University of Kansas Medical Center
RESEARCH PROTOCOL INVOLVING HUMAN SUBJECTS TEMPLATE

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Principal Investigator: Neil Segal, MD, MS

Study Title: An open-label pilot study of the effect of intra-articular bilateral knee injections of Zilretta on OARSI recommended physical performance measures in adults with symptomatic knee osteoarthritis.

Co-Investigator(s): Mayank Kothari, MBBS

I. Purpose, Background, and Rationale

A. Aim and Hypotheses

This study will be an open-label trial to determine the functional effects of bilateral IA injections of Zilretta into knee joints of 70 subjects with bilateral KL grade 2-4 symptomatic knee osteoarthritis (OA). Measurement and evaluation of outcomes at baseline, 6, 12 and 24 weeks will allow assessment of short and long-term effects, consistent with Osteoarthritis Research Society International (OARSI) and Outcome Measures in Rheumatology (OMERACT) recommendations.

The **primary** aim of the proposed study is to demonstrate the effect of IA injection of Zilretta on physical performance measures in adults with bilateral knee OA. We will test this primary study endpoint by assessing the change in OARSI recommended physical performance tests[1] (30-second chair standing test, 40m fast-paced walking test, stair ascent) 6, 12 (primary) and 24 weeks after treatment.

The secondary outcome will be the change in Knee Injury and Osteoarthritis Outcome Score (KOOS-PS) at 6, 12 (main) and 24 weeks after treatment. This will be captured using the patient-reported physical function short form.

The tertiary outcomes will be the change in KOOS-Quality of life subscale (QoL) and Numerical Rating Scale (NRS) for pain. While the primary outcomes will be defined at the 12-week follow-up, all outcomes will be assessed at the 6- and 24-week follow-up as well to define the course and trajectory of effects.

We hypothesize that people with bilateral symptomatic knee OA will experience significant improvement in physical performance, physical function and quality of life for at least 12 weeks following treatment. Based on research done using Zilretta in patients with knee OA, we anticipate that it will have a greater magnitude and duration of analgesic effect along with significantly fewer adverse events when compared to the rates reported for standard of care therapy (i.e. immediate release corticosteroid injection).[2]

Specific Aim 1: To determine the extent to which intra-articular (IA) injection of Zilretta in patients with bilateral knee OA improves physical performance (OARSI recommended physical performance measures).

> **Primary Hypothesis 1:** Bilateral knee intra-articular injection of Zilretta results in improved physical performance detectable at: a) 6 weeks, b) 12 weeks (primary), and c) 24 weeks.

Specific Aim 2: To determine the extent to which IA injection of Zilretta in patients with bilateral knee OA improves physical function (KOOS-PF).

> **Hypothesis 2:** Bilateral knee IA injection of Zilretta results in improved patient-reported physical function (KOOS-PS) at: a) 6 weeks, b) 12 weeks, and c) 24 weeks.

Specific Aim 3: To determine the extent to which bilateral knee IA injection of Zilretta in patients with bilateral knee OA improves quality of life (KOOS-QoL).

> **Hypothesis 3:** Intra-articular (IA) bilateral knee injection of Zilretta in patients with bilateral knee osteoarthritis results in improved quality of life (KOOS-QoL) detectable at: a) 6 weeks, b) 12 weeks, and c) 24 weeks.

B. Background and Significance

Osteoarthritis (OA) is the most common disabling disease in older adults, affecting over 27 million Americans, resulting in significantly impaired function and mobility and a societal economic burden. OA is a joint disorder characterized by structural pathology that involves the whole joint, including cartilage lesions, bone remodeling, osteophyte formation, and joint inflammation, among others, leading to symptoms and loss of normal joint function. OA typically becomes symptomatic later in life, usually after age 50, though it may start earlier, such as when a joint injury has occurred or in familial forms. It is the most common form of arthritis worldwide, affecting an estimated 250 million people; about 80% of people over 65 having radiographic evidence of OA.

OA is a leading cause of disability among elderly in the US. In recent estimates of global years lived with disability, musculoskeletal-related conditions ranked second, with low back pain, neck pain, and knee OA being the three most common conditions[3]. There is a high prevalence of knee OA globally[4] and it ranked within the top 10 causes of disability and non-communicable diseases for global disability-adjusted life years worldwide (*i.e.,* years of life lost and years lived with disability)[3,5]. The public health impact of OA is significant on both individuals and society due to the absence of a cure, making symptomatic treatment essential.

The greatest challenge, affecting patients with OA of the hips and knees, is pain and stiffness in these joints, which often leads to significant disability requiring surgical intervention. In a longitudinal panel survey conducted by the US Census Bureau, arthritis or rheumatism was the most commonly reported cause of disability, and difficulties related to lower extremity functioning or activities were the most commonly reported limitations among all respondents. In particular, the most widely recognized limitation was in walking 3 city blocks, which influenced an estimated 22.5 million US adults, and difficulty with climbing stairs, influencing an estimated 21.7 million US adults[6].

Zilretta is an extended-release PLGA microsphere preparation of triamcinolone acetonide which is FDA approved for treatment of knee pain due to osteoarthritis. Zilretta (triamcinolone acetonide extended-release) is formulated using proprietary microsphere technology combining triamcinolone acetonide with a polylactic-co-glycolic acid (PLGA) matrix. Zilretta received Fast Track Designation from the FDA. This designation is reserved for drugs in clinical development that is intended to treat a serious condition and addresses an unmet medical need.

Preliminary Data: In previous clinical trials, Zilretta (triamcinolone acetonide extended-release) provided an extended and predictable release of triamcinolone acetonide as the biocompatible PLGA microspheres slowly degraded. A single IA dose of ZIlretta resulted in less systemically bioavailable triamcinolone acetonide when compared to a matched dose of triamcinolone acetonide crystalline suspension (TAcs)[7] due to slower release and absorption into the systemic circulation. By providing prolonged local exposure to triamcinolone acetonide while minimizing systemic exposure, IA injection of Zilretta is expected to have sustained anti-inflammatory and analgesic efficacy while minimizing adverse effects[8]. A prior clinical trial provided evidence for analgesic efficacy for knee OA, with a significant reduction in average daily pain (ADP) sustained through 12-week follow-up[9]

Therefore, the **primary** aim of the proposed study is to demonstrate the effects of IA injection of Zilretta on physical performance in adults with bilateral knee OA. We propose to determine the change in OARSI recommended[10] physical performance measures (30-second chair stand time, 40m fast-paced walking time, stair ascent time) along with patient-reported physical function (KOOS-PS) and quality of life (KOOS-QoL) at baseline and at intervals following bilateral knee injection of Zilretta. The ***expected deliverables*** of this research will be evidence regarding the effects of Zilretta on objective and patient-reported physical functional outcomes in patients with bilateral symptomatic knee OA.

C. Rationale

Disability due to knee OA is based on the inter-relationships of pathology (e.g. knee cartilage degeneration), impairments (e.g. pain), functional limitations (e.g. difficulties with standing from a chair, walking or using stairs), and disability (e.g. inability to participate in community). Biomedical research frequently focuses primarily on pathology and sometimes on impairments. However, there is an imperfect correlation between impairments and functional limitations. While the effect of Zilretta on pain has been explored, the effects on physical function and performance are incompletely understood.

II. Research Plan and Design

A. Study Objectives: The goal of this study is to evaluate the efficacy of Zilretta for improving physical performance, physical function, and quality of life.

B. Study Type and Design:

This will be an open-label study to determine the functional effects of IA injection of Zilretta into bilateral knees of 70 subjects with KL grade 2-4 symptomatic knee OA. Measurement of OARSI recommended physical performance measures[10], patient-reported physical function, quality of life and pain at baseline, 6, 12 and 24 weeks will allow assessment of short and long-term effects, consistent with OARSI and OMERACT recommendations. Adverse events will be monitored.

Each subject will participate for up to 24 weeks following the intra-articular Zilretta injection. Enrollment is expected to take approximately 6 months. Total study duration is expected to require at least 12 months.

C. Sample size, statistical methods, and power calculation

In this open-label study, all subjects will receive a single IA Zilretta injection into each knee. Outcome measures will be collected at baseline, 6, 12 (primary) and 24 weeks post-treatment. A 24-week follow-up duration was selected to detect meaningful differences from standard of care (IA corticosteroids - TAcs) and long-term effects, while minimizing the variability that may occur further out from treatment. The overall type I error rate will be controlled with an alpha level of 0.0167 to account for three comparisons at the primary time point.

Based on the respective MDC_{95} calculated from the standard error of the mean change for OARSI recommended physical performance[1], the effect size for this minimal detectible change is 0.392. To detect an effect size at least this large, with an adjusted alpha of 0.0167 and 80% power, a sample size of 60 would be required. To accommodate up to 14% dropout, we plan to recruit 70 subjects.

D. Subject Criteria (See Vulnerable Populations appendix, if applicable):

Enrollment will be comprised of 70 subjects age 30 and over with symptomatic bilateral knee OA. There will be no exclusions based on sex or ethnic group. Potential subjects will be identified through hospital records, referrals from physicians in the surrounding community, mass mailings, and advertisements posted in area clinics and businesses. Any study-related advertisements will be approved by the IRB prior to use. Subjects will be recruited based upon inclusion and exclusion criteria detailed below.

1. **Inclusion Criteria**

- Men and women age 30 years or older with symptomatic bilateral knee OA.
- Symptomatic Knee OA will be defined as the presence of a definite osteophyte or joint space narrowing (KL Grade ≥2) on posteroanterior (PA) fixed flexion knee radiographs in subjects limited by bilateral pain in each knee, rated on a Numerical Rating Scale as ≥ 4/10 on more than half of the days over the past month. Radiographic change must be visible at standard image size, irrespective of capability to detect more subtle changes through digital enhancement.
- Bilateral knee symptoms for ≥ 3 months prior to screening.
- Undergone at least one prior conservative osteoarthritis treatment (e.g. physical therapy, analgesics).
- Body Mass Index ≤ 40 kg/m^2.
- Ambulatory.
- Willing and able to comply with the study procedures and visit schedules and ability to follow verbal and written instructions.
- Willing to abstain from the use of protocol-restricted medications during the study after signing Informed Consent (Refer to §II.5) and also willing to abstain from use of all analgesics other than acetaminophen 1 week prior to the beginning of treatment.

2. Exclusion Criteria

- Current consumption of more than 14 alcoholic drinks per week.
- Clinical signs and symptoms of active knee infection or crystal disease of either knee within 1 month of screening.
- Diagnosed with rheumatoid arthritis, Reiter's syndrome, psoriatic arthritis, gout, ankylosing spondylitis, or arthritis secondary to other inflammatory diseases; HIV, viral hepatitis; chondrocalcinosis, Paget's disease, or villonodular synovitis.
- Diagnosed with leukemia, known presence of metastatic malignant cells, or ongoing or planned chemotherapeutic treatment.
- Diseases of the spine, hip or other lower extremity joints judged by the investigator to be contributing to the pain in either knee (i.e. sciatica, nerve pain, hip OA). Note: Patients with hip replacement in either hip may be enrolled provided there is sufficient pain relief after hip replacement that analgesics are not required
- Untreated symptomatic injury of either knee (e.g., acute traumatic injury, anterior cruciate ligament injury, clinically symptomatic meniscus injury characterized by a mechanical issue such as locking or catching).
- Uncontrolled diabetes.
- Women who report pregnancy or childbearing potential and not using acceptable contraceptive measures (oral contraceptive, long acting reversible contraceptive therapy) (due to the potential for change in body mass and distribution to alter knee symptoms over the period of follow-up).
- Presence of surgical hardware or other foreign body intended to treat arthritis or cartilage-related pathology in either knee.
- Arthroscopy or open surgery of either knee within 6 months of screening.
- Planned/anticipated surgery of either knee during the study period.
- Use of systemic immunosuppressant within 6 weeks of screening.
- Oral corticosteroids (investigational or marketed) within 2 weeks of screening.
- IA corticosteroid (investigational or marketed) in either knee within 3 months of screening.
- IV or IM corticosteroid (investigational or marketed) within 3 months of screening.
- Any other IA drug/biologic use within 6 months of screening or 5 half-lives (whichever is longer) (e.g., hyaluronic acid, platelet-rich plasma (PRP) injection, stem cells, prolotherapy and amniotic fluid injection).
- Any documented clinically significant degree of cognitive impairment or other condition, finding, or psychiatric illness at screening which, in the opinion of the investigator, could compromise subject safety.
- Any condition other than OA of the knee which, in the opinion of the investigator, affects the ability to ambulate to a sufficient degree to interfere with the assessment of the safety and treatment effects of the study injection.
- Participated in any investigational drug or device trial within 30 days prior to screening or concurrent participation in another research study that could complicate interpretation of the study findings.

3. Withdrawal/Termination criteria:

Subjects may discontinue the study at any time. The investigator may discontinue a subject at any time for subject safety or noncompliance. Subjects who are discontinued for safety will be referred to their primary care physician for follow-up.

The Investigator/Coordinator will complete a study exit form in the CRF for any subject who prematurely discontinues from the study. If discontinuation was the result of an AE, the AE also will be recorded in the CRF.

4. Allowable Medications/Nonpharmacological Therapies

The following medications and nonpharmacological therapies may be taken or used throughout the study:

- Aspirin for cardio-protection at a maximum stable dose provided the dose was stabilized over 3 months prior to study entry.
- Physical therapy/bracing for the knee joint if the program was initiated and consistent prior to study entry.
- Acetaminophen may be taken (up to 3000 mg per day) as rescue medication, if needed. Rescue medication is used only to treat worsening pain, whether or not that pain is associated with knee (e.g, may be used to treat headache, acute injury, etc.) and is not used for prophylaxis.
- Use of acetaminophen must be discontinued 48 hours prior to the procedure visit and each follow-up visit.

5. Restricted Medications/Nonpharmacological Therapies

Volunteers will be advised that participation in the study will require them to abstain from certain medications and therapies. The following medications and nonpharmacological therapies should not be taken or used beginning immediately upon enrollment (after signing informed consent) until subject reaches the end of the study:

- Oral non-steroidal anti-inflammatory drugs (unless on a chronic stable dose for ≥3 months prior to enrollment).
- Topical analgesics applied to the skin over the knee (e.g., NSAID's, capsaicin, lidocaine, heat patches).
- Orally administered systemic corticosteroids.
- Other Intra-articular injection into the knee joint.
- Centrally acting pain medications (e.g., pregabalin, gabapentin, duloxetine, milnacipran) (unless on a chronic stable dose for ≥3 months prior to enrollment).

E. Specific methods and techniques used throughout the study

Investigational Product

Zilretta kits will be received from Flexion Therapeutics and stored in a refrigerator between 2-8 degrees Celsius upon receipt. An 'Investigational Drug Accountability Record' will be used to record the product use for each subject. The kit label number, date of receipt, dates of dispense and preparation, and the date of destruction of the remaining product will be tracked and logged.

Treatment Procedure:

Zilretta (triamcinolone acetonide extended-release injectable suspension) is indicated as an intra-articular injection for the management of pain due to knee OA. To prepare the injectate, we will dilute 32 mg Zilretta in a 5 ml diluent for each knee, per manufacturer instructions. After application of sterile chlorhexidine and topical lidocaine (optional) for skin anesthesia, we will locate the superior recess of each knee joint, aspirate excess joint fluid and then inject the 5ml injectate into each knee joint capsule (bilateral injections).

Primary Outcome

OARSI recommended physical performance measures

OARSI (Osteoarthritis Research Society International) recommended physical performance measures[1] are a consensus-derived set that test physical function, which is complementary to patient-reported measures for

use in people diagnosed with knee osteoarthritis or following knee replacement. They are recommended for use prospectively as outcome measures in research, and also in clinical practice to make treatment decisions based on the results[11].

1. 30-second chair standing test

The 30-second chair standing test evaluates the maximum number of repetitions that a subject can stand from a seated position in a 30 second period[1]. From the sitting position, the subject will stand up completely up so hips and knees are fully extended, then completely back in the seated position. This will be repeated for 30 seconds and we will record the total number of chair stands (up and down equals one stand). The tester will stand close to the side of the chair for safety and also ensure that the subject comes to a full standing and sitting position during the test.

The study coordinator or designee will enter the data collected into the CRF after completion of the test.

2. 40m fast-paced walking test

The 40-meter fast-paced walking test is a test that is timed over 4 x 10m (33 ft) paths for a total of 40 m (132 ft)[1]. We will mark out a 10 m (33 ft) walkway with bright colored tape at each end. A cone will be placed approximately 2 meters before the start mark and 2 meters beyond the finish mark of the 10m walkway. Enough space will be left to turn safely around at each end.

A practice trial of 1-2 turns will be done before testing to help subjects to understand the procedure. We will begin timing subjects after giving the signal to start at the start line and stop the timer once the subject crosses back over the start line after completing the course. When the subject crosses the 10m mark, timing will be paused while the subject turns around the cone and then it will be resumed once they cross the 10m mark again. The same will be repeated for the following turns and timing will be stopped once the subject crosses the start line for the final time. The duration of one trial will be recorded to the nearest 100th of a second. Time of one test trial will be recorded and expressed as speed (m/s) by dividing distance (40m) by total time. Regular walking aid will be allowed and its use will be recorded.

For subjects in whom safety is of concern, the tester will follow slightly behind and off to one side of the subject, while taking care not to pace or impede them. If there is no excess concern for safety, the tester will follow well to the side so as to view the crossing at the 10m walkway at both ends. The study coordinator or designee will enter the data into the CRF.

3. Stair negotiation

Stair negotiation is also a common activity limitation and rehabilitation goal in people with symptomatic knee OA. We will time the subjects ascending and descending a flight of 9 stairs as quickly as they can accomplish in a safe manner. We will conduct the nine-step stair test in the staircase adjoining our research space (individual step height of 17 cm).

Timing will begin on the signal to start and terminate when the subject returns with both feet to the ground level. Total time to ascend and descend steps for 1 test trial will be recorded to the nearest 100th of a second.

Use of a handrail and walking aid will be permitted if required[1]. The study coordinator or designee will enter the data into the CRF.

<u>Secondary Outcome</u>

Patient-reported physical function (KOOS-PS).

In addition to the performance-based primary outcome measures, KOOS-PS, a self-reported measure of mobility will be included.

KOOS-Physical Function (KOOS-PS) Short Form is a parsimonious measure of physical function derived from the KOOS[12]. The KOOS-Physical Function Short Form also has good internal consistency and high

test-retest reliability.

A working group tasked with constructing a composite measure of OA severity sponsored by the Osteoarthritis Research Society International (OARSI) and Outcome Measures in Rheumatology (OMERACT) developed the KOOS-PS. Respondents are asked to indicate the degree of difficulty experienced in the last week due to a knee problem in 7 tasks: Rising from Bed, Putting on socks/stockings, Rising from sitting, Bending to the floor, Twisting/pivoting on the injured knee, Kneeling, and Squatting. Responses are measured on a Likert scale ranging from 'None' to 'Extreme'.

Tertiary Outcomes

KOOS QoL

KOOS-QoL is a self-reported measure[13] (4 questions) of quality of life, which is part of the five patient-relevant subscales of KOOS. A Likert scale is used and all four questions have five possible answer options scored from 0 (No Problems) to 4 (Extreme Problems). Each of the five scores is calculated as the sum of the items included. Scores are transformed to a 0-100 scale, with zero representing extreme knee problems and 100 representing no knee problems as is common in orthopedic assessment scales and generic measures. Scores between 0 and 100 represent the percentage of total possible score achieved.

Numerical Rating Scale (NRS) for Pain

The Numeric Rating Scale for Pain (NRS for Pain) is a measure of pain intensity. The investigator or designee will verbally introduce the 11-point scale to the research subject to rate pain in each knee. The investigator or designee will ask the following questions,

- "On a scale of 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable, how would you rate your knee pain IN THE PAST 7 DAYS?"

The subject will complete the NRS for Pain worksheet after receiving instructions from the study coordinator. The NRS for Pain will be completed by the subject at baseline evaluation and at each follow-up visit (6,12 and 24 weeks). The study coordinator or designee will enter the data into the CRF.

The table below details the schedule of study assessments and procedures, for each visit.

Assessment	Visit 1 Screening	Visit 2 Day 1- Zilretta Treatment‡	Visit 3 6-Week Follow-up	Visit 4 12-Week Follow-up	Visit 5 24-Week Follow-up
Informed Consent	X				
Eligibility	X	X‡			
Medical history	X	X‡			
Radiograph (if not previously done)	X				
Concomitant medications	X	X‡	X	X	X
Prior/Concurrent Therapy	X				
Study Treatment		X			
BMI Assessment	X				
Physical Exam		X	X	X	X
KOOS-PS & QoL Questionnaire		X	X	X	X
NRS for Pain	X	X‡	X	X	X
Physical Performance Measures			X	X	X
AE/SAE Assessment		X	X	X	X

*Each visit must be within ±5 days of the scheduled visit; ‡ may be combined with screening visit.

F. Risk/benefit assessment

The reference safety information for Zilretta for this study is the Zilretta USPI.

1. Physical risk:

Zilretta (triamcinolone acetonide extended-release injectable suspension) is indicated as an intra-articular injection for the management of pain due to knee OA. The most commonly reported adverse reactions (incidence ≥1%) in clinical studies included sinusitis (2%), cough (2%), contusions (2%) and joint swelling (3%). For additional details, please see Package Insert (http://www.zilrettalabel.com/PI.pdf). The website for Zilretta additionally lists: joint pain, headache, back pain, sore throat and runny nose, and bruising as common side effects of receiving a Zilretta injection.

The following is a list of other adverse events that can occur following injection of corticosteroids, although not reported following Zilretta.

- **Hypersensitivity Reactions:** Rare instances of anaphylaxis, including serious cases, have occurred in patients with hypersensitivity to corticosteroids. Serious cases of anaphylaxis, including death, have been reported in individuals receiving triamcinolone acetonide injection, regardless of the route of administration.
- **Joint Damage:** Intra-articular administration may result in damage to joint tissues.
- **Increased Risk of Infections:** Infection with any pathogen in any location of the body may be

associated with corticosteroids. Corticosteroids may increase the susceptibility to new infection and decrease resistance and the ability to localize infection. A marked increase in pain accompanied by local swelling, restriction of joint motion, fever, and malaise are suggestive of septic arthritis.

- **Alterations in Endocrine Function:** Corticosteroids can produce reversible hypothalamic-pituitary-adrenal axis suppression, with potential for adrenal insufficiency after withdrawal of treatment, which may persist for months.
- **Cardiovascular and Renal Effects:** Corticosteroids can cause blood pressure elevation, salt, and water retention, and an increase potassium excretion.
- **Increased Intraocular Pressure:** Corticosteroid use may be associated with increased intraocular pressure.
- **Gastrointestinal Perforation:** Corticosteroid administration may increase the risk of gastrointestinal perforation in a patient with certain GI disorders and fresh intestinal anastomoses.
- **Alterations in Bone Density:** Corticosteroids decrease bone formation and increase bone resorption.

2. **Psychological risk:** Corticosteroids may cause adverse psychiatric reactions. Although this has not been reported following Zilretta injection, we will advise patients to immediately report any behavior or mood disturbances.
3. **Social risk:** N/A
4. **Economic risk:** N/A
5. **Potential benefit of participating in the study*:*** Participation in this study may produce possible benefits for the individual subjects such as improved pain, stiffness or function in your knee joint.

G. Location where study will be performed: The study will be performed at the University of Kansas Medical Center, Sudler 1st Floor (primarily Rooms 1023-1024). All data forms and records will be kept on site at the University of Kansas Medical Center in locked drawers and password-protected computers in a locked office. Only members of the research team will have access to the data.

H. Collaboration (with another institution, if applicable): N/A

I. Single IRB Review for a Multi-site study (if applicable): N/A

J. Community-Based Participatory Research (if applicable): N/A

K. Personnel who will conduct the study, including:

1. Indicate, by title, who will be present during study procedure(s):
    a. Dr. Neil Segal, Principal Investigator
    b. Dr. Mayank Kothari, Sub-Investigator
2. Primary responsibility for the following activities, for example:
    a. Determining eligibility: Segal/Kothari/Deas
    b. Obtaining informed consent: Deas/Segal
    c. Providing on-going information to the study sponsor and the IRB: Deas/Segal
    d. Maintaining subject's research records: Deas/Segal
    e. Completing physical examination: Segal
    f. Measuring height, weight: Kothari/Deas/Segal
    g. Drawing / collecting laboratory specimens: N/A

h. Performing / conducting tests, procedures, interventions, questionnaires: Segal/Deas

i. Completing study data forms: Deas/Segal/Kothari

j. Managing study database: Deas/Kothari

L. Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

Compliance reports which include telephone screening forms for eligibility, radiographs, medication amenability and safety logs will be reviewed by the Principal Investigator on a weekly basis. Reports containing the proportion of subjects completing the intervention or prematurely terminating participation will be generated monthly.

Adverse event information will be collected at each visit and during all contacts with subjects. If an adverse event is reported to study staff, data including the type of event, onset/end dates, duration, and outcome will be collected and reported to the Principal Investigator. All adverse event information will be assessed by the Principal Investigator for severity, seriousness, and relationship to Zilretta, per the definitions below.

The Investigator is required to follow SAEs until resolution or withdrawal of consent. Resolution is defined as:

- A return to baseline for a pre-existing condition; or
- Resolved with or without residual effects; or
- The Investigator does not expect any further improvement or worsening of the event.

Reporting Serious Adverse Events:

An event that is serious must be recorded on the AE worksheet and requires expeditious handling to comply with regulatory requirements. Any adverse event classified as "serious" by the Investigator must be reported to the IRB within 24 hours of becoming aware of its occurrence. All serious adverse events in patients treated with Zilretta must also be reported to Flexion within 3 calendar days. The Investigator should attempt to collect as much information about the adverse event as needed to get a complete medical understanding and forward that information to Flexion as it becomes available.

All serious adverse events which are considered causally related to Zilretta (see definitions below) must be assessed for whether it is expected per the USPI. An unexpected event means that it is not described within the reference safety information (the Zilretta USPI) as associated with Zilretta. If an event is assessed as serious, related to Zilretta, and unexpected, it must be reported to the FDA according to 21CFR314.80

Safety Definitions:

Adverse Event (AE): An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product, any clinically significant abnormality found on an ECG, laboratory test, or physical examination, or any worsening (i.e., any clinical significant adverse change in frequency and/or intensity) of a preexisting condition, which is temporally associated with the use of the medicinal (investigational) product

Serious Adverse Event (SAE): An SAE is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening, ("Life-threatening" refers to an event in which the patient was at substantial risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization.

- Note: Adverse events requiring hospitalizations that are less than 24 hours in duration do not meet this criterion. A scheduled hospitalization for an elective procedure or a pre-existing condition that has not worsened during participation in the study does not meet this criterion.

- Results in permanent or significant disability/incapacity; a substantial disruption of the patient's ability to carry out normal life functions.
- Is a congenital anomaly/birth defect.
- Is an important medical event: event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above.

Severity: the intensity of the adverse event. This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. The severity of AEs will be assessed according to the following definitions:

- Mild: the AE is noticeable to the patient and/or the Investigator, but does not interfere with routine activity.
- Moderate: the AE interferes with routine activity, but responds to symptomatic therapy or rest.
- Severe: the AE significantly limits the patient's ability to perform routine activities despite symptomatic therapy.

Causal Relationship: A medically-qualified Investigator must assess the relationship of any AE (including SAEs) to the use of the investigational product, as related or not related, based on clinical judgment and using all available information, and may include consideration of possible alternative causes of the AE, including the disease under treatment, pre-existing conditions, concomitant use of other drugs, and presence of environmental or genetic factors, the temporal association between drug exposure and onset of the AE, and whether the manifestations of the AE are consistent with known actions or toxicity of the investigational product. The causal relationship between the study medication and the AE will be assessed using one of the following categories:

- **Not Related:** An AE is not associated with study medication if:
    - Temporal relationship is lacking (e.g., the event did not occur within a reasonable time frame following administration of the study medication); or
    - Other causative factors more likely explain the event (e.g., a pre-existing condition, other concomitant treatments).
- **Related:** An AE is attributed to the study medication if:
    - There is a positive temporal relationship (e.g., the event occurred within a reasonable time frame following administration of study medication); and
    - The AE is more likely explained by the investigational product than by another cause (i.e., the AE shows a pattern consistent with previous knowledge of the investigational product or the class of the investigational product).

Data and Safety Monitoring Plan

The data will be reviewed by the study team and the Principal Investigator. The reviewed data include eligibility, data reconcilation and safety logs. There will also be reports containing the proportion of subjects completing the intervention or prematurely terminating participation . This will be done on a monthly basis.

III. Subject Participation

A. Recruitment:

Potential study subjects will be recruited from the clinics of the principal investigator and referring physicians, and using local advertising. Any study-related advertisements will be approved by the governing IRB prior to use. Subjects will be recruited based upon inclusion and exclusion criteria detailed above. Subjects will be compensated for their time and effort at follow-up visits.

Each subject will participate for up to 24 weeks. Enrollment is expected to take up to 6 months. Total study duration is expected to be at least 12 months.

B. Screening Interview/questionnaire:

Potential subjects who express interest in the study will be pre-screened by telephone. A script will be used to determine eligibility for the first screening clinic visit by study staff. Please reference Appendix A.

C. Informed consent process and timing of obtaining consent

Potential subjects who pass the initial screening criteria (telephone screen) will be asked to schedule a screening visit to provide written consent and possibly undergo screening procedures during a screening visit. Subjects will be informed that the study is voluntary and choosing not to participate will not affect their current treatment. They may request the opportunity to review the consent form with their physician and/or family members. Moreover, they will be fully informed of the purpose of the study and of the possible risks involved before providing their consent. Written, informed consent will be obtained and documented by an investigator or a study coordinator prior to initiation of study procedures. All subjects will be provided with a copy of the signed consent form.

D. Alternatives to Participation:

The alternative to study participation is choosing not to participate and to continue with their current treatment plan.

E. Costs to Subjects:

There will be no cost to subjects to participate in the study and no insurance will be billed as a part of the study procedures.

F. How new information will be conveyed to the study subject and how it will be documented:

If any new information is to be provided to the study subject the informed consent form (ICF) will be amended and approved by the IRB. Each subject will then be reconsented using the newest revision of the ICF.

G. Payment, including a prorated plan for payment:

Subjects will be provided $20 at Visit #2, $20 at Visit #3, $30 at Visit #4, and $30 at the final Visit #5 (up to $100 per subject) as compensation for their time. They additionally will be provided with parking validation for in-person visits. Moreover, subjects will be provided with encouragement during the study period, especially prior to and after each study visit by letting them know that their contributions are appreciated.

H. Payment for a research-related injury:

If the subject is injured because of study participation, there will be no compensation for physical or non-physical injuries. No compensation will be available for any extra expenses that may be the result of research-related physical injuries including additional hospital bills, lost wages, travel expenses, etc. No compensation will be available for any non-physical injuries that may occur because of research participation, such as legal problems, problems with your finances or job, or damage to your reputation.

IV. Data Collection and Protection

A. Data Management and Security:

OARSI recommended[10] physical performance measures (30-second chair stand time, 40m fast-paced walking time, stair ascent time) along with patient-reported physical function (KOOS-PS), quality of life (KOOS-QoL) and NRS pain data will be collected at baseline and 6, 12 and 24 weeks post-treatment. All data collected will remain confidential and accessible only to the research team. This data will be collected using a paper-based

CRF that will be transferred into two databases. These two databases contain the same collection points so reconciliation can be done to detect data entry errors.

Data regarding subject demographics, knee radiographs, OARSI recommended physical performance measures, and KOOS subscale responses and NRS pain will be gathered from subjects and numerically coded in sequential order with no patient identifiers. A master list with patient information and assigned study IDs will be stored in a separate password-protected file available only to the Principal Investigator and the Research Coordinator. Analyses will be performed on coded files. Physical files (e.g. questionnaires) will be stored in locked file cabinets in the offices of the Principal Investigator.

B. Sample / Specimen Collection: N/A

C. Tissue Banking Considerations: N/A

D. Procedures to protect subject confidentiality:

Data regarding subject's demographic, knee radiographs, OARSI recommended physical performance measures, and KOOS subscale responses and NRS pain data will be obtained directly from subjects. All data will be coded with no information that could identify a subject. A master list with patient information and assigned study ID's will be stored in a password-protected file available only to the Principal Investigator and the Research Coordinator. Analyses will be performed on coded files. Physical files (e.g. questionnaires) will be stored in locked file cabinets in the office of the Principal Investigator.

E. Quality Assurance / Monitoring

Study staff will have proper qualifications for data entry and will receive training in data entry procedures to ensure data entered is of highest quality. Data will be entered by researchers into two separate databases developed for this study. Each database will contain the same collection points and then be reconciled to clean the data for analysis and outcome reports to the Principal Investigator. Data will be backed-up daily.

V. Data Analysis and Reporting

A. Statistical and Data Analysis:

The primary analysis will regard the change in the 3 OARSI-recommended physical performance measures between baseline and 12 weeks following injections. The three pre/post change scores will be assessed with one-sample t-test for matched pairs, using an alpha level of (.05/3) to determine statistical significant change.

Differences between baseline and other timepoints and secondary/tertiary outcome measures will be assessed with repeated measures analyses, with time as a repeated factor to account for multiple comparisons over time. A soft-lock of the database will occur after the 6- and 12-week time points. The analyses of secondary and tertiary outcomes will involve the analyses of longitudinal data, collected at baseline, 6 weeks, 12 weeks, and 24 weeks. These response variables will be modeled using the framework of longitudinal mixed models. Each model will be based on two main effects: a random subject effect, and a fixed time effect consisting of four levels (baseline, 3 follow-up time points). The distribution of the secondary and tertiary outcome measures will be examined and transformation to a normal distribution will be performed if needed. We will use the Akaike information criterion to determine an appropriate variance/covariance structure. Each of the hypotheses of interest will be tested by examining appropriate contrasts and estimable linear forms in the overall mean and the main effects for time. Analyses will be conducted using SAS (Version 9.1.2). PROC MIXED will be used to fit the longitudinal models.

B. Outcome: We expect statistically significant and clinically meaningful improvement in physical performance, physical function, pain and quality of life 12 weeks following bilateral treatment with Zilretta.

C. Study results to subjects: The study will be registered on www.clinicaltrials.gov and results posted upon completion of the study.

D. Publication Plan: Results will be submitted for presentation at an appropriate conference and for publication in an appropriate journal for the topic of study.

VI. Bibliography / References / Literature Cited

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APPENDIX I: VULNERABLE POPULATIONS

I. Recruitment will not include any vulnerable population.