter of blood or >450,000 per microliter of blood

Data Storage and Confidentiality

Data will be collected on paper and entered by a research team member assigned to the study who is blinded to group allocation. All paper forms will be identified by the random number assigned to each patient except the informed consent form which will contain the patient's name as required. The data forms will be stored by the primary investigator or a research team member in a locked cabinet in an on-site office. REDCap (developed and first deployed at Vanderbilt University) will be used for data entry, and then the database will be pulled into SAS 9.4 (SAS Institute Inc., Cary, NC, USA) for analysis. The primary investigator and study staff will have access to the database through a password protected computer. This includes clinical, laboratory, and radiographic data. The information in the database will be linked to each patient by the random number assigned to that specific patient. The key used to link each patient to the assigned random number will be stored on a password protected computer. All publically available files (i.e., resulting publications or presentations) will contain only de-identified information.

Standard clinical protocol regarding patient confidentiality will be followed. Information about study patients will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed patient authorization informing the patient of the following:

- What protected health information (PHI) will be collected from patients in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research patient to revoke their authorization for use of their PHI

Dual Enrollment, Risks & Benefits, Bias

Participation in this study will preclude enrollment into other trials. The potential risks with participation include loss of confidentiality and those risks outlined above specific to each treatment intervention. Risks of confidentiality will be minimized by limiting access to data (as outlined above) and following institutional protocol when releasing or destroying protected health information.

Potential benefits of participation in this study include improvement in daily pain, function, and quality of life. The information learned from this study will be used to contribute to the literature regarding the treatment intervention outlined.

We recognize that there is risk for inherent bias within our study as patients are unable to be completely blinded to type of treatment he/she receives because each treatment procedure is distinctly different. Risk of selection bias is minimized by use of a randomized allocation schedule. Risk of influence on the patient when completing clinical outcome scales will be negated as the research team member administering the clinical outcome tools at 3, 6, and 12 months post-treatment will be blinded to group allocation.

ClinicalTrials.gov

As this study meets the FDAAA 801 definition of an "Applicable Clinical Trial" based on a drug and biologic, it is required that the study be registered on ClinicalTrials.gov. The Principal Investigator will register and activate the study account prior to enrollment, and all results will be entered upon study completion, followings statistical analysis, per the Results Database Guidelines.

2.5 – Statistical Analysis

Aim 1: Change in mean KOOS subscale scores over time based on treatment group

Total scores will be calculated for each patient using the KOOS subscales. The validated analysis guidelines for the KOOS instrument will be used. Patient's total scores will be compared at baseline, 3 months, 6 months, and 12 months post treatment and compared by treatment group.

The statistical method that will be used is a Repeated Measures ANOVA (RM-ANOVA). This statistical strategy will allow us to control for the correlation in each individual's measurements over time. The use of the standardized scales ensures that the outcome variable will be normally distributed, which will meet the underlying assumptions of the RM-ANOVA analysis. We will use the KOOS subscale score as the dependent variable, and the treatment assignment as the independent variable. If the distribution of gender is unequal across treatment groups after randomization, this statistical method will allow us to control for gender because changes in score will differ by gender. We will also be able to control for clinically meaningful variables such as patient age or BMI if there are differences in these variables between the two treatment groups at baseline.

Aim 2a: Change in mean NPRS score over time based on treatment group

Total scores will be calculated for each patient using the NPRS. The NPRS instrument will be processed using the tool's validated analysis guidelines. Patient's total scores will be compared at baseline, 3 months, 6 months, and 12 months post treatment and compared by treatment group.

The statistical method that will be used is a RM-ANOVA. This statistical strategy will allow us to control for the correlation in each individual's measurements over time. The use of the standardized scales ensures that the outcome variable will be normally distributed, which will meet the underlying assumptions of the RM-ANOVA analysis. We will use the NPRS as the dependent variable, and the treatment assignment as the independent variable. If the distribution of gender is unequal across treatment groups after randomization, this statistical method will allow us to control for gender

because changes in score will differ by gender. We will also be able to control for clinically meaningful variables such as patient age or BMI if there are differences in these variables between the two treatment groups at baseline.

Aim 2b: Change in mean PROMIS – Global Health score based on treatment group

Total scores will be calculated for each patient using the PROM Global Health scale (short form, version 1.1). The PROMIS global health instrument will be processed using the tool's validated analysis guidelines. Patient's total scores will be compared at baseline, 3 months, 6 months, and 12 months post treatment and compared by treatment group.

The statistical method that will be used is a RM-ANOVA. This statistical strategy will allow us to control for the correlation in each individual's measurements over time. The use of the standardized scales ensures that the outcome variable will be normally distributed, which will meet the underlying assumptions of the RM-ANOVA analysis. We will use the PROMIS global heath scale as the dependent variable, and the treatment assignment as the independent variable. If the distribution of gender is unequal across treatment groups after randomization, this statistical method will allow us to control for gender because changes in score will differ by gender. We will also be able to control for clinically meaningful variables such as patient age or BMI if there are differences in these variables between the two treatment groups at baseline.

All data will be analyzed with an intention to treat approach. Any potential drop out patterns will be analyzed to determine whether it is ignorable or informative.

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4.0 – APPENDICES

4.1 Knee injury and Osteoarthritis Outcome Score (KOOS)

4.2 Numeric Pain Rating Scale (NPRS)

4.3 Patient Reported Outcome Measurement Information System (PROMIS) Global Health scores (short form, version 1.1)

4.4 Relevant past medical history regarding the patient's knee

4.5 Study Calendar

4.6 Post-Procedure Care Instructions

4.7 Adverse Event Collection Form

4.8 Other relevant therapies since study treatment

4.9 Patient recruitment information