CLINICAL STUDY PROTOCOL

An Open-Label Study to Evaluate the Effect of the Administration of FX006 on Synovial Inflammation in Patients with Osteoarthritis of the Knee

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STUDY MEDICATION(S): FX006

INDICATION: Osteoarthritis of the knee

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SIGNATURE PAGE

Clinical Study Protocol Version 6.0 (dated 12 July 2019)

Sponsor Safety Officer Approval

Signature: _____ **Date:** 15 Jul 2019

Name (print): *Scott Kelley, MD*

Title: Chief Medical Officer

Principal Investigator Agreement: I have read the protocol and agree to conduct the study as outlined herein.

Signature: _____ **Date:** _____

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1. ABBREVIATIONS AND DEFINITION OF TERMS

ACR	American College of Rheumatology
ADP	Average Daily Pain
AE	Adverse Event
AUE	Area Under the Effect Curve
BMI	Body Mass Index
CFR	Code of Federal Regulations
CGIC	Clinical Global Impression of Change
CI	Confidence Interval
cm	Centimeter
CMC	Carboxymethylcellulose Sodium
CSR	Clinical Study Report
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
EULAR	European League Against Rheumatism
FBR	Foreign Body Response
FDA	Food and Drug Administration
FOV	Field of View
GCP	Good Clinical Practice
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic-pituitary-adrenal
IA	Intra-articular
IB	Investigator's Brochure
IL-1β	Interleukin 1 beta
IRB/EC	Institutional Review Board/Ethics Committee
IM	Intramuscular
IV	Intravenous
JSN	Joint Space Narrowing
kg	Kilogram
KOOS	Knee injury and Osteoarthritis Outcome Score
LK	Likert
LSM	Least Square Mean
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter

mm	Millimeter
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid (RNA)
msec	Millisecond
NaCl	Sodium Chloride
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
PD	Pharmacodynamic(s)
PGIC	Patients' Global Impression of Change
PI	Principal Investigator
PLGA	Poly[lactic-co-glycolic acid]
PK	Pharmacokinetic(s)
PRP	Platelet Rich Plasma
QOL	Quality of Life
QTc	QT interval corrected for heart rate
RBC	Red Blood Cells
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TEAE	Treatment-emergent Adverse Event
TA[1]	Triamcinolone Acetonide
TAcs[2]	Triamcinolone Acetonide Injectable Suspension, Immediate-Release (commercially available)
US	United States of America
USP	United States Pharmacopeia
w/w	weight by weight
WBC	White Blood Cells
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

[1] Abbreviated in past protocols and documents as TCA
[2] Abbreviated in past protocols and documents as TCA-IR

2. SYNOPSIS

Title of Study: An Open-Label Study to Evaluate the Effect of the Administration of FX006 on Synovial Inflammation in Patients with Osteoarthritis of the Knee

Study Centers: Approximately 10 centers

Study Phase: 3b

Objectives:

The primary objective of this study is to assess the effect of a single intra-articular (IA) injection of FX006 32 milligrams (mg) to control inflammation as measured by a reduction in Magnetic Resonance Imaging (MRI) assessed synovial volume in patients with symptomatic osteoarthritis (OA) of the knee and defined synovial volume at baseline.

Secondary objectives of the study include the following:

- To assess the effect of FX006 on pain, stiffness, function and quality of life
- To assess changes in bone area and cartilage thickness of the knee

Exploratory objectives of the study include the following:

- To assess structural changes in bone, cartilage and meniscus
- To assess changes in contrast enhancement of tissue other than synovial tissue
- To identify Responders and Non-Responders by assessing structural and contrast changes in bone, cartilage and other tissues
- Through assessment of blood IL-1b Messenger Ribonucleic Acid (mRNA) levels, to segment patients into inflammatory and non-inflammatory phenotypes, and to examine the response to therapy of these phenotypes

Study Design and Methodology:

This is an open-label study assessing the effect of the administration of a single IA injection of FX006 32 mg on synovial volume in patients with OA of the knee. The study will be conducted in male and female patients who are ≥ 40 years of age.

Eligible patients who provide written consent and meet all entry criteria will undergo initial ultrasound examination and MRI with contrast of the index knee. For each MRI, a pretreatment synovial volume measurement will be derived by quantitative image analysis. Patients will then receive a single IA injection of FX006 administered to the index knee at Baseline/Day 1. Patients will return to the clinic at Weeks 6 and 24 for an MRI with contrast of the index knee and other assessments. Patients must also have a blood sample drawn for Estimated Glomerular Filtration Rate (eGFR) testing within 30 days prior to the scheduled MRIs. In addition, a patient questionnaire will be administered and adverse events (AEs) and concomitant medication updates will be collected via telephone at Weeks 12 and 18.

The study is expected to enroll over approximately 15 months.

Number of Patients:

Approximately 100 patients will be enrolled and treated with a single IA injection of 32 mg FX006 to ensure a minimum of 70 evaluable Synovitis Imaging patients, as defined by pre-treatment MRI synovial volume measurement.

Test Product, Dose and Mode of Administration:

FX006 – extended release formulation of triamcinolone acetonide (TA) in 75:25 poly(lactic-co-glycolic) acid (PLGA) microspheres: Nominal 32 mg TA, IA injection, administered as a 5 milliliter (mL) injection

Reference Compound(s), Dose and Mode of Administration:

Not applicable

Duration of Dosing:
Single IA injection of FX006

Inclusion Criteria:

To be included in the trial, patients must fulfill the following criteria:

1. Written consent to participate in the study
2. Male or female ≥ 40 years of age
3. Body mass index (BMI) ≤ 40 kg/m^2
4. Ambulatory and in good general health
5. Willing and able to comply with the study procedures and visit schedules and able to follow verbal and written instructions
6. Willing to abstain from use of the following protocol-restricted medications during the study:
    a. Topical or oral NSAIDs (from 5 days prior to Pre-treatment MRI through Week 6 MRI, and again from 5 days prior to Week 24 MRI)
    b. Intravenous (IV), intramuscular (IM) and oral corticosteroids (Note: inhaled, intranasal, and topical corticosteroids are allowed)
    c. IA corticosteroids in any joint other than the study medication
    d. Any IA intervention in the index knee including aspiration or the injection of any approved or investigational agent, including viscosupplementation (hyaluronic acid)
    e. Any investigational drug, device, or biologic
    f. Immunomodulators, immunosuppressives, or chemotherapeutic agents
    g. Live or attenuated vaccines
7. Symptoms associated with OA of the index knee for ≥ 6 months prior to Screening (patient self-report is acceptable)
8. Currently meets American College of Rheumatology (ACR) Criteria (clinical and radiological) for OA (Altman et al, 1986) as follows:
    a. Knee pain
    b. at least 1 of the following:
        i. Age > 50 years
        ii. Stiffness < 30 minutes
        iii. Crepitus
    c. Osteophytes (as determined by central reading facility of index knee X-ray obtained at Screening)
9. Kellgren-Lawrence (K-L) Grade 2 or 3 in the index knee based on X-ray performed during Screening (centrally read)
    a. Grade 2: definite osteophytes and possible narrowing of joint space
    b. Grade 3: moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends
10. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC A) total sum score of ≥ 6 at Screening and Baseline/Day 1
11. Index knee pain on most days over the last month (as reported by the patient).
12. If bilateral OA exists, pain in the contralateral knee must be less than pain in the index knee as reported by the patient; if pain is equal, index knee will be identified based on knee with highest level of inflammation as determined by ultrasound
13. Sexually active males and females of child-bearing potential (defined as not surgically sterile or post-menopausal [defined as 12 consecutive months with no menses without an alternative medical cause] for at least 1 year as documented in medical history) agree to use one of the following highly effective methods of contraception: abstinence; oral, injected or implanted hormonal methods of contraception; intrauterine device or intrauterine system; condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository; or monogamous intercourse with a partner who is surgically sterile (post-vasectomy, post-hysterectomy, or tubal ligation). Such contraceptive measures should be used throughout the duration of the study.

Exclusion Criteria:

Patients fulfilling at least one of the following criteria may not be included in the study:

Disease-related criteria

1. Any inflammatory arthritis including reactive arthritis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, arthritis associated with inflammatory bowel disease, gout or secondary OA from gout
2. History of infection or crystal disease in the index knee joint
3. Unstable index knee joint (such as a torn anterior cruciate ligament) within 12 months of Screening

Previous or concomitant treatment-related criteria

4. Presence of surgical hardware or other foreign body in the index knee
5. Surgery or arthroscopy of the index knee within 12 months of Screening
6. IA corticosteroid (investigational or marketed) in any joint within 3 months of Screening
7. IA treatment of the index knee with hyaluronic acid (investigational or marketed) within 6 months of Screening
8. IV or IM corticosteroids (investigational or marketed) within 3 months of Screening
9. Oral corticosteroids (investigational or marketed) within 1 month of Screening
10. Any other IA drug/biologic in the index knee within 6 months of Screening or 5 half-lives (whichever is longer) (e.g., platelet rich plasma (PRP) injection, stem cells, prolotherapy, and amniotic fluid injection)
11. Prior administration of FX006 (Zilretta®)
12. Planned/anticipated surgery of the index knee during the study period

Patient-related criteria

13. Suspected hypersensitivity to any form of triamcinolone
14. History of sarcoidosis or amyloidosis
15. Active or history of malignancy within the last 5 years, with the exception of resected basal cell carcinoma, squamous cell carcinoma of the skin, or effectively managed cervical carcinoma
16. History or evidence of active or latent systemic fungal or mycobacterial (including tuberculosis), or of ocular herpes simplex
17. Any bacterial or viral infection requiring IV antibiotics within 4 weeks of Screening or oral antibiotics within 2 weeks of Screening
18. History of osteomyelitis in the index leg at any time, or of other areas within 5 years
19. Laboratory evidence of infection with human immunodeficiency virus (HIV), positive test for hepatitis B surface antigen (HbsAg) or positive serology for hepatitis C virus (HCV) with positive test for HCV Ribonucleic Acid (RNA)
20. Any electrocardiogram (ECG) abnormality judged clinically significant by the Investigator.
21. A medical history suggesting the patient will or is likely to require a course of parenteral or oral corticosteroids during the study period
22. eGFR results < 40 mL/minute (reported as Creatinine Clearance)
23. Any contraindication to MRI Scanning (e.g., presence of certain ferromagnetic foreign bodies or electronic devices including most cardiac pacemakers, claustrophobia)
24. Known hypersensitivity to any form of MRI contrast
25. History of or active Cushing's syndrome
26. Active substance abuse (drugs or alcohol), or history of substance abuse within 12 months of screening
27. Skin breakdown at the knee where the injection would take place
28. Females who are pregnant or nursing or plan to become pregnant during the study; men who plan to conceive during the study

29. Use of immunomodulators, immunosuppressives, or chemotherapeutic agents within 5 years of Screening
30. Has received a live or live attenuated vaccine within 3 months of Screening
31. Use of any other investigational drug, biologic or device within 3 months of Screening
32. Any other clinically significant acute or chronic medical conditions (e.g., bleeding disorder) that, in the judgment of the Investigator, would preclude the use of an IA corticosteroid or that could compromise patient safety, limit the patient's ability to complete the study, and/or compromise the objectives of the study

Procedures and Assessments:

The study will involve a Screening period (up to 28 days), a pretreatment day when an Ultrasound and MRI of the index knee will be performed, dosing at Baseline/Day 1 and two additional clinic visits at Weeks 6 and 24. In addition, patients must also have a blood sample drawn for eGFR testing within 30 days prior to the scheduled MRIs. Follow-up via telephone will occur at Weeks 12 and 18.

At specified times throughout the study, patients will undergo physical examinations, index knee assessments, imaging of the index knee (MRI with contrast), 12-lead ECG; blood samples will be collected for laboratory safety tests and vital signs will be collected or measured. Information regarding AEs and concomitant medications will be collected, and patient questionnaires (Western Ontario and McMaster Universities (WOMAC®) Osteoarthritis Index Likert (LK) 3.1, the Knee injury and Osteoarthritis Outcome Score (KOOS) Quality of Life (QOL) Subscale), and a set of questions exploring pain and stiffness of the knee will be completed.

Patients will have index knee synovial fluid collected at Baseline/Day 1. These samples will be preserved for potential future OA biomarker analyses.

Refer to Schedule of Study Assessments for full details.

Blinding:

Not applicable; this is an open-label study.

Study Drug Administration Procedure:

IA injections will be performed by the assigned injector. The injector may choose the position of the knee (e.g., extended or flexed), the approach for the injection (e.g., medial or lateral) and the numbing agent to be used (e.g., ethyl chloride, subcutaneous lidocaine; IA anesthetics are not allowed) based on standard of care. Sterile technique should be used.

Prior to injection, the index knee should be thoroughly cleansed using a bactericidal solution. The index knee will be aspirated in all cases prior to administration of study medication. Following attempted aspiration, 5 mL of the reconstituted FX006 will be injected into the synovial space. *Refer to the Pharmacy binder for detailed instructions on how to prepare FX006.*

The same needle used for IA injection of the study medication may also be used for synovial fluid aspiration, thereby allowing for a single injection with syringe replacement. The injector will use a 21 gauge or larger needle for injection and aspiration of fluid.

Injector will record injection procedure and any product related issues relating to reconstitution or administration of FX006 (e.g. inability to achieve an appropriate suspension, inability to administer the full dose due to clogging of the needle) and report the occurrence to the Site Monitor, as detailed in the Pharmacy Binder.

Post-Injection Care

Patients should be advised to avoid strenuous activities or prolonged weight-bearing activities for approximately 24 to 48 hours following the injection and to also maintain a stable lifestyle with regard to physical activity throughout the duration of the study.

If the patient has an immediate reaction (e.g., tenderness, increased pain, swelling, effusion, decreased mobility of the index joint), the patient should be treated according to local clinical guidelines and physician experience.

Concomitant Medications:

The exclusion criteria indicate that a patient may not be enrolled if he/she has used any of the following within the specified windows. In addition, these medications should not be taken or used throughout the study:

- IV, IM, or oral corticosteroids
- IA corticosteroids in any joint
- Any IA intervention in index knee including aspiration or the injection of any approved or investigational agent, including viscosupplementation (hyaluronic acid).
- Any investigational drug, device, or biologic
- Immunomodulators, immunosuppressives, or chemotherapeutic agents
- Live or attenuated vaccines
- If patient is currently using other analgesics for pain relief, continued use is permitted; however, patient must abstain from use of topical or oral NSAIDs from 5 days prior to Pre-treatment MRI through Week 6, and again from 5 days prior to Week 24 MRI.

Criteria for Evaluation:

Imaging Variables

Synovial volume and structural changes in various tissues (bone, cartilage, meniscus) and change in contrast enhancement of tissues other than synovium will be evaluated based on MRI by a central imaging vendor.

Efficacy Variables

Efficacy will be evaluated based on the results of the WOMAC®LK 3.1, 5-point scale): pain, stiffness and function domains independently and collectively (Bellamy et al, 1988), the KOOS QOL and a set of questions exploring pain and stiffness of the knee.

Safety Variables

Safety and tolerability will be evaluated on the basis of AEs spontaneously reported by the patient or discovered by the Investigator and findings from the following assessments: physical examinations, index knee assessments, vital signs, and clinical laboratory evaluations.

Biomarker Variables

Synovial fluid samples will be preserved for a maximum of 5 years for potential future analyses of biomarkers that may contribute to the pathogenesis of OA and/or be associated with responsiveness to FX006 treatment. No genomic analyses (gene sequencing studies) will be performed using these samples. Patients will be able to withdraw consent throughout the duration of storage. Once analysis has begun, consent will no longer be able to be withdrawn.

Whole blood samples will be taken from patients at Baseline/Day 1. Total Ribonucleic Acid (RNA) will be isolated from these samples for analysis of messenger RNA (mRNA) levels of the pro-inflammatory cytokine interleukin 1 beta (IL-1β). The expression of baseline IL-1β among patients will be compared to several other parameters, such as Index Knee X-rays and MRI images, with the goal of determining if baseline mRNA IL-1β expression contributes to the pathogenesis of OA and/or is associated with responsiveness to FX006 treatment.

Sample Size Considerations:

Approximately 100 patients will be enrolled to ensure a minimum of 70 evaluable Synovitis Imaging patients, as defined by pre-treatment MRI synovial volume measurement of greater than 3000 mm^3 of gadolinium enhancement as determined by quantitative image analysis, with quality Pretreatment and Week 6 MRIs. Previous trials had similar sample sizes (Gait et al, 2016) (O'Neill et al., 2016).

Statistical Methods:

Complete details of the statistical analyses will be specified in the statistical analysis plan (SAP). Four analysis populations are planned for the study as follows:

- The Safety Population will include all patients who receive study drug. The Safety Population will be used to assess safety and tolerability.
- The Synovitis Imaging Population will include all patients from the Safety Population who have a pre-treatment synovial volume measurement of greater than 3000 mm^3 of gadolinium enhancement as determined by quantitative image analysis, as well as quality pre-treatment and Week 6 MRIs. In addition, the patients included in this population will have no major protocol deviations and/or imaging variations (i.e. change in coil, machine, or software) deemed to potentially impact the imaging analysis.
- The Imaging Population will include all patients from the Safety Population, who have quality MRI data available for pre-treatment and at least one post- baseline timepoint and with no major protocol deviations and/or imaging variations (i.e. change in coil, machine or software) deemed to potentially impact the imaging analysis.
- The Efficacy Population will include all patients from the Safety Population, who have at least one post-Baseline/Day 1 WOMAC or KOOS assessment.

Demographic and baseline characteristics will be listed by study site and patient and will be summarized. Frequencies and proportions will be presented for the categorical variables and descriptive statistics will be presented for continuous variables.

Primary efficacy analysis will be the mean standardized change from baseline at 6 weeks in synovial volume will be determined and the 95% confidence interval (CI) will be calculated. Secondary efficacy analyses include an absolute change (mm^3) from baseline at 6 weeks in synovial volume will also be calculated and the 95% CI will be presented. Standardized and absolute change from baseline will also be analyzed for synovial volume at 24 weeks.

Descriptive statistics and the 95% CIs will be calculated on pain, stiffness, function and quality of life. Other efficacy variables such as structural and contrast changes in bone, cartilage and other tissues based on the MRI results will be provided in the SAP and described in the Imaging Manual. A responder/non-responder analysis will be conducted to describe associations between physiological and functional/QOL measures and MRI results

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary. Incidences (number and percent) of treatment-emergent adverse events (TEAEs), those events that started after dosing or worsened after dosing, will be presented. Additional analyses of incidences of TEAEs will also be presented by maximum severity and by relationship to study medication.

Similar presentations will be provided for serious AEs, AEs leading to death, AEs leading to withdrawal from the study, and for AEs related to the index knee.

Clinical laboratory data and vital sign information will be summarized as summary statistics for value and change from Day 1 at each individual time point. Summary statistics will include n, mean, median, standard deviation, minimum, and maximum.

3. ETHICS

The study will be conducted according to the Declaration of Helsinki, and in accordance with local laws and regulations.

3.1. Institutional Review Board/Ethics Committee

This study will be conducted in compliance with current Good Clinical Practices (GCP), and in accordance with local laws and regulations.

This study protocol and other related study documents will be submitted to the Institutional Review Board/Ethics Committee (IRB/EC) by the site or the Sponsor for review and approval as dictated by local regulations. IRB/EC approval must be obtained before commencement of any study procedures. The study will be conducted only at sites where IRB/EC approval has been obtained.

3.2. Ethical Conduct of Study

This study will be conducted in accordance with the protocol, GCP guidelines and applicable national regulatory requirements.

GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that have originated in the Declaration of Helsinki and that the clinical study data are credible.

3.3. Patient Information and Consent

Prior to initiation of any study related procedures, patients will give their written consent to participate in the study after having been informed about the nature and purpose of the study, participation and termination conditions, and risks and benefits.

An IRB/EC-approved informed consent document must be signed by the patient before his or her participation in the study. A copy of the informed consent document must be provided to the patient. If applicable, it will be provided in a certified translation of the local language.

Signed informed consent forms must remain in each patient's study file and must be available for verification by study monitors at any time.

4. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

4.1. Investigators

A Principal Investigator (PI) will be responsible for study conduct at each center and may delegate study-related activities to appropriately qualified and trained staff. This delegation will be documented in a study-specific Delegation of Responsibilities form.

The contact information for all PIs participating in the trial will be kept in the Trial Master File.

4.2. Study Administrative Structure

The study will be managed by the Sponsor with specific responsibilities delegated to contract research organizations.

5. INTRODUCTION

5.1. Osteoarthritis

Osteoarthritis (OA) is a painful and debilitating musculoskeletal disease that is characterized by intra-articular (IA) inflammation, deterioration of articular cartilage, and degenerative changes to peri-articular and subchondral bone (Creamer and Hochberg, 1997; Goldring and Goldring, 2006). Arthritis is the most common cause of disability in the United States of America (US), and OA is the most common joint disease, affecting 27 million Americans, with numbers expected to grow as a result of aging, obesity and sports injuries. Recent data suggest that OA accounts for over $185 billion of annual healthcare expenditures in the US, which does not include loss of productivity costs. It is estimated that by 2030, 45 million people will have OA. OA commonly affect large weight-bearing joints like the knees and hips, but also occurs in the shoulders, hands, feet and spine. Patients with OA suffer from joint pain, tenderness, stiffness and limited movement. As the disease progresses, it becomes increasingly painful and debilitating, culminating, in many cases, in the need for total joint arthroplasty.

Current Guidelines from the American College of Rheumatology (ACR), Osteoarthritis Research Society International (OARSI) and the European League against Rheumatism (EULAR) recommend the use of IA corticosteroids for short-term acute pain relief (Hochberg et al, 2012; Jordan et al, 2003; Menge et al, 2014).

While historically OA has been considered a non-inflammatory disease, it is increasingly being recognized that chronic synovitis occurs in all stages of knee OA (Benito et al, 2005; Sellam and Berenbaum, 2010; Wenham and Conaghan, 2010). As synovial inflammation is correlated with clinical symptoms and joint degeneration, it should be an important target for therapeutic intervention. The inflamed synovium may well be the target for IA corticosteroids which are widely used in knee OA (Ayral et al, 2005).

5.2. Background

5.2.1. Investigational Medicinal Product: FX006

FX006 is an extended-release formulation of triamcinolone acetonide (TA) for IA administration. It is approved in the US under the trade name ZILRETTA® (triamcinolone acetonide extended-release injectable suspension) for the management of pain of osteoarthritis of the knee. FX006 is intended to deliver TA to the synovial and peri-synovial tissues for a period of approximately 3 months depending on the dose administered (Bodick et al, 2013).

FX006 contains TA, United States Pharmacopeia (Ph. Eur/USP), formulated in 75:25 poly(lactic-co-glycolic acid) (PLGA) microspheres with a nominal drug load of 25% (weight by weight [w/w]) and is provided as a sterile white to off-white powder for reconstitution. The drug product is reconstituted with diluent containing an isotonic, sterile aqueous solution of sodium chloride (NaCl; 0.9% w/w), carboxymethylcellulose sodium (CMC; 0.5% w/w) and polysorbate-80 (0.1% w/w) to form a suspension prior to IA injection.

Further details of the physiochemical properties of FX006 can be found in the Investigator's Brochure (IB).

5.2.2. Rationale for FX006 in OA of the Knee

Available clinical and nonclinical data indicate that FX006 is safe and well tolerated when administered as a single injection into one knee and provides pain relief that is meaningfully better and more persistent than that provided by triamcinolone acetonide injectable suspension, immediate-release (commercially available) (TAcs). Nonclinical data and the literature suggest that this potential could extend to limiting structural progression in patients with inflammatory joint disorders. The near term clinical development program for FX006 focuses on analgesics effects in patient with OA (bilateral knee, hip, and shoulder) as well as other shoulder maladies.

5.2.3. Toxicology

Overall, single or repeat administration of FX006 at the proposed clinical dose of 32 mg has no new safety liabilities compared to TAcs:

- Systemic findings were similar among TAcs and FX006 groups following single and repeat dosing and were generally reversible. Initial effects on clinical pathology parameters were more pronounced for the immediate-release form. The incidence and/or intensity of steroid-associated systemic histopathological findings at the later time points were slightly higher for high dose FX006 than for TAcs at the same dose level of TA (18.75 mg/mL/joint), as expected based on the sustained release of TA. Microspheres were not detected in tissues outside of the synovial space.
- Local findings were similar among the TAcs and the FX006 groups and were reversible. The single and repeat dose dog toxicity studies recapitulated known, previously published, effects of TA in normal animal joints following prolonged exposure. These include decreased Safranin O staining (single or repeat dose) and changes in structure and cellularity of articular cartilage (repeated dosing only).
- An expected, mild, reversible Foreign Body Response (FBR) was noted to the PLGA component of FX006 microspheres.
  - The local tissue response to the presence of blank microspheres as well as FX006 microspheres consisted of an expected FBR of macrophage and multinucleated giant cell infiltration involving the synovium. Following a single dose, the FBR was evident at Day 4, peaked at approximately 6 weeks and was completely resolved by 6 months in all FX006-dosed animals. Occasional lymphocyte and plasma cell infiltrates and sporadic focal-to-multifocal areas of minimal-to-slight fibrosis resolved by 9 months. Following repeat IA dosing, a similar local, reversible FBR was noted.
  - Further, the dogs in these studies showed no local signs of inflammation on or around the joint and did not display pain, discomfort or difficulty in ambulation in any treatment group; hence, this local response was considered to be nonadverse.

Information available for TA from the literature, corticosteroid product labels and clinical experience suggest that the potential of genetic toxicity, reproductive toxicity and carcinogenic potential of TA are well understood. Similarly, the biocompatibility and local safety of PLGA microspheres, and genotoxic, reproductive toxicological and carcinogenic potential of PLGA have been described in a combination of literature and product information packages. Therefore, no new risks relative to TAcs are presented by FX006 as intended for use.

5.2.4. Systemic and Local Pharmacokinetics (PK) in Patients with Osteoarthritis of the Knee

Overall, FX006 displayed a favorable plasma PK profile relative to that of TAcs.

PK observations resulted in a controlled and stable release of TA from PLGA microspheres into synovial tissues, where concentrations remained high relative to plasma concentrations for at least 12 weeks. TA was absorbed systemically, with a plateau in plasma TA concentrations occurring in the first 24 hours post-dose, and slow elimination from the systemic circulation observed in the weeks thereafter.

Relative to TAcs, 32 mg FX006 produced substantially lower peak plasma and systemic exposure to TA. FX006 performed as expected, prolonging the residence of TA in the joint while minimizing systemic exposure to TA.

5.2.5. Pharmacodynamics (PD) in Patients with Osteoarthritis of the Knee

In a Phase 2 PK/ PD study evaluating three dose levels of FX006 (10 mg, 40 mg, 60 mg) administered as a 3 mL injection, suppression of cortisol in the days following injection produced by the 10 and 40 mg dose of FX006 was less than that produced by injection of TAcs; the 60 mg dose of FX006 produced effects similar to 40 mg TAcs. Cortisol suppression subsequent to Day 1-2 associated with all doses of FX006 would not be expected to be of clinical consequence in adult patients without otherwise compromised hypothalamic-pituitary-adrenal (HPA) axis function.

In a Phase 2 study in diabetic patients with knee OA, treatment with 32 mg FX006 resulted in a statistically significant ($p=0.0452$) reduction in blood glucose elevation relative to TAcs over a 72-hour period following IA injection. The time in glycemic target range (70-180 mg/dL) (American Diabetes Association, 2016) was greater for FX006 as compared to TAcs over the 48 hours post IA injection, providing another indication of the improvement in glycemic control. Over the entire time course of the 15-day post injection glucose monitoring period, blood glucose levels associated with FX006 remained at levels similar to or lower than those produced by TAcs. This observation is consistent with PK studies demonstrating low systemic exposure to TA associated with FX006.

5.2.6. Efficacy in Patients with Osteoarthritis of the Knee

Efficacy data from three studies provide substantial evidence supporting the effectiveness of 32 mg FX006 in the management of OA pain. (Bodick et al, 2015; Conaghan et al, 2017)

Results of the primary endpoint from the Phase 3, multi-center, adequate, and well-controlled trial, showed that patients treated with 32 mg FX006 had a rapid, durable, and meaningful analgesic response that was statistically significantly better than placebo treated patients ($P<0.0001$). This finding was supported by a second smaller Phase 2b study, where a highly similar pattern of response to 32 mg FX006 was demonstrated.

Robustness of the primary outcome in the Phase 3 study was further supported by the internal consistency demonstrated in favor of 32 mg FX006 through secondary analyses utilizing the primary outcome data (average daily pain [ADP]) to evaluate durability and magnitude of response. These included least square mean (LSM) testing at each week and area under the effect curve (AUE) analyses for Weeks 1 through 12 and Weeks 1 through 24. Results demonstrated

that the analgesic effect of 32 mg FX006 is significant at Week 1, increases through Week 7, and is sustained through at least Week 16. Responder analyses further suggested that FX006 provides clinically relevant improvement from Weeks 1 through 16 relative to placebo.

Analyses utilizing data collected from other instruments or measures, i.e., Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Patients' Global Impression of Change (PGIC), Clinical Global Impression of Change (CGIC), and Knee injury and Osteoarthritis Outcome Score (KOOS) Quality of Life (QOL), provided additional insight into effects on pain relief as well as physical function and global well-being. FX006 32 mg provides clinically relevant improvement relative to placebo through Week 12 for WOMAC and KOOS QOL and through at least Week 16 for PGIC and CGIC. Additionally, significant reduction of rescue medication utilization in patients treated with FX006 32 mg is of potential important clinical consequence and adds a meaningful element to the overall effectiveness profile of FX006 32 mg. Collectively, these results provide substantial evidence to support FX006 32 mg as an effective therapy for the management of OA knee pain.

5.2.7. Systemic and Local Safety in Patients with Osteoarthritis of the Knee

The evaluation of 687 patients treated with a single IA injection of FX006 at any dose in the FX006 clinical studies suggests that it was well tolerated with systemic and local safety profiles similar to those of TAcs and placebo.

The safety data from the FX006 clinical studies are largely consistent.

- The number of TEAEs reported was similar across groups (FX006 46.0%; placebo 49.2%; TAcs 51.0%).
- The majority of TEAEs in FX006-treated patients were mild or moderate (Grade 1 or 2). Severe or life-threatening events occurred in the FX006-treated patients at a rate of 3.0% as compared to 5.0% and 2.6% in the placebo and TAcs groups, respectively.
- In the FX006-treated patients (n=687), the most common TEAEs were:
  - Arthralgia (in any joint) 9.8% (n=67)
  - Headache 5.4% (n=37)
  - Upper Respiratory Tract Infection 3.1% (n=21)
  - Joint swelling 2.8% (n=19)
  - Contusion and back pain 2.3% (n=16)
  - Nasopharyngitis 2.2% (n=15)
- The rate of serious adverse events (SAEs) was low and consistent across groups (FX006 1.9%; placebo 1.1%; TAcs 2.3%); none were considered related to the study drug.
- Across all studies there were no deaths.

In the Phase 3 study, qualitative assessments based on X-rays of the index knee at 24 weeks post injection included joint space narrowing (JSN), subchondral bone changes, osteonecrosis, and insufficiency fracture.

- The overall rate of JSN worsening of at least 1-grade between baseline and Week 24 was low and similar among treatment groups (5.0% [7/140], 4.1% [6/148], and 3.5% [5/145] of patients with both baseline and Week 24 X-rays in the 32 mg FX006, placebo, and TAcs groups respectively); for all but 1 of these 18 patients, JSN worsened by 1-grade only. The remaining patient (in the placebo group) had a 2-grade worsening in JSN (from 0 at baseline to Grade 2 at Week 24).
- No FX006-treated patient had X-ray evidence of treatment-emergent insufficiency fracture, subchondral bone changes, or osteonecrosis at Week 24.
- Eighteen patients discontinued the study prior to Week 24 and completed a final X-ray as part of early termination visit. Of these, 2 patients, 1 in the 32 mg FX006 group and 1 in the placebo group, had a 1-grade increase in JSN. There were no reports of insufficiency fracture, subchondral bone changes, or osteonecrosis.

5.2.8. Conclusion

These data provide bases for continued clinical study of FX006.

6. STUDY OBJECTIVES

6.1. Primary Objective

The primary objective of this study is to assess the effect of a single IA injection of FX006 32 mg to control inflammation as measured by a reduction in MRI-assessed synovial volume in patients with symptomatic OA of the knee and defined synovial volume at baseline.

6.2. Secondary Objectives

The secondary objectives of this study are:

- to assess the effect of FX006 on pain, stiffness, function, and quality of life.
- to assess changes in bone area and cartilage thickness of the knee.

6.3. Exploratory Objectives

The exploratory objectives of this study are:

- to assess structural changes in bone, cartilage, and meniscus.
- to assess changes in contrast enhancement of tissue other than synovial tissue.
- to identify Responders and Non-Responders by assessing structural and contrast changes in bone, cartilage, and other tissues.
- through assessment of blood IL-1b mRNA levels, to segment patients into inflammatory and non-inflammatory phenotypes, and to examine the response to therapy of these phenotypes.

7. INVESTIGATIONAL PLAN

7.1. Overall Study Design and Plan

This is an open-label study assessing the effect of the administration of a single IA injection of FX006 32 mg on synovial volume in patients with OA of the knee. The study will be conducted in male and female patients who are ≥ 40 years of age.

Eligible patients who provide written consent and meet all entry criteria will undergo initial ultrasound examination and MRI with contrast of the index knee. For each MRI, a pre-treatment synovial volume measurement will be derived by quantitative image analysis. Patients will then receive a single IA injection of FX006 administered to the index knee at Baseline/Day 1. Patients will return to the clinic at Weeks 6 and 24 for an MRI with contrast of the index knee and other assessments. Patients must also have a blood sample drawn for eGFR testing within 30 days prior to the scheduled MRIs. In addition, a patient questionnaire will be administered, and AEs and concomitant medication updates will be collected via telephone at Weeks 12 and 18.

Refer to Table 1, Schedule of Study Assessments for full details.

The study is expected to enroll over approximately 15 months.

7.2. Site Staffing Requirements

The PI is responsible for overseeing the conduct of the study at his/her site, ensuring that sufficient and appropriately experienced staff are available to conduct the trial, and ensuring that activities are appropriately delegated and documented. Any delegation of responsibilities will be documented in a study-specific Clinical Site Responsibilities and Signature log. The term 'Principal Investigator' is used throughout this protocol to refer to the actual PI and/or his/her delegated team member(s) for the specific responsibility being described.

Pharmacist/Coordinator

- Must be a registered pharmacist or an individual with the qualifications and training required to handle and prepare study medications
- Is responsible for handling and preparing all study medications and maintaining investigational product accountability records

Injector/Aspirator

- Must be a medical doctor, a physician's assistant, or nurse practitioner experienced in administering IA injections and performing synovial fluid aspirations of the knee
- Is responsible for performing IA injections of study medication and synovial fluid aspirations of the knee

Assessor

- Must be either the PI or sub-investigator listed on the FDA Form 1572
- Must be a medical doctor, doctor of osteopathic medicine, a physician's assistant, or nurse practitioner

- Must have relevant OA experience
- Is responsible for performing the physical examination and index knee assessments
- Can be responsible for assessing ultrasound images for the presence of synovitis only if a rheumatologist or orthopedic specialty
- Is responsible for assessing all adverse event (AE)s including seriousness, severity, and causality

Radiologist

- Can be responsible for assessing ultrasound images for the presence of synovitis only if a medical doctor specializing in radiology
  - Must be either the PI or physician sub-investigator

Imaging Technician

- Must be an experienced technician in performing required imaging
- Is responsible for performing the required imaging

The same individual may serve in multiple roles (e.g., a physician sub-investigator may serve as both the Injector and/or Assessor).

7.3. Discussion of Study Design

7.3.1. Rationale for Study Population

Patients with pain associated with OA of either the knee as defined by clinical and radiologic criteria that are otherwise in good health or that have chronic conditions (for example, hypertension) that are well controlled are eligible. In general, this population tolerates IA injections of commercially available corticosteroids (Habib 2009). In prior clinical studies of FX006 in this population, single injections of up to 60 mg of FX006 were well tolerated.

7.3.2. Rationale for Dose Selection

The US Food and Drug Administration (FDA) approved dose of FX006 as an approved treatment for pain associated with OA of the knee is 32 mg; therefore, this dose was selected for further study in investigational uses.

7.3.3. Rationale for Study Design

The current trial design is substantially similar to synovitis studies previously conducted in OA of the knee (Gait et al, 2016; O'Neill et al, 2016). The design applied in this protocol has proved reliable for evaluating changes in synovitis and synovial tissue volume.

7.3.4. Rationale for Study Parameters

Synovial volume and structural changes in various tissues (bone, cartilage, meniscus) and change in contrast enhancement of tissues other than synovium evaluated on the basis of MRI results directly support the assessment of the effect on synovial inflammation following IA

administration of FX006, and the analyses are substantially similar to those conducted for assessment of synovitis and changes in synovial tissue volume.

The clinical safety parameters to be assessed (adverse events, physical examinations, index knee examinations, vital signs, and clinical laboratory evaluations) are standard safety and tolerability assessments and support the clinical monitoring necessary based on the safety profile for FX006.

7.3.5. Rationale for Control Type

Not applicable. This is a single arm open-label study.

7.4. Selection of Study Population

7.4.1. Number of Patients

Approximately 100 patients will be enrolled and treated with a single IA injection of 32 mg FX006 to ensure a minimum of 70 evaluable Synovitis Imaging patients, as defined by pre-treatment MRI synovial volume measurement.

7.4.2. Inclusion Criteria

To be included in the trial, patients must fulfill the following criteria:

1. Written consent to participate in the study
2. Male or female ≥ 40 years of age
3. Body mass index (BMI) ≤ 40 kg/m^2
4. Ambulatory and in good general health
5. Willing and able to comply with the study procedures and visit schedules and able to follow verbal and written instructions
6. Willing to abstain from use of the following protocol-restricted medications during the study:
    - Topical or oral NSAIDs (from 5 days prior to Pre-treatment MRI through Week 6, and again from 5 days prior to Week 24 MRI)
    - Intravenous (IV), intramuscular (IM) and oral corticosteroids (Note: inhaled, intranasal, and topical corticosteroids are allowed)
    - IA corticosteroids in any joint (other than the study medication)
    - Any IA intervention in the index knee including aspiration or the injection of any approved or investigational agent, including viscosupplementation (hyaluronic acid)
    - Any investigational drug, device or biologic
    - Immunomodulators, immunosuppressives, or chemotherapeutic agents
    - Live or attenuated vaccines
7. Symptoms consistent with OA of the index knee for ≥ 6 months prior to Screening (patient self-report is acceptable)

8. Currently meets ACR Criteria (clinical and radiological) for OA (Altman et al, 1989) as follows:

- Knee pain
- at least 1 of the following:
    - Age > 50 years
    - Stiffness < 30 minutes
    - Crepitus
- Osteophytes (as determined by central reading facility of index knee X-ray obtained at Screening)

9. Kellgren-Lawrence (K-L) Grade 2 or 3 in the index knee based on X-ray performed during Screening (centrally read)
    a) Grade 2: definite osteophytes and possible narrowing of joint space
    b) Grade 3: moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends
10. WOMAC A total sum score of ≥ 6 at Screening and Baseline/Day 1
11. Index knee pain on most days over the last month (as reported by the patient)
12. If bilateral OA exists, pain in the contralateral knee must be less than pain in the index knee as reported by the patient; if pain is equal, index knee will be identified based on knee with highest level of inflammation as determined by ultrasound
13. Sexually active males and females of child-bearing potential (defined as not surgically sterile or post-menopausal [defined as 12 consecutive months with no menses without an alternative medical cause] for at least 1 year as documented in medical history) agree to use one of the following highly effective methods of contraception: abstinence; oral, injected, or implanted hormonal methods of contraception; intrauterine device or intrauterine system; condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository; or monogamous intercourse with a partner who is surgically sterile (post-vasectomy, post-hysterectomy, or tubal ligation). Such contraceptive measures should be used throughout the duration of the study.

7.4.3. Exclusion Criteria

Patients fulfilling at least one of the following criteria may not be included in the study:

Disease-related criteria

1. Any inflammatory arthritis including reactive arthritis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, arthritis associated with inflammatory bowel disease, gout, or secondary OA from gout
2. History of infection or crystal disease in the index knee joint

3. Unstable index knee joint (such as a torn anterior cruciate ligament) within 12 months of Screening.

Previous or concomitant treatment-related criteria

4. Presence of surgical hardware or other foreign body in the index knee
5. Surgery or arthroscopy of the index knee within 12 months of Screening
6. IA corticosteroid (investigational or marketed) in any joint within 3 months of Screening
7. IA treatment of the index knee with hyaluronic acid (investigational or marketed) within 6 months of Screening
8. IV or IM corticosteroids (investigational or marketed) within 3 months of Screening
9. Oral corticosteroids (investigational or marketed) within 1 month of Screening
10. Any other IA drug/biologic in the index knee within 6 months of Screening or 5 half-lives (whichever is longer) (e.g., platelet rich plasma (PRP) injection, stem cells, prolotherapy, and amniotic fluid injection)
11. Prior administration of FX006 (Zilretta®)
12. Planned/anticipated surgery of the index knee during the study period

Patient-related criteria

13. Suspected hypersensitivity to any form of triamcinolone
14. History of sarcoidosis or amyloidosis
15. Active or history of malignancy within the last 5 years, with the exception of resected basal cell carcinoma, squamous cell carcinoma of the skin, or effectively managed cervical carcinoma
16. History or evidence of active or latent systemic fungal or mycobacterial infection (including tuberculosis), or of ocular herpes simplex
17. Any bacterial or viral infection requiring IV antibiotics within 4 weeks of Screening or oral antibiotics within 2 weeks of Screening
18. History of osteomyelitis in the index leg at any time, or of other areas within 5 years
19. Laboratory evidence of infection with human immunodeficiency virus (HIV), positive test for hepatitis B surface antigen (HbsAg), or positive serology for hepatitis C virus (HCV) with positive test for HCV Ribonucleic Acid (RNA)
20. Any electrocardiogram (ECG) abnormality judged clinically significant by the Investigator
21. A medical history suggesting the patient will or is likely to require a course of parenteral or oral corticosteroids during the study period
22. eGFR results < 40 mL/minute (reported as Creatinine Clearance)
23. Any contraindication to MRI Scanning (e.g., presence of certain ferromagnetic foreign bodies or electronic devices including most cardiac pacemakers, claustrophobia)

24. Known hypersensitivity to any form of MRI contrast
25. History of or active Cushing's syndrome
26. Active substance abuse (drugs or alcohol) or history of substance abuse within 12 months of Screening
27. Skin breakdown at the knee where the injection would take place
28. Females who are pregnant or nursing or plan to become pregnant during the study; men who plan to conceive during the study
29. Use of immunomodulators, immunosuppressives, or chemotherapeutic agents within 5 years of Screening
30. Has received a live or live attenuated vaccine within 3 months of Screening
31. Use of any other investigational drug, biologic, or device within 3 months of Screening
32. Any other clinically significant acute or chronic medical conditions (e.g., bleeding disorder) that, in the judgment of the Investigator, would preclude the use of an IA corticosteroid or that could compromise patient safety, limit the patient's ability to complete the study, and/or compromise the objectives of the study.

7.4.4. Screen Failures

Minimal data for patients who fail screening such as demographic information and the reason for screen failure will be collected.

Patients that fail to meet eligibility criteria may be re-screened at the discretion of the Medical Monitor. The Medical Monitor will clearly document the rationale for any re-screening decision. Patients that are allowed to re-screen will be assigned a new screening number, re-consented and may have screening assessments repeated if necessary.

7.5. Treatment Administered

7.5.1. Study Medication Treatment Arm(s)

Investigational Medicinal Product Arm:

- FX006: an extended-release formulation of TA in 75:25 PLGA microspheres. Nominal 32 mg TA, administered as a single 5 mL IA injection.

7.5.2. Identity of Investigational Product(s)

FX006 is supplied as a sterile, white to off white powder in a single unit dose 5 mL vial with a butyl rubber stopper, aluminum seal and plastic cap. FX006 is reconstituted in diluent containing an isotonic, sterile aqueous solution of sodium chloride (NaCl; 0.9% w/w), carboxymethylcellulose sodium (CMC; 0.5% w/w) and polysorbate-80 (0.1% w/w) to form a suspension prior to IA injection. Diluent will be supplied as a sterile liquid in a 5 mL vial with a butyl rubber stopper, aluminum seal and plastic cap. FX006 will be reconstituted in 5.0 mL of diluent to form a suspension immediately prior to IA injection. FX006 will be administered as a single 5 mL IA injection.

7.5.3. Receipt, Preparation, Dispensing, and Storage

Study medication will be shipped to the site from the drug supply distribution center. Receipt and dispensation of study medication will be properly documented within the Drug Tracking Module. Any temperature excursions should be documented in the Drug Tracking Module and on specified documents for Sponsor assessment and authorization for continued use.

Dispensing, preparation and administration of investigational products occurs under the supervision of the Principal Investigator or a qualified sub-investigator. The Principal Investigator may only delegate these activities in accordance with state licensing board requirements, local institutional policies, and applicable law. Before delegating this activity, the Principal Investigator should also ensure that the delegate is trained on and understands the requirements of the protocol.

The packaged kits of FX006 will be stored in a secure area and will be stored refrigerated at 2 to 8 °C.

7.5.4. Packaging and Labeling of Study Medication

The packaged kit of FX006 will contain one (1) vial of FX006, one (1) vial of Diluent, and a vial adapter. The FX006 and diluent vials will be labelled with their respective unique lot numbers within the packaged kit, which will be affixed with its own label and kit number.

7.5.5. Return of Study Medication

All study medications (packaged kits/used and unused vials) will be returned to the drug supply distribution center. Return of study medications will be properly documented.

7.5.6. Method of Assigning Patients to Treatment Groups

This is a single arm open-label study. All patients will receive a single IA injection of FX006 32 mg.

7.5.7. Blinding

This is an open-label study.

7.5.8. Study Drug Administration Procedure

IA injections of study drug will be performed by the assigned injector. The injector may choose the position of the knee (e.g., extended or flexed), the approach for the injection (e.g., medial or lateral) and the numbing agent to be used based on standard of care. Sterile technique should be used.

Prior to injection, the index knee should be thoroughly cleansed using a bactericidal solution. The index knee will be aspirated in all cases prior to administration of study medication. Following attempted aspiration, 5 mL of the reconstituted FX006 will be injected into the synovial space. Refer to the Pharmacy Binder for detailed instructions on how to prepare FX006.

The same needle used for synovial fluid aspiration may also be used for IA injection of study drug, thereby allowing for a single injection with syringe replacement. The injector will use a 21 gauge or larger needle for injection and aspiration of fluid.

Injector will record injection procedure and any product related issues relating to reconstitution or administration of FX006 (e.g. inability to achieve an appropriate suspension, inability to administer the full dose due to clogging of the needle) and report the occurrence to the Site Monitor, as detailed in the Pharmacy Binder.

Post-Injection Care

Patients should be advised to avoid strenuous activities or prolonged weight-bearing activities for approximately 24 to 48 hours following the injection and to also maintain a stable lifestyle with regard to physical activity throughout the duration of the study.

If the patient has an immediate reaction (e.g., tenderness, increased pain, swelling, effusion, decreased mobility of the index joint) the patient should be treated according to local clinical guidelines and physician experience. Refer to protocol Section 8.4.3.

7.5.9. Treatment Compliance

Study medication will be administered by the injector in the clinic. Details regarding study medication administration will be documented in the electronic Case Report Form (eCRF). The receipt, dispensation, and return/destruction of any study medication will be properly documented per the instructions within the Drug Tracking Module.

If for any reason the administration of study medication is stopped before the entire volume is injected, the injector should document the reason for stopping administration and record the volume delivered.

7.5.10. Removal of Patients from Therapy or Assessments

Each patient in this study receives study medication as a single IA injection. Therefore, discontinuation from treatment is not applicable. However, each patient may discontinue from the study for further assessments and study visits.

Each patient will be informed of his/her right to discontinue from the study at any time for any reason and without prejudice to alternative treatment. The PI may also discontinue a patient from the study at any time if, for example, he/she considers the patient's health to be compromised by remaining in the study, or the study is prematurely terminated. In these cases, the PI will:

1. Attempt to arrange for a formal Early Termination Visit and complete the study assessments specified for the End-of-Study scheduled for Week 24.
2. Determine whether the patient is willing to be contacted to follow ongoing or new AEs through Week 24 (if reason for discontinuation is not "subject withdrew consent").
3. Document patient consent in the source document for continued follow-up.
4. Contact the patient as necessary (via phone or in-person) to follow ongoing or new AEs through Week 24 (concomitant medications associated with any AE will also be captured).

Data collected from discontinued patients will be included in the clinical study report. Patients who discontinue from the study may be replaced at the discretion of the Sponsor.

7.6. Prior and Concomitant Medications and Therapies

During the study, all existing (prior to study entry), new, or changes in concomitant medications and the associated reasons for use or change will be documented and reported.

7.6.1. Allowable Medications and Therapies

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

- Any treatment for a pre-existing condition or for an AE, including the study indication (e.g., analgesic medications), that is not listed below as restricted
- Physical therapy for index knee
- Bracing of index knee

7.6.2. Restricted Medications

Per the exclusion criteria, a patient is not eligible for this study if he/she has received any of the indicated treatments within the specified windows detailed in the Exclusion criteria (Section 7.4.3). In addition, the following medications should not be taken or used from the time of obtaining consent to the End of Study visit:

- Topical or oral NSAIDs (from 5 days prior to Pre-treatment MRI through Week 6, and again from 5 days prior to Week 24 MRI)
- IV, IM, or oral corticosteroids
- IA corticosteroids in any joint
- Any IA intervention in index knee including aspiration or the injection of any approved or investigational agent, including viscosupplementation (hyaluronic acid)
- Any investigational drug, device or biologic
- Immunomodulators, immunosuppressives, or chemotherapeutic agents
- Live or attenuated vaccines

7.7. Study Variables

7.7.1. Safety Variables

Safety and tolerability will be evaluated based on AEs spontaneously reported by the patient or discovered by the Investigator and findings from the following assessments: physical examinations, index knee assessments, vital signs, and clinical laboratory evaluations.

7.7.2. Imaging Variables

Synovial volume and structural changes in various tissues (bone, cartilage, meniscus) and change in contrast enhancement of tissues other than synovium will be evaluated on the basis of MRI by a central imaging vendor.

7.7.3. Efficacy Variables

Efficacy will be evaluated on the basis of the results of the WOMAC® Osteoarthritis Index LK 3.1, 5-point scale): pain, stiffness and function domains independently and collectively (Bellamy et al, 1988), the KOOS QOL Subscale (http://www.koos.nu/) and a set of questions exploring pain and stiffness of the knee.

7.7.4. Biomarker Variables

Synovial fluid samples will be preserved for a maximum of 5 years for potential future analyses of biomarkers that may contribute to the pathogenesis of OA and/or be associated with responsiveness to FX006 treatment. No genomic analyses (gene sequencing studies) will be performed using these samples. Patients will be able to withdraw consent throughout the duration of storage. Once analysis has begun, consent will no longer be able to be withdrawn.

Whole blood samples will be taken from patients at Baseline/Day 1. Total RNA will be isolated from these samples for analysis of messenger RNA (mRNA) levels of the pro-inflammatory cytokine IL-1β. The expression of baseline IL-1β among patients will be compared to several other parameters, such as index knee X-rays and MRI images, with the goal of determining if baseline mRNA IL-1β expression contributes to the pathogenesis of OA and/or is associated with responsiveness to FX006 treatment.

7.8. Schedule of Study Assessments

A summary of the schedule of study assessments is provided in Table 1. Refer to Section 7.9 for details of each assessment.

Table 1: Schedule of Study Assessments

Procedures	Screening[1]	Pre-Treatment[2]	Baseline / Day 1	Week 6[3]	Week 12[3,4]	Week 18[3,4]	Week 24 / End of Study[3]
Informed consent	X[5]						
Inclusion/Exclusion Review	X		X[6]				
Medical History/Update	X		X[6]				
OA Medical History/Update	X		X[6]				
Prior Treatment & Medications[7]	X		X[6]				
Physical Examination	X						X
Index Knee X-ray[8]	X						
Index Knee Assessment[9]	X		X[6]	X			X
Ultrasound examination of index knee[10]		X					
MRI with contrast of index knee[11]		X		X			X
12-Lead ECG	X						
Vital Signs	X		X[6]				X
Height	X						
Weight and BMI	X						X
Hematology & Chemistry[12]	X						X
HIV, Hepatitis B/C[12]	X						
IL-1β mRNA levels[12]			X[6]				
eGFR[12, 13]	X			X			X
Serum Pregnancy Test[14]	X						
Urine Pregnancy Test [14]			X[6]				X
WOMAC[15]	X		X[6]	X	X	X	X
Supplemental pain and stiffness questionnaire[15]			X[6]	X			X
KOOS QOL[15]			X[6]	X			X
Index knee aspiration and collection of synovial fluid[8]			X[6]				
Treatment administration			X				
AEs & ConMeds [16]			----------X----------				
SAEs[17]	----------X----------						

[1] Screening may occur up to 28 days prior to Day 1
[2] The Pre-Treatment visit must be within 10 days of Baseline/Day 1 and once eligibility is confirmed.
[3] Visit should be conducted within +/- 7 days from scheduled date
[4] Via telephone
[5] Consent must be obtained prior to performing any study-specific procedures
[6] Complete assessment prior to dosing
[7] Record any medications received within 30 days prior to the Screening visit
[8] Standing, fixed flexion PA view, weight bearing X-ray of the index knee will be taken using a standardized knee positioning device. The Screening X-ray will be read centrally for KL grade, the presence of osteophytes and the measurement of joint space width.
[9] Index knee will be assessed for tenderness, heat/redness, swelling, effusion, and Baker's cyst. New clinically significant findings or findings that worsen from the patient's baseline condition should be recorded as AEs.
[10] Ultrasound must be performed within -10 days prior to Baseline/Day 1 and using a linear transducer and with the knee in a semi-flexed position. Synovial proliferation will be confirmed in a transverse view but measured in a longitudinal view only.
[11] MRI must be performed within -10 days prior to Baseline/Day 1 and +/- 7 days of Weeks 6 and 24 and only if eGFR results are ≥ 40 mL/min per the central laboratory. MRI images should follow the vendor specific knee sequences as detailed in MRI Acquisition Manual.
[12] Via Central Laboratory
[13] Must be performed within 30 days prior to Week 6 and Week 24 MRI with contrast.
[14] Conduct for females of childbearing potential only. Serum pregnancy test to be performed via central laboratory at Screening; urine pregnancy test to be performed locally at Baseline/Day 1 and results available prior to dosing.
[15] Patient questionnaires to be completed prior to all other assessments
[16] AEs and Concomitant Medications will be captured from Day 1 (from start of study drug administration) to Week 24/Final Visit
[17] SAEs will be recorded from Informed Consent to the end of participation in the study or Week 24/Final Visit

7.9. Study Procedures

7.9.1. Informed Consent

Prior to initiation of any study related procedures, patients will be informed about the nature and purpose of the study, participation and termination conditions, and risks and benefits. Patients will be given the opportunity to ask questions of site personnel and to discuss with family or friends if they wish. After a patient has had ample opportunity to consider the information provided, they will be asked to sign the study's informed consent form to participate in the study.

7.9.2. Review of Eligibility, Medical History, Prior Treatment, and Medications

Eligibility criteria (inclusion and exclusion criteria), medical history (including OA history), and prior treatment and medications are reviewed during screening and again at Day 1.

OA medical history includes ACR diagnosis details, OA diagnosis date (if available), number of days with pain in both knees in the last month, and previous IA steroid or hyaluronic injections.

7.9.3. Physical Examination

The physical exam will assess the following body systems:

1. General Appearance
2. Skin
3. Lymphatics
4. HEENT (head, ears, eyes, nose, throat)
5. Cardiovascular
6. Respiratory
7. Abdominal
8. Musculoskeletal
9. Neurological

Any clinically significant findings, outside of the typical disease state, must be documented in the source and added to the medical history if found at Screening or recorded as an AE if new or worsened from pre-dosing on Day 1.

7.9.4. Index Knee X-ray

A diagnostic quality X-ray of the index knee is required (standing, fixed flexion PA view; weight-bearing; use of standardized knee positioning device is required) at Screening. The X-ray will be sent to the central imaging vendor for K-L grading, the presence of osteophytes (both required for eligibility), and joint space width. An image acquisition manual will be distributed to sites with simple instructions for radioanatomic positioning for reliable grading.

7.9.5. Index Knee Ultrasound

A diagnostic quality ultrasound of the index knee is required within -10 days prior to Day 1/Baseline. The ultrasound will be read locally for inflammation. Please refer to Schedule of Study Assessments for specific timing requirements.

7.9.5.1. Machine Capabilities

The machines used in the study should be able to distinguish the edge of proliferated synovium from the surrounding periarticular fat. For the patient with a higher BMI, a lower frequency transducer capability e.g., 7.5-10 MHz should be available, with a normal scanning frequency expected between 10MHz and 15 MHz being optimal. All scanning will be performed with a linear transducer.

7.9.5.2. Scanning Method

The knee will be scanned in a semi-flexed position, approximately 30 degrees from horizontal, with the knee supported if needed. The transducer will be held in a longitudinal, midline position superficial to the quadriceps tendon. The suprapatellar recess will be located below the tendon. The pouch will be examined to its most proximal extreme. Synovial proliferation will be confirmed in a transverse view but measured in a longitudinal view only.

The measurement will be an average of synovial thickening of the opposite anterior and posterior surfaces of the pouch.

To measure, gentle pressure will be applied by the transducer in order to displace any fluid. Once the anterior and posterior surfaces are gently touching, a total measurement of synovial thickness is made using the machine's built-in electronic callipers. This measurement is recorded in mm. This is then divided by two to give an average single membrane measurement. The use of ≥ 2mm thickening synovium will define abnormal synovial thickening.

7.9.5.3. Image Capture

The best representative image should be captured, correctly labelled (left or right knee), and saved in both a longitudinal and transverse view. The same longitudinal image used to show the electronic callipers will be saved. The thickness of the synovium will be displayed on the screen in mm. For further external verification, a 5 second video sweep of the pouch should also be captured.

7.9.6. Index Knee MRI with Contrast

A diagnostic quality MRI with a gadolinium-based contrast agent of the index knee is required within 10 days prior to Day 1/Baseline, and then again at Week 6 and Week 24 (+/- 7 days).

Once eligibility is confirmed, a pre-treatment MRI will be obtained and submitted to the central imaging facility to confirm image quality. Documentation of a quality MRI is needed prior to Day 1/Baseline. If the initial pre-treatment MRI fails quality control (QC), another MRI will need to be completed prior to Day 1/Baseline but at least 3-4 days after the initial MRI to ensure that that contrast agent has washed out.

All subsequent images (Week 6 and 24) will need to be completed on the same machine with the same coil and software as the Pre-treatment image and will need to be submitted to the central imaging vendor to confirm image quality. If the image does not pass QC, another MRI will need

to be completed within the +/- 7 day visit window but at least 3-4 days after the initial MRI to ensure that that contrast agent has washed out.

An MRI image acquisition manual will be distributed to sites with detailed technical instructions and specifications for imaging sequences, imaging coil and Field of View (FOV) positions.

The MRI will be analyzed centrally for the primary endpoint as well as the secondary and exploratory endpoints based on imaging.

7.9.7. Index Knee Assessment

The index knee assessment will be performed by the designated assessor at the days indicated in the Schedule of Study Assessments. The index knee will be assessed for tenderness, heat/redness, swelling, effusion, and Baker's cyst. If there is a clinically significant finding outside of the patient's typical disease state at the Screening or Day 1 Visit (pre-injection), add to the Medical History. At time points post-injection, if there are new clinically significant findings or findings that worsen for the patient's baseline condition, record as AEs.

7.9.8. 12-lead ECG

At Screening, a 12-lead ECG will be obtained in the supine position. Measures of heart rate, PR interval, RR interval, QT interval, QT interval corrected for heart rate (QTc) (corrected for Bazett's or Fridericia's) interval, and QRS duration will be obtained. ECGs will be locally read and a copy of each recording will be kept with the patient's source documentation. If any abnormality is determined to be clinically significant by the Investigator, the patient would be a screen failure.

7.9.9. Vital Signs

Vital signs are to be taken at the days indicated in the Schedule of Study Assessments. The following measurements will be taken: sitting blood pressure, heart rate, respiration rate, and temperature.

7.9.10. Height, Weight, and BMI Determination

Height and weight are to be taken at the days indicated in the Schedule of Study Assessments. Height will be measured in centimeters (cm) or inches. Weight will be measured in kilograms or pounds. BMI will be calculated using the formulas in Table 2 (reference: www.cdc.gov):

Table 2: BMI Calculations

Measurement Units	Formula and Calculation
Kilograms and meters (or centimeters)	**Formula: weight (kg) / [height (m)]^2** With the metric system, the formula for BMI is weight in kilograms divided by height in meters squared. If, as common, height is measured in centimeters, divide height in centimeters by 100 to obtain height in meters. Example: Weight = 68 kg, Height = 165 cm (1.65 m) Calculation: $68 \div (1.65)^2 = 24.98$
Pounds and inches	**Formula: weight (lb) / [height (in)]^2 x 703** Calculate BMI by dividing weight in pounds (lbs) by height in inches (in) squared and multiplying by a conversion factor of 703. Example: Weight = 150 lbs, Height = 5'5" (65") Calculation: $[150 \div (65)^2] \times 703 = 24.96$

7.9.11. Synovial Fluid Aspiration

Synovial fluid samples for potential future biomarker evaluation will be obtained from all patients via aspiration on Day 1 prior to study medication administration. The injector/aspirator will attempt to aspirate synovial fluid from the knee using sterile technique. The volume of the synovial fluid obtained will be recorded in the eCRF. If no synovial fluid is obtained then 0 mLs should be recorded. Procedures for sample collection, handling, storage, and shipment will be described in the Central Laboratory Manual. The synovial fluid samples will be preserved for potential future analyses of biomarkers that may contribute to the pathogenesis of OA and/or be associated with responsiveness to FX006 treatment.

7.9.12. Central Clinical Laboratory Evaluations

Blood samples will be taken at the days indicated in the Schedule of Study Assessments. The specific laboratory panels to be run can be found in Table 3. Follow the Central Laboratory Manual for detailed sample collection, handling, storage, and shipping instructions.

Table 3: Clinical Laboratory Panel

Hematology	**Clinical Chemistry**
Hemoglobin	Sodium
Hematocrit	Potassium
Erythrocyte count (red blood cells [RBC])	Bicarbonate
Mean cell volume	Chloride
Leukocytes (white blood cells [WBC])	Calcium
Absolute counts of:	Total bilirubin
• Neutrophils	Alkaline phosphatase
• Lymphocytes	Alanine aminotransferase
• Monocytes	Aspartate aminotransferase
• Eosinophils	Blood urea nitrogen
• Basophils	Creatinine
• Platelets	Uric acid
	Glucose
Infectious diseases	Total protein
Hepatitis B Surface Antigen	Albumin
Hepatitis C Virus Antibody[1]	
HIV[2]	
Other	
IL-1β mRNA levels	
eGFR	
Pregnancy tests (females of child-bearing potential only) **Serum:** submitted to and performed by Central Laboratory **Urine:** test provided by central laboratory but performed and read at the site	

1. Patients positive for HCV Antibody will have reflex testing for circulating HCV RNA.
2. HIV screening will use a current 4th generation test for both antibody and viral antigen.

7.9.13. Patient Questionnaires

The following questionnaires will be completed by patient prior to all other assessments scheduled for the visit day at the days indicated in Table 1.

- WOMAC® LK 3.1
- KOOS - Quality of Life Subscale (Q1-Q4)
- Supplemental Pain and Stiffness Questions

7.9.14. Treatment Administration

At Day 1, and after completion of all required assessments, the following will occur:

- Study medication will be prepared by the pharmacist/coordinator. Refer to the Pharmacy Binder for FX006 dose preparation instructions.
- Synovial fluid will be aspirated from the index knee just prior to administration of study medication (refer to Section 7.9.11 for more details of the synovial fluid aspiration).

- The injector/aspirator will perform the IA injection of the study medication (refer to Section 7.5.8 for instructions).

7.9.15. Review of Adverse Events and Concomitant Medications

Subjects will be monitored for adverse events from signing of informed consent through the end of their participation in the study. Refer to Section 8.4.1 for further information about reporting of AEs.

Review of any Concomitant Medications will also be performed and documented in source documentation. Refer to Section 7.6 for further information regarding allowed and restricted concomitant medications and therapies.

8. ADVERSE EVENTS

Subjects will be monitored for adverse events from signing of informed consent through the end of their participation in the study.

Between the signing of the informed consent and initiation of study medication, only serious adverse events will be recorded on the eCRF. Following the start of study drug administration, all treatment-emergent adverse events, including serious adverse events, will be collected through the Final Visit. Results of clinical safety assessments are to be recorded in the patient's medical records and transcribed to the appropriate eCRF, including the AE eCRF for clinically significant findings.

8.1. 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.
- 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 planned 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.

Planned Hospitalization:

A hospitalization planned prior to first dose of study medication is considered a therapeutic intervention. If the planned hospitalization or procedure is executed as planned, it should be recorded in the patient's medical history. However, if complications arise during the planned hospitalization or procedure or the patient experiences an AE during the planned hospitalization or procedure, it must be reported as an AE.

8.2. Monitoring of Adverse Events

At each visit, all AEs and SAEs that occur from the time of treatment and throughout a patient's study participation that are observed or reported by the patient, will be recorded in the source documentation and appropriate section of the AE or SAE eCRF.

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;
- Resolved with or without residual effects;
- The Investigator does not expect any further improvement or worsening of the event;
- Fatal outcome: If an autopsy is performed on a deceased patient, the autopsy report should be provided to the Sponsor as soon as it is available.

8.2.1. Monitoring of Laboratory Assessments and other Diagnostic Tests

The Investigator will review results of laboratory and other diagnostic tests for clinical significance and consideration as an AE.

8.3. Assessment of Adverse Events

8.3.1. Assessment of Severity

Each adverse event should be evaluated for severity or intensity. 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.

8.3.2. Assessment of Relationship to Study Medication

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 the following factors:

- 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.
- 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).

8.4. Recording of Adverse Events

All AEs, regardless of seriousness, severity, or causal relationship to study medication, will be recorded on the AE page of the eCRF.

If discernible at the time of completing an AE eCRF, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Investigator and recorded on the appropriate AE eCRF. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the Investigator to be a component of a specific disease or syndrome, then it should be recorded as a separate AE on the appropriate AE eCRF.

8.4.1. Reporting of Serious Adverse Events

When an SAE occurs, the Investigator or designee, must log into the electronic data capture (EDC) system and complete the SAE report form within 24 hours of becoming aware of the SAE. The EDC system will notify the Medical Monitor and other appropriate study personnel of the SAE.

If the EDC SAE form is not available, the Investigator should complete and sign the paper SAE form and email it to the Sponsor. When the EDC system is available again, the SAE should be input into the SAE form.

Follow up information relating to an SAE must be reported to the Sponsor within 24 hours of receipt by the Investigator by entering new or updated information into the EDC SAE form.

All SAEs that occur at your site should, in addition, be reported by the Investigators to the responsible IRB/EC without undue delay, if applicable according to IRB/EC requirements.

During the conduct of the study, the Sponsor will provide expedited safety reports (AEs classified as serious, unexpected and related to study medication) to the investigative sites. If this occurs, the investigative site must report the information to their IRB per local guidelines (may be submitted by the Sponsor or designee for sites that use a central IRB).

8.4.2. Safety Monitoring Roles

The site personnel will carefully monitor each patient throughout the study for possible AEs. All AEs will be reviewed and assessed by the Investigator.

The Sponsor must promptly review all information relevant to the safety of an investigational new product received from any source including AE data individually and in aggregate throughout the course of the study.

Investigators will receive prompt notification of any adverse experience associated with the use of the study medication that is both serious and unexpected, or any finding that suggests a significant risk for patients. The Investigator will promptly inform the IRB/EC of the notification and insert the notification in the Investigator's Regulatory Binder in accordance with local regulations.

The medical monitor and the Sponsor will review the study's safety data approximately on a quarterly basis, in order to identify potential safety issues/trends that may not be apparent through individual AE reporting. If systematic review identifies a concern, the Sponsor will take steps to address the issues including, but not limited to, modifying the protocol or Informed Consent, and/or notifying investigators, authorities and IRB/ECs.

8.4.3. Clinical Management of Index Knee Related Events

If the patient has an immediate reaction following administration of study medication or returns to the clinic with an acute exacerbation (e.g., tenderness, increased pain, swelling, effusion, decreased mobility of the index knee), the patient should be treated according to local clinical guidelines and physician experience.

If the index knee is aspirated at any time after administration of study medication for any reason, the volume of synovial fluid aspirated must be documented, synovial fluid should be (1) cultured, (2) evaluated for presence of crystals and (3) assessed for white cell count at a local laboratory, and the results should be documented.

Any event that is a change from the patient's baseline status (new or worsening case) should be reported as an AE and those meeting the definition of serious must be reported in accordance with Section 8.4.

8.4.4. Pregnancy

All pregnancies, female patients or female partners of male patients during the study, must be reported within 24 hours on the Pregnancy Report Form. The Investigator must continue to follow the pregnancy until the completion of the pregnancy, including the outcome and the condition of the newborn (if applicable). If not all information on the Pregnancy Report Form is available at the time of the initial report, follow-up reports should be provided to the Sponsor in a timely manner. Additional subsequent follow-up is not needed when a newborn baby is healthy.

9. STATISTICAL CONSIDERATIONS

9.1. Statistical and Analytical Plans

Key aspects of the proposed statistical analyses are summarized below. A comprehensive Statistical Analysis Plan will be written and approved prior to database lock for this study. If, after the study has been completed, changes are made to the statistical analysis plan referenced below, these deviations to the plan will be listed in the Clinical Study Report (CSR), along with an explanation as to why they occurred.

9.1.1. Interim Analysis

Once all enrolled patients in the Synovitis Imaging Population have completed the Week 6 visit the mean standardized change from baseline at 6 weeks in synovial volume (the primary endpoint) and other efficacy measures, will be analyzed. Safety analyses may be included and will be detailed in the SAP. Full results of this interim analysis will be presented to the Sponsor.

9.1.2. Final Analyses

Once all enrolled patients have completed the Week 24 visit, all final analyses specified in the SAP will be completed following database lock and reported in the CSR. Post-hoc, exploratory analyses, may also be performed to further understand and elucidate study results; these analyses will be clearly identified as such in the CSR.

9.2. General Considerations and Methods

Data collected in this study will be presented using summary tables, figures, and data listings. Continuous variables will be summarized using descriptive statistics, including the mean, median, standard deviation, minimum and maximum. Categorical variables will be summarized by frequencies and percentages. CIs may also be provided. Figures will be used to support the presentation of certain data. Sensitivity analyses may be performed to examine the effect of missing data, as well as the effect of any baseline imbalance.

All CIs, statistical tests, and resulting p values will be reported as 2-sided. Significance will be assessed at $\alpha = 0.05$ level and the significance level will not be adjusted for the secondary endpoint analyses.

9.2.1. Analysis Populations

Complete details of the statistical analysis will be specified in the SAP. Four analysis populations are planned for this study as follows:

- The Safety Population will include all patients who receive study drug. The Safety Population will be used to assess safety and tolerability.
- The Synovitis Imaging Population will include all patients from the Safety Population, who have a pre-treatment synovial volume measurement of greater than 3000 mm^3 of gadolinium enhancement as determined by quantitative image analysis, as well as quality pre-treatment and Week 6 MRIs. In addition, the patients included in this population will have no major protocol deviations and/or imaging variations

(i.e. change in coil, machine or software) deemed to potentially impact the imaging analysis.

- The Imaging Population will include all patients from the Safety Population, who have quality MRI data available for pre-treatment and at least one post baseline timepoint and with no major protocol deviations and/or imaging variations (i.e., change in coil, machine or software) deemed to potentially impact the imaging analysis.
- The Efficacy Population will include all patients from the Safety Population, who have at least one post-Baseline WOMAC or KOOS assessment.

9.2.2. Study Data

Study data identified in this protocol are collected, and source verified, on electronic Case Record Forms (eCRF) at sites completing the study. All study data will be formulated into data sets to provide transparency, traceability, and integrity of trial analysis results from collection source.

9.2.3. Study Variables for Assessment

Please refer to Section 7.7 for study variables.

9.2.4. Sub-Groups and Covariates

Sub-groups may be defined and explored and will be documented in the Statistical Analysis Plan.

9.3. Determination of Sample Size

Approximately 100 patients will be enrolled to ensure a minimum of 70 evaluable Synovitis Imaging patients, as defined by pre-treatment MRI synovial volume measurement of greater than 3000 mm^3 of gadolinium enhancement as determined by quantitative image analysis, with quality Pretreatment and Week 6 MRIs. Previous trials had similar sample sizes (Gait et al., 2016) (O'Neill et al., 2016).

9.4. General Statistical Methods

9.4.1. Demographics and Baseline Characteristics

Demographic and baseline characteristics will be listed by study site and patient, and will be summarized. Frequencies and proportions will be presented for the categorical variables and descriptive statistics will be presented for continuous variables.

9.4.2. Exposure

Treatment exposure will be listed by patient and will be summarized.

9.4.3. Efficacy Analyses

Primary efficacy endpoint:

- Mean standardized change from baseline at 6 weeks in synovial volume

Secondary efficacy endpoints:

- Mean absolute change (mm^3) from baseline at 6 weeks in synovial volume
- Mean standardized change from baseline at 24 weeks in synovial volume
- Mean absolute change (mm^3) from baseline at 24 weeks in synovial volume

Analysis of additional secondary and exploratory endpoints will be defined in the SAP.

9.4.4. Safety Analyses

9.4.4.1. Analysis of Adverse Events

AEs will be coded using MedDRA. Incidences (number and percent) of TEAEs; (those events that started after dosing or worsened after dosing), will be presented. Additional analyses of incidences of TEAEs will also be presented by maximum severity and by relationship to study medication. Similar presentations will be provided for serious AEs, AEs leading to death, AEs leading to withdrawal from the study, and for AEs related to the index knee.

9.4.4.2. Other Safety Analyses

Clinical laboratory data and vital sign information will be summarized as summary statistics for value and change from Day 1 at each individual time point. Summary statistics will include n, mean, median, standard deviation, minimum, and maximum.

Details for the additional safety endpoints will be provided in the SAP.

10. DATA QUALITY ASSURANCE

At the time the study is initiated, the clinical study monitor will thoroughly review the final protocol and the eCRF with the PI and staff. During the study, the clinical study monitor will visit the clinical site regularly to check the completeness of the patient records, the accuracy of entries into the eCRF, the adherence to the final protocol and to International Conference on Harmonisation GCP, the progress of enrollment, and the storage, dispensing and accountability of study medication. The PI and key study personnel should be available to assist the clinical study monitor during these visits.

The PI will give the monitor, auditor(s), Sponsor, Sponsor designee and regulatory authorities direct access to relevant clinical records. No information in these records about the identity of the patients will leave the clinical site. The Sponsor will maintain the confidentiality of all patient records.

Source data is all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Independent clinical quality assurance audits may be performed at any time during or following completion of the Study by the Sponsor, or its authorized agents, regulatory authorities, Competent Authorities and/or the IRB/EC.

11. DATA HANDLING AND RECORDKEEPING

11.1. Case Report Forms

The eCRF will be supplied by the Sponsor or designee and should be handled in accordance with the instructions provided. All study data should initially be documented in source documents (e.g., patient charts, notes, laboratory reports, ECG recordings, etc.) and then subsequently entered from the source into the eCRF. All eCRFs should be filled out completely by examining personnel or the study coordinator. The eCRF is reviewed, signed, and dated electronically by the PI.

11.2. Study Medication Accountability

All study medication required for completion of this study will be provided by the Sponsor or designee. Study medication will be acknowledged upon receipt indicating shipment content and condition. Damaged supplies will be replaced.

Accurate records of all study medications received by, dispensed from or returned to the study site should be maintained per instructions in the Pharmacy Binder.

In the event of a temperature excursion, refer to the Pharmacy Binder for instructions.

In the event of a product complaint, complete the Product Complaint Form located in the Pharmacy Binder and send to the assigned monitor or clinical manager who will coordinate with the Sponsor for further guidance.

11.3. Confidentiality of Data

Patient medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.

Upon the patient's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare.

Data generated by this study must be available for inspection by representatives of Competent Authorities, the Sponsor or their representative, and the IRB/EC.

To maintain patient privacy, all eCRFs, study drug accountability records, study reports, and communications will identify the patient by initials where permitted and/or by the assigned patient number. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

11.4. Retention of Records

In accordance with United States of America Code of Federal Regulations (21 CFR 312.62[c]), the Sponsor requires that records and documents pertaining to the conduct of this study and the distribution of study medications, including eCRFs, consent forms, laboratory test results and medical inventory records, must be retained by the PI for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the investigation is discontinued and the regulatory authorities are notified. The Sponsor or their representative will notify the PI of these events. If local regulations are more stringent than that specified above, the local regulations will be adhered to.

11.5. Protocol Adherence

The PI must adhere to the protocol as detailed in this document and agrees that any changes to the protocol must be approved by the Sponsor or their representative prior to seeking approval from the IRB/EC. When the changes involved are only logistical and administrative in nature to the trial this may not require prior approval by the IRB/EC. The PI will be responsible for enrolling only those patients who have met protocol eligibility criteria.

12. PUBLICATION POLICY

12.1. Sponsor's Publication Policy

Sponsor or its designee shall have the right to publish or otherwise publicly disclose the information contained in or related to the Study Drug, the Study Data, or other Confidential Information in any form without the written consent of Site, the PI or any other person. Each of Site and PI further agrees that Sponsor shall have the exclusive right to commercialize any products or services that are based upon, or derived from the Study Drug, the Study Data, or other Confidential Information.

12.2. Site Publication

After the Study is completed, which means that all completed eCRFs have been received by Sponsor, and the database has been locked at all participating sites and Study closeout visits have taken place at all participating sites, then Site shall have the right, subject to the Health Insurance Portability and Accountability Act (HIPAA) Rules, to publish or otherwise make public data resulting from the conduct of the Study at the Site upon the earlier of (a) the date of publication of a multi-center publication coordinated by Sponsor with respect to the data resulting from the Study, and (b) the date of submission of the data resulting from the Study by Sponsor to the FDA for regulatory approval; provided that Site shall furnish Sponsor with a copy of any proposed publication or release at least 90 days in advance of the proposed submission or presentation date. Within this 90-day period, the Sponsor shall review such proposed publication or release to determine whether it contains any Confidential Information (other than Study Data), or whether Sponsor desires to file patent applications on subject matter contained therein, and to ensure the accuracy of the information contained in the publication or release. Upon receiving any notification from Sponsor requesting deletion of Confidential Information (other than Study Data), requesting correction of inaccuracies, or requesting a delay in publication of up to 90 days to allow the filing of patent applications before publication or release, Site shall take the requested action. The parties acknowledge and agree that Site shall be solely responsible for the editorial content of any such publication or release. In a manner consistent with customary practice, Site shall acknowledge the support and contributions of Sponsor, if requested by Sponsor, in connection with the Study, in any and all publications and presentations reporting and data resulting from the Study. Site and the PI shall comply with all applicable federal and state laws and other applicable rules and requirements regarding disclosure of industry support (financial or otherwise) in connection with such publications and presentations.

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