BIOMEDICAL RESEARCH PROTOCOL
UNIVERSITY OF MISSOURI

Project Title: Biological Response to Platelet-rich Plasma and Corticosteroid Injections
IRB Number: 2092036
Version Number: 2.0
Version Date: 10 September 2022
Principal Investigator: Dr. James Keeney
Funding Source: Department of Orthopaedic Surgery

Clinical Trial Phase: Phase 1/Phase 2
Clinicaltrials.gov Number: pending
Study Biologic: Platelet-rich Plasma

I. Background:

Knee osteoarthritis (OA) is an extremely common cause of disability, with a global prevalence of 22.9% in adults aged 40 and over[1]. OA is a whole-joint disease characterized by progressive degradation of articular cartilage, chronic inflammation of joint tissue, and subchondral bone remodeling, resulting in severe pain and decreased mobility in patients[2].

No cure currently exists for OA, and treatment is aimed at symptomatic management and prevention of disease progression[2,3]. Currently, this consists of:

1. Initial conservative treatment for osteoarthritis across all levels of radiographic disease severity includes activity modification, oral analgesic or anti-inflammatory medications, non-supervised or supervised (e.g., physical therapy) exercise, and occasionally bracing.

2. Injection therapies have been used in the treatment of osteoarthritis for more than 60 years. Medical corticosteroids have served as a gold standard for symptom management as an intra-articular injection, but concerns have always existed around the potential for either the steroid medication (which suppresses both repair and inflammation processes) or the local anesthetic co-administered with the steroid to contribute to degradation of joint cartilage over time. Alternative substances have been developed to address the joint environment – with an intent to improve symptoms, while decreasing the potential for joint degeneration. These alternative medications include viscosupplements (hyaluronic acid analogues) and biological agents (platelet-rich plasma, or stem cell therapies).

3. Surgical interventions include arthroscopy for concurrent symptomatic meniscus tears or unstable cartilage that contribute to mechanical symptoms, osteotomy (realignment) surgery for active patients with single compartment arthritis, and arthroplasty (joint replacement) for

patients with more limited activity goals, severe arthritis, and temporary—but not sustained---pain relief with the conservative treatments described in #1 and #2.

Intra-articular injection of a corticosteroid has been shown to be effective in providing short-term relief of knee OA symptoms, possibly due to anti-inflammatory and immunosuppressive effects[4]. Repeated corticosteroid injections have thus become the standard of care for patients with mild to moderate knee OA.

Intra-articular injection of platelet-rich plasma (PRP) has emerged as a promising alternative to corticosteroid injection in knee OA patients. Studies have indicated that PRP is safe and may provide benefits such as pain relief, improved knee function, and enhanced quality of life[2]. Moreover, PRP injection has been shown to provide longer-lasting symptomatic attenuation, with clinically significant improvement observed for as long as 12 months post-injection[5].

Previous studies have indicated that concentrations of inflammatory and degradative biomarkers in patient serum, urine, and synovial fluid may provide insight into OA pathophysiology[6]. To our knowledge, no study has been performed to assess the impact of intra-articular PRP injection upon fluid concentrations of a comprehensive panel of proposed OA-related biomarkers. In this study, we will evaluate the impact of intra-articular PRP injection upon markers of cartilage matrix turnover, inflammatory mediators, degradative enzymes, inhibitors of degradative enzymes, and markers of bone metabolism in serum, urine, and synovial fluid of knee OA patients.

Objectives:

Primary objective:

- To evaluate the effect of intra-articular PRP injection upon serum, urine, and synovial fluid MCP-1 concentrations in knee OA patients
    - Previous studies[7] have indicated that this inflammatory biomarker may be significantly decreased from baseline in the serum of knee OA patients after receiving intra-articular PRP injections, when measured three months after a patient's final injection. Monitoring fluid biomarker concentration provides insight into the joint environment and may correspond to clinical outcomes.

Secondary objectives:

- To examine the effect of PRP injection upon serum, urine, and synovial fluid concentrations of additional pro-inflammatory biomarkers in knee OA patients
    - Other pro-inflammatory markers of interest in fluids of knee osteoarthritis patients include RANTES, IL-1b, IL-6, IL-8, TNF-a, MIP-1a, and PGE2. Each of these proteins plays a unique role in the inflammatory cascade and can provide insight into the inflammatory status of the joint.

- To evaluate the effect of PRP injection upon serum, urine, and synovial fluid concentrations of anti-inflammatory biomarkers in knee OA patients
    - In contrast, IL-1RA, IL-4, and IL-10 act through pathways to reduce inflammation. Measuring levels of these proteins affords increased insight into inflammatory status.
- To assess the effect of PRP injection upon serum, urine, and synovial fluid concentrations of other pro-degradative biomarkers in knee OA patients
    - Cartilage degradation by matrix metalloproteinases (MMPs) is a significant component of OA progression, and analysis of these enzymes is critical in understanding this component of OA pathophysiology.
- To evaluate the effect of PRP injection upon serum, urine, and synovial fluid concentrations of anti-degradative biomarkers in knee OA patients
    - TIMP-1 and TIMP-2 are proteins that inhibit degradation within the joint and are therefore relevant to the monitoring of OA progression.
- To examine the effect of PRP injection upon serum, urine, and synovial fluid concentrations of cartilage matrix proteins in knee OA patients
    - Collagen and proteoglycan are key components of the extracellular matrix of cartilage. Assessing COMP, CTX-I, CTX-II, PIICP, and HA concentrations provides further insight into the balance between anabolism and catabolism of these components.
- To assess differences in patient serum, urine, and synovial fluid biomarker concentrations between single- and double-PRP injections
- To evaluate the impact of PRP injection upon patient-reported outcome measures
- To evaluate relationships between baseline patient biomarker concentrations and treatment efficacy, based on patient-reported outcome measures, following PRP or corticosteroid injection

II. Drugs/Biologics/Devices

The biologic under investigation in this study is platelet-rich plasma (PRP), a concentrated solution of platelets of autologous origin. This product will be produced from approximately 15 milliliters of whole blood by a venous blood draw performed by a clinical medical staff within the patient exam room. This sample will be placed into a centrifuge located in the clinical office to collate the platelets within the sample for proper collection of PRP. The 15 milliliters of whole blood will produce approximately 4-6 milliliters of PRP. The volume of PRP administered will be approximately 4-6 milliliters via intraarticular injection in the affected knee of patients with moderate-to-severe knee OA. This amount of PRP is indicated because standard knee injections (a large joint injection) range from 5-10 milliliters of medical substance. The PRP will be administered during the initial visit.

The current standard of care for treatment of moderate-to-severe knee OA involves serial, intra-articular injection of a corticosteroid, with injections performed, at minimum, three months apart. This modality has been shown to provide short-term relief of knee OA symptoms, possibly by modulating the immune response and associated inflammatory cascade and will be used as the reference therapy in this study. However, a limitation of this treatment is a lack of understanding of its impact upon OA pathophysiology, and specifically cartilage degeneration. PRP has been

selected for this study because it has been shown to provide pain relief and increase functionality when administered as described. Moreover, its beneficial effect may persist longer than that of corticosteroid injection, as well as remove the potential for cartilage degradation associated with intraarticular corticosteroid treatment. A corticosteroid study arm is also implemented to compare standard of care treatment to the PRP treatment population.

The medication used in the corticosteroid study arm is Triamcinolone Acetonide 40 mg/1 ml. The corticosteroid is to be ordered and regulated through the Investigational Pharmacy. The corticosteroid vials are to be stored in a lock box within the medical supplies room in the principal investigator's clinic. The medical supply room is up to IDS standards. Monthly temperature logs and expiration logs of the medication will be sent to IDS. The injection will consist of Triamcinolone 40 mg/1 mL (Kenalog) combined with 5 mL of 1% lidocaine and will be administered. The injection at the initial visit is the only established Triamcinolone injection within the study protocol. The participant may elect to receive an additional corticosteroid injection after 3 months after the initial injection if it is indicated by pain scores and physician clinical evaluation. This process may be repeated at other 3-month intervals within the study period, which is standard of care and an FDA approved use of the medication to control pain and treat the knee osteoarthritis.

An Investigational New Drug Application (IND) is not necessary for the products utilized in this study. We do not intend to report the investigation to the FDA as a well-controlled study in support of a new indication and with no intent to use it to support any other significant change in the labeling of PRP or triamcinolone. The use of these products does not involve a route of administration, dose, patient population, or other factors that significantly increases the risk associated with the use of the products. This investigation is not intended to promote or commercialize any of the products utilized.

III. Recruitment Process:

The clinical visit record for the Department of Orthopaedics will be screened for patients with osteoarthritis who generally meet the study requirements and who have a history of recurrent knee injections that have found clinical benefit. This patient population will be contacted via telephone to survey the need for another injection. If the patient requests to schedule a visit for injection, the research staff will inform the patient of the research opportunity. Once in the office, recruitment will take place in a private space during the clinic visit. Eligible patients who agree to participate in the study will be required to sign an Informed Consent Document prior to any study-specific procedures being done. After signing the Informed Consent, study Subjects are defined as "enrolled."

IV. Consent Process

Once the potential participant has been contacted and is in the clinic, a member from the research team will explain the whole research study and answer all their questions in a private room in the clinic. Once everything has been explained to the potential participant, ensuring that they

comprehend everything in the consent document, and all the potential participant's questions have been answered, then the participant will be asked to sign the consent form. The participants will also be given a copy of the form they just signed to keep for themselves.

V. Inclusion/Exclusion:

Inclusion criteria:

1. Patients aged 40 and over, presenting with a knee disorder of at least one knee
2. Patients eligible for use of either corticosteroid or biological agent for treatment of moderate or severe (but not end-stage) knee osteoarthritis
3. KL grade of 2-3

Exclusion criteria:

1. Subjects less than 40 years of age
2. Previous reconstructive knee surgery
3. Participating in another clinical trial
4. Unable to receive corticosteroid injections (i.e., allergies, adverse reactions, etc.)
5. Unable to sign informed consent
6. Pregnant or plan to become pregnant

VI. Number of Subjects:

Approximately 70 participants will be enrolled. 35 to each arm if funding allows.

VII. Study Procedures/Methods:

Enrollment/Randomization/Treatment Visit:

Eligible patients with primary osteoarthritis in at least one knee will be enrolled on a voluntary basis. Prior to the injection, patient-reported outcomes will be quantified using a visual-analog scale (VAS) to assess pain level, a Knee Injury and Osteoarthritis Outcome Score-Joint Replacement (KOOS-JR), and UCLA Activity score to assess functional activity level as part of standard care[7, 8]. These results will be deidentified and analyzed in the laboratory.

The patient will be randomized into one of two treatment arms. If the patient has a history of receiving bilateral knee injections for osteoarthritis and meets study requirements, randomization will occur for both knees. Randomization will be employed using a computer-generated random assignment sequence and results kept in concealed envelopes. The participant will be blinded to the injection regime received.

Enrolled patients will be randomized to one of two treatment arms:

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Treatment Groups	First Injection

Steroid Group	Triamcinolone 40 mg/1 mL (Kenalog) injection with 5 mL of 1% lidocaine
PRP Group	Platelet-rich plasma

Blinding:

Syringes will be prepared and masked with opaque tape by the clinic nurses, thus providing blinding for subjects.

A specimen of serum (from ~5mL of whole blood) and urine will be collected from the subject. If the subject's knee is aspirated prior to the injection (as commonly practiced), the synovial fluid will be collected, as well. These specimens will be deidentified and analyzed in the laboratory.

If the subject is randomized to the PRP group, the remainder of the PRP mixture not injected into the knee will be deidentified and analyzed in the laboratory.

Follow-Up/Clinic Visits:

Subjects will be required to complete VAS, KOOS-JR, and UCLA activity score surveys at baseline, 2 weeks, 4 weeks, 8 weeks, 12 weeks, 6 months, and 1 year. At the 4-week follow-up visit, urine and blood specimens will be collected as they were at the baseline visit. Synovial fluid collection will occur at this time point by a knee aspiration procedure.

Clinic follow-up evaluations will include a general assessment of appearance, range of motion, and function. If the subject schedules another clinic visit within the one-year study timeline, subjects will be unblinded to which treatment arm they had received. At this visit or any time after, if an injection is indicated by participant pain and functionality scores, the participant has the choice to receive steroid or PRP injection, based on preference. If subjects in the PRP study arm experience severe pain, it is recommended to follow up with their physician for assessment and possible rescue therapy with corticosteroid injection. At any point during the study, the subject has the right to request rescue surgery and may proceed to do so with physician approval.

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The visits and all research activities are outlined below:

Baseline Visit:

- Consent/Randomization
- Objective Measures:
  - Serum collection
  - Urine collection
  - Synovial fluid collection
  - General assessments
- Subjective Measures:

- VAS Pain
- KOOS-JR
- UCLA Activity

- Intervention:
  - Initial injection based on treatment arm

2 Weeks (+/- 6 days) Surveys:

- Subjective Measures:
  - VAS Pain
  - KOOS-JR
  - UCLA Activity

4 Weeks (+/- 1 week) Follow-Up:

- Objective Measures:
  - Serum collection
  - Urine collection
  - Synovial fluid collection
  - General assessments
- Subjective Measures:
  - VAS Pain
  - KOOS-JR
  - UCLA Activity

8 Weeks (+/- 13 days) Surveys:

- Subjective Measures:
  - VAS Pain
  - KOOS-JR
  - UCLA Activity

12 Weeks (+/- 2 weeks) Surveys:

- Subjective Measures:
  - VAS Pain
  - KOOS-JR
  - UCLA Activity

6 Months (+/- 2 month) Surveys:

- Subjective Measures:
  - VAS Pain
  - KOOS-JR
  - UCLA Activity

12 Months (+/- 2 month) Surveys:

- Subjective Measures:
  - VAS Pain
  - KOOS-JR
  - UCLA Activity

	Initial Visit	2 Weeks Surveys	4 Weeks Clinic Visit	8 Weeks Surveys	12 Weeks Surveys	6 Months Surveys	12 Months Surveys
Consent	X						
Randomization	X						
Serum Collection	X		X				
Urine Collection	X		X				
Synovial Fluid Collection	X		X				
General Assessments	X		X				
VAS Score (pain scale)	X	X	X	X	X	X	X
KOOS-JR	X	X	X	X	X	X	X
UCLA Activity	X	X	X	X	X	X	X
Injection	X						

Surgical Specimen Collection:

If it is indicated for a subject to undergo surgery (total knee arthroplasty), the meniscus and synovium that is normally resected and discarded after surgery will be collected for analysis. After collection, these tissues and data will be deidentified from the subject and assigned a number for use in study analysis. We seek to only collect tissues from subjects that are normally

removed and discarded during total knee arthroplasty procedures. None of the subjects will undergo additional surgical procedures for the collection of tissues in this study**.** Potential subjects must agree to the collection of these specimens to participate in the study, even if they do not end up needing surgical intervention.

VIII. Potential Risks:

We do not expect any significant medical risks associated with the treatments presented in this study. Most of the risk associated with this study is related to piercing the skin by injection with synovial fluid collection and the venous blood draw. These can include the following: pain, bruising, bleeding, infection, and damage to surrounding structures, such as nerves and blood vessels. The risks of PRP injections include local infection and pain at injection site. The risks of corticosteroid injections include pain at injection site, skin discoloration, elevated blood sugar, infection, cartilage deterioration, and allergic reaction.

Synovial fluid collection in this study is part of the injection process and a stand-alone procedure at the 4-week visit. It has the same risk as the risk for injections listed previously. If there is a concern with the risks associated with the study, the participants are recommended to ask their provider for more information regarding the risks of the injection to their specific health condition. At any point, the participant has the right to withdraw from the study without recourse.

To help lower these possible risks, only professionally trained individuals will participate in the collection of the specimens. The participant will also be monitored for 5-10 minutes after injection.

All adverse events reported spontaneously by the participant or in response to questioning or observation by the investigator/research staff will be assessed for subject safety, acted upon as needed per situation and be recorded. If the events meet the criteria for IRB reporting, they will be reported to the IRB within five days.

Any adverse event associated with the intervention or deviation from the standard protocol will be evaluated related to subject safety and reporting requirements as well as any potential breach of confidentiality.

IX. Potential Benefits:

Potential benefits include relief of their knee pain and return of function. Steroid injections have been shown to provide symptom relief for patients suffering from osteoarthritis. We also hope to demonstrate a differential efficacy between different corticosteroid and PRP injections, which would help guide future clinical decision-making.

X. Compensation:

The subjects will not be compensated for their participation in the study.

XI. Costs:

Most costs to run this study, including the cost of the injections, the equipment to draw blood and collect urine, the containers to store the blood until the tests are run, and the assays run in the laboratory, will be covered by the department. Participants will not occur any of these costs.

The subjects will be financially responsible for all their standard of care treatment. The initial visit is a standard of care, and the participant will be responsible for covering the cost of the visit itself, not the injection procedure or other testing that may occur during the visit. The 4-week visit is not standard of care and will be covered through an internal research fund. Any clinical visit scheduled due to a reoccurrence of knee pain is standard of care and the participant may choose to receive another injection if indicated. The participant is unblinded during this visit and may choose their preference of injection type if indicated. The participant will accrue the cost of the visit and for the injection if the participant chooses to receive the procedure.

All assays and tests will be completed in the laboratory of the same hospital where samples are acquired, so there will be no transportation outside of the hospital.

XII. Data Safety Monitoring Plan

Patient confidentiality during this study will be protected in compliance with HIPAA (Health Insurance Portability and Accountability) requirements as well as the requirements of the University of Missouri Health-Sciences IRB. All subjects will have a de-identified Subject Study ID number. All subject information in electronic format will be kept in password-protected storage. All subject information in paper format will be kept in locked cabinets in a secured suite at the Missouri Orthopedic Institute with access granted only to the designated research personnel. Data will be stored on the Department of Orthopaedic Surgery shared drive and/or Patient IQ, a HIPAA-compliant cloud-based platform that is contracted with the Department of Orthopaedic surgery.

The investigator and research coordinators will monitor the study data. They will review the conduct of clinical studies to assure that the clinical investigators abide by their obligations to conduct clinical studies properly. The investigator will also ensure that the research staff is given access to all study-related documents and study-related facilities, as applicable, and has adequate space to conduct the monitoring, when applicable. The coordinator will review all source documents and compare them to the data contained in the electronic case report forms (eCRF). Staff members will ensure data integrity at three-month intervals during the study.

Adverse Event

An AE is any untoward medical occurrence in a clinical investigation subject, which changes the medical baseline of the subject. An AE can be an unfavorable and unintended sign, symptom, or disease, whether related to the study device (AEs may also be referred to as complications).

Anticipated Adverse Event

An anticipated AE is an AE, of which the nature, severity or degree of incidence is known and identified in applicable product labeling, published literature or the study protocol. The list of anticipated events is provided in section VIII, Potential Risk.

Serious Adverse Event

A SAE meets one or more of the following definitions:

- Resulted in in-patient hospitalization
- Resulted in prolonged existing hospitalization
- Resulted in persistent or significant disability/incapacity
- Resulted in permanent impairment of a body function or permanent damage to a body structure
- Necessitated medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure
- Was a life-threatening situation
- Resulted in participant death

General Physical Examination Findings

At screening for inclusion into the study, any clinically significant abnormality should be recorded as a preexisting condition. From the time of consent forward, any new clinically significant findings or abnormalities that meet the definition of a protocol defined AE must also be recorded and documented as an AE.

The PI of the study, Dr. Keeney, and study coordinators will review participant charts and study data (including patient reported outcome measures through Patient IQ) at every clinical follow up visit. During the follow up visit, investigators and coordinators will seek information relating to the participant's health by asking a series of questions and ask as to whether they have seen a doctor for any reason, been hospitalized for any reason or have a current impediment to their function. If any event that is deemed a SAE (listed above) will implement a pause in the participant's study routine until further work up can be obtained.

The adverse event (AE) reporting requirements for this study are as follows:

- All AEs that meet the definition of serious and occur within the period of injection to 90 days following the date of injection.
- All AEs related to the injection site, regardless of seriousness or time of occurrence.

Adverse Event Reporting

All unanticipated problems (UAPs) involving risks to the participants will be reported to the IRB promptly, but no later than two weeks or 10 business days from the time of identification. An UAP is defined as the following:

- Unexpected (in terms of nature, severity, or frequency) given the information provided in research-related documents and the characteristics of the subject population being studied
- Related or possibly related to participation in the research
- Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized

Any AE or SAE that is expected, as identified in the study documentation, but is occurring at greater frequency or severity, as determined by the principal investigator's assessment, will be reported to the IRB as a UAP. However, adverse events determined to be unrelated to the study will not be submitted to the IRB.

The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. The start of study procedures is the point of consent. Any AEs which fit the protocol defined reportable events must be reported from the time of consent until study completion.

Information on protocol defined AEs should be recorded immediately in the source document and in the appropriate AE module of the eCRF. All clearly related signs, symptoms and abnormal diagnostic procedure results should be recorded in the source document and grouped under one diagnosis, as appropriate. The clinical course of each event should be followed until resolution or until it is determined at the end of the study that the AE will not resolve.

All injection site events occurring at any time as well as all serious adverse events (SAEs) occurring within 90 days post injection will be collected and compared to published data. It is expected that the AE rates reported for the study will be comparable to those reported in the literature for other corticosteroid and PRP injections.

XIII. Statistics/Power:

The number of subjects per group was determined from a pre-study power analysis using previously published data on prevalence of OA biomarkers and reported symptomatology

XIV. References:

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