STATISTICAL ANALYSIS PLAN

Protocol FX006-2017-012

A Randomized, Open-label, Parallel Group Study in Patients with Bilateral Knee Osteoarthritis Comparing the Systemic Exposure of Triamcinolone Acetonide Following Administration into Both Knees of either Extended-release FX006 or Immediate-release TAcs (Triamcinolone Acetonide Suspension)

Protocol Number: (Version Date)	FX006-2017-012 Version 2.0 (10 November 2017)
Name of Test Drug:	FX006
Phase:	2a
Methodology:	Randomized, open label, parallel group, two intraarticular injections (one in each knee) study
Sponsor:	Flexion Therapeutics 10 Mall Road, Suite 301 Burlington, Massachusetts, USA Tel: 781-305-7777
Sponsor Representative:	Joelle Lufkin Vice President Clinical Operations
Document Date:	08 March 2018
Document Version:	Final Version 1.0

SIGNATURE PAGE

Protocol Title:	A Randomized, Open-label, Parallel Group Study in Patients with Bilateral Knee Osteoarthritis Comparing the Systemic Exposure of Triamcinolone Acetonide Following Administration into Both Knees of either Extended-release FX006 or Immediate-release TAcs (Triamcinolone Acetonide Suspension)
Sponsor:	Flexion Therapeutics 10 Mall Road, Suite 301 Burlington, Massachusetts, USA Tel:781-305-7777
Protocol Number:	FX006-2017-012 Version 2.0 (10 November 2017)
Document Date / Version	08 March 2018 / Version 1.0
Cytel, Inc. Author: Teresa Curto (Biostatistician)	Signature: _____ Date: 19-Mar-2018
Contributing Author: Katherine Kacena, PhD BioBridges Biostatistician	Signature: _____ Date: _____ Digitally signed by Katherine Kacena, Phd DN: cn=Katherine Kacena, Phd, o, ou, email=kk@kkstats.com, c=US Date: 2018.03.13 22:33:58 -04'00'

Sponsor Approval

By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol, clinical development plan, and all applicable regulatory guidances and guidelines.

I have discussed any questions I have regarding the contents of this document with the biostatistical author.

I also understand that any subsequent changes to the planned statistical analyses, as described herein, may have a regulatory impact and/or result in timeline adjustments. All changes to the planned analyses will be described in the clinical study report (CSR).

Sponsor Signatory:
Joelle Lufkin
Vice President – Clinical Operations

Signature: _____
Date: 12 March 2018

Scott Kelley, MD
Chief Medical Officer

Signature: _____
Date: 12 Mar 2018

TABLE OF CONTENTS

Section **Page**

LIST OF IN-TEXT TABLES

Table **Page**

ABBREVIATIONS

Abbreviation	Definition
AE	Adverse events
ACR	American College of Rheumatology
ADaM	Analysis Data Model
ANOVA	Analysis of variance
ATC	Anatomic Therapeutic Class
AUC	Area Under the Plasma Concentration Curve
$AUC_{(0-24)}$	Area Under the Plasma Concentration Curve from 0 to 24 hours
$AUC_{(0-inf)}$	Area Under the Plasma Concentration Curve from 0 extrapolated to infinity
$AUC_{(0-t)}$	Area Under the Plasma Concentration Curve from 0 to last quantifiable concentration (t)
BLOQ	Below Limit of Quantification
BMI	Body Mass Index
CI	Confidence Interval
C_{last}	Last quantifiable plasma concentration
CL	Total Body Clearance
C_{max}	Maximum Plasma Concentration
CRF	Case Report Form
CSR	Clinical study report
CV	Coefficient of variation
ECG	Electrocardiogram
EMA	European Medicines Agency
EULAR	European League Against Rheumatism
FDA	Food and Drug Administration
GM	Geometric Mean
HbA1c	Hemoglobin A1C
HIV	Human Immunodeficiency Virus
IA	Intra-articular
ICH	International Conference on Harmonization
IMP	Investigational Medicinal product
IxRS	Interactive Voice/Web Response System
kg	Kilogram
LSMD	Least Square Mean Difference

Abbreviation	Definition
m	Meter
mg	Milligram
mL	Milliliter
MedDRA	Medical Dictionary for Regulatory Activities
MRT	Mean Residence Time
ms	millisecond
NCA	Non-Compartmental pharmacokinetic analysis
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
PK	Pharmacokinetic
PT	Preferred Term
Q1	1st quartile
Q3	3rd quartile
REML	Residual Maximum Likelihood
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SE	Standard Error
SOC	System Organ Class
TA[1]	Triamcinolone Acetonide
TAcs[2]	Triamcinolone Acetonide Injectable Suspension, Immediate-Release (commercially available) (Reference Compound, Kenalog®-40)
TEAE	Treatment Emergent AE
t_{last}	Time of Last Quantifiable Plasma Concentration
t_{max}	Time from Dosing to Peak Exposure
$t_{1/2}$	Terminal Half-Life
US	United States
Vz_F	Apparent volume of distribution during terminal phase after non-intravenous administration, which approximates a steady state parameter estimate
WHO	World Health Organization
WHO-DD	World Health Organization-Drug Dictionary
λ_z	Terminal Elimination Rate Constant (aka: Lambda_z)

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

1. INTRODUCTION AND OBJECTIVES OF ANALYSIS

1.1. Introduction

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 (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. We estimate 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).

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).

FX006 is an extended-release formulation of triamcinolone acetonide (TA) for IA administration. It is approved under the trade name ZILRETTA™ for the management of pain of OA of the knee, however, injection in both knees has not been studied and is an investigational use. 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)

1.2. Objectives of Statistical Analysis

The primary objectives of this study are:

- to compare the plasma pharmacokinetics (PK), including systemic exposure, of triamcinolone acetonide between extended-release (FX006) and immediate release (TAcs) formulations.
- To assess the safety and general tolerability

when FX006 is administered as two 5 mL IA injections (one to each knee) for a total dose of 64 mg or TAcs is administered as two 1 mL injections (one to each knee) for a total dose of 80 mg in patients with bilateral knee OA.

This statistical analysis plan (SAP) is designed to outline the methods to be used in the analysis of study data in order to answer the study objective(s). Populations for analysis, data handling rules, statistical methods, and formats for data presentation are provided. The statistical analyses and summary tabulations described in this SAP will provide the basis for the results sections of the clinical study report (CSR) for this trial, as well as used for regulatory filings and manuscripts and presentations.

This SAP also will outline any differences in the currently planned analytical objectives relative to those planned in the study protocol.

2. STUDY DESIGN

2.1. Synopsis of Study Design

This randomized, parallel group study will be conducted in male and female patients ≥ 40 years of age with bilateral knee OA.

Approximately 24 patients will be randomized to one of two treatment groups (1:1) and treated with two single IA injections of either:

1. extended-release FX006 64 mg total dose (approximately 12 patients) or
2. immediate-release TAcs 80 mg total dose (approximately 12 patients).

Each patient will be evaluated for a total of 6 weeks following the two IA injections. Following screening, PK and safety will be evaluated at 6 out-patient visits scheduled on Study Days 1 [calendar day of injection], 2, 8, 15, 29, and 43.

2.2. Randomization Methodology

Patients will be assigned to treatment groups by randomization using a central system accessed directly by the sites after the patient is assessed as eligible. Randomization will be continuous across the study (i.e., no stratification), using a block size of 4 (four). This is an open-label study design and no patients or study personnel are blinded to treatment assignment.

2.3. Stopping Rules and Unblinding

Unblinding is not applicable to this study since this is an open label-study.

There will be no data review committee for this study.

Furthermore, discontinuation from treatment is not applicable to this study as each treated patient receives study medication at a single study visit as two IA injections, one in each knee, except in the case in which only one knee is injected.

2.4. Study Procedures

Patients participating in this study will complete 7 scheduled visits for a total study duration of up to 8 weeks (including Screening period). The study will involve a Screening period (up to 14 days), a Baseline/Day 1 visit (Day 1) and 5 outpatient visits following Day 1. At each visit following Screening, patients will provide blood samples for TA drug concentration measurements for PK analysis.

At specified times throughout the study, patients will undergo safety assessments such as physical examinations, vital signs, hematology and chemistry blood collection for analyses and, adverse event and concomitant medication review.

Blood samples for TA drug concentration measurements will be obtained from all patients prior to administration of study medication on Day 1, and at hours 1, 2, 3, 4, 5, 6, 8, 10, and 12 (± 10 minutes) after the first knee injection, on Day 2 at 24 hours (± 2 hours) after the first injection of study medication, and at each of the subsequent scheduled visits: Day 8, 15, 29 and 43 (time as

convenient during the scheduled visit). The date and time that each blood sample was obtained will be recorded.

Blood samples for morning cortisol levels will be obtained between 7-9 am from all patients at Screening, prior to administration of the study medication on Day 1, and then at each of the subsequent scheduled visits.

The schedule of assessments, as outlined in the study protocol, is provided in Table 1

Table 1 Schedule of Assessments

Procedures	**Screening[1]**	**Day 1**	**Day 2**	**Day 8[2]**	**Day 15[2]**	**Day 29[2]**	**Day 43[2] (End of Study)**
Informed consent	X[3]						
Inclusion/Exclusion Review	X	X[4]					
Medical History/Update	X	X[4]					
OA Medical History	X						
Prior Treatment & Medications[5]	X	X[4]					
Physical Examination	X	X[4]					X
Bilateral Knee X-rays	X[6]						
Bilateral Knee Assessments[7]	X	X[4]					X
12-Lead ECG	X						
Vital Signs	X	X[4]	X	X	X	X	X
Height	X						
Weight and BMI	X						X
Hematology & Chemistry[8]	X	X[4]					X
HIV, Hepatitis B/C, HbA1c[8]	X						
Pregnancy Test[9]	X	X[4]					X
Blood for Drug Concentrations		X[10]	X[11]	X	X	X	X
Blood for Morning Serum Cortisol[12]	X	X[4]	X	X	X	X	X
Knee Aspiration and Collection of Synovial Fluid		X[4]					
Treatment Administration[13]		X					
Adverse Event Monitoring[14]		---------- X----------					
Concomitant Medications[14]		----------X----------					

1 Screening may occur up to 14 days prior to Day 1 (calendar day of dosing).
2 Visit should be conducted +/- 2 days from scheduled date
3 Consent must be obtained prior to performing any study-specific procedures.
4 Complete assessment prior to dosing.
5 Record any medications received within 30 days prior to Screening.
6 Obtain new x-rays if >6 months since prior x-ray.
7 Both knees will be assessed for tenderness, heat/redness, swelling, effusion, and Baker's cyst. Clinically significant findings (new or worsening from baseline) should be recorded as AEs.
8 Via Central Laboratory

9 Conduct for females of childbearing potential only. Serum pregnancy test to be performed via central laboratory at Screening and End-of-Study visit (scheduled for Day 43); urine pregnancy test to be performed locally on Day 1 and results available prior to dosing.
10 On Day 1, blood for plasma drug concentration measurements will be collected prior to dosing and at Hours 1, 2, 3, 4, 5, 6, 8, 10, and 12 post-injection of the first knee (±10 minutes).
11 On Day 2, blood for plasma drug concentration measurements will be collected at 24 hours post-injection of the first knee (± 2 hours), and on subsequent visits as convenient.
12 Blood for morning cortisol measurements will be collected between 7 and 9 am.
13 The injection of the second knee should take place within 15 minutes of the first knee injection.
14 AEs and Concomitant Medications will be captured from Day 1 (post-injection) to End of Study Visit.

2.5. Safety, Pharmacodynamic and Pharmacokinetic (PK) Safety Variables

2.5.1. 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, knee assessments, vital signs, and clinical laboratory evaluations.

2.5.2. Pharmacokinetic Variables

Blood samples (4 mL per sample) for TA drug concentration measurements will be obtained from all patients at the following nominal, or planned, times:

- On Day 1, within 1 hour prior to administration of study medication (Nominal Time -1),
- On Day 1, at Hours 1, 2, 3, 4, 5, 6, 8, 10, and 12 (± 10 minutes) after injection of the first knee,
- On Day 2, at 24 (± 2 hours) after injection of the first knee,
- On Days 8, 15, 29, and 43 (time as convenient during the scheduled visit).

These represent a total of 15 samples from each patient, each sample representing 4 mL of blood, for a maximum estimated total volume of 60 mL of blood collected from each patient for drug concentration measurement.

Procedures for sample collection, handling, storage and shipment will be described in the Laboratory Manual. Plasma TA concentrations will be measured using an established validated LC-MS/MS method.

PK parameter endpoints are described in section 4.8.4.

2.5.3. Pharmacodynamic Variables

Blood samples for serum cortisol levels will be collected between 7 am and 9 am at Screening, prior to administration of the study medication on Day 1, and on Study Days 1, 2, 15, 29, 43.

Synovial fluid samples will be preserved for a maximum of 5 years for potential future analyses of biomarkers that may have utility in defining pathogenesis of OA and/or be associated with responsiveness to FX006 treatment. No analysis is planned at this time.

3. PATIENT POPULATIONS

3.1. Population Definitions

The following patient populations will be evaluated and used for presentation and analysis of the data:

Safety population:

All patients who received study medication (injection in at least one knee). Patients will be analyzed according to the treatment they have received ("analyses as treated").

Pharmacokinetic Population:

All patients from safety population who receive two IA injections (one to each knee), complete scheduled sampling, and have sufficient plasma concentration data to allow calculation of PK parameters will be included in the PK population. Review of the plasma drug concentration data and eligibility for inclusion into the PK population will be completed by the study Pharmacokineticist. The rationale for inclusion or exclusion in the PK population will be documented and listed. Patients will be analyzed according to the treatment they have received ("analyses as treated").

3.2. Protocol Deviations

All protocol deviations will be presented in the data listings.

4. STATISTICAL METHODS

4.1. Sample Size Justification

4.1.1. Sample Size Considerations

In this study, it is expected that the systemic exposure in plasma of TA from extended-release FX006 should not exceed that of the immediate-release TAcs formulation for the key parameters of C_{max}, $AUC_{(0-t)}$, and $AUC_{(0-inf)}$.

In a previous PK study with knee OA patients (FX006-2015-009) the ratio of the mean exposure parameters for FX006 32 mg (N=60) and TAcs 40 mg (N=18) for C_{max} was 0.10 with the upper limit of its 90% CI being 0.15 and for $AUC_{(0-inf)}$ was 0.52 with the upper limit of its 90% CI being 0.86. The pooled coefficients of variation for the parameters was between 0.53 (C_{max}) and 0.68 (AUC_{0-inf}).

4.1.2. Sample Size Estimate

In this study, it is expected that the ratio of exposure means (FX006/TAcs) will be less than 1.0 when administered to treat bilateral knee OA. A sample size of 12 in each treatment arm (24 in total) is estimated. The sample size of 24 achieves approximately 90% power, with a two-sided alpha 0.05, to detect a ratio less than 1.0 of the exposure PK parameter means (FX006 / TAcs), with a pooled coefficient of variation estimate of 0.68 (PASS 15 Power Analysis and Sample Size Software (2017). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass). The sample size of 12 per treatment arm assumes a 10% noncompliance sampling rate (a drop-out rate) for providing complete blood samples for PK analysis, and is sufficient to characterize the comparative PK of FX006 and TAcs in this study. The total sample size is estimated to be 24 patients (12 in each treatment arm) for the study.

4.2. General Statistical Methods and Data Handling

4.2.1. General Methods

All output will be incorporated into Microsoft Excel or Word files, sorted and labeled according to the International Conference on Harmonization (ICH) recommendations, and formatted to the appropriate page size(s).

Tabulations will be produced for appropriate demographic, baseline, pharmacodynamics, PK and safety parameters. Summary tables will present data by treatment arm.

For categorical variables, summary tabulations of the number and percentage within each category (with a category for missing data) of the parameter will be presented.

For continuous variables, the number of non-missing values (n), the mean, median, standard deviation (SD), minimum and maximum values will be presented. 95% Confidence Intervals (CI) may be provided. In addition, for concentration-based parameters, the coefficient of variation (CV %), the geometric mean (GM) and log-scale standard deviation will be provided. Additional statistics may be presented for certain endpoints as described below.

All collected data will be presented in by-patient listings sorted by treatment arm and patient number.

All data listings that contain an evaluation date will contain relative analysis day (Rel Day). Pre-treatment and post treatment analysis days are numbered relative to the day of the first dose of study treatment (i.e., injection in the first knee), which is designated as Day 1. The preceding day is Day-1; the day before that is Day-2, etc.

For data listings relative to PK assessments where several assessments are made on the same day, there will be also a relative study day and time.

The sections below describe the intended analysis of the endpoints. Additional sensitivity analysis may be employed in the event of any unforeseen data anomalies or data issues not known at the time of writing this analysis plan.

4.2.2. Computing Environment

All descriptive statistical analyses will be performed using SAS® statistical software (Version 9.4 or higher), unless otherwise noted.

Non-compartmental PK analyses (NCA) will be conducted using Phoenix 7, WinNonlin® Version 7 or higher (Certara Corporation).

Adverse events will be coding using Medical Dictionary for Regulation Activity (MedDRA) Version 20.1 (or higher).

Concomitant medications will be coded using World Health Organization (WHO) Drug Dictionary (DD) (Version B3 Mar 2014 or Higher).

4.2.3. Methods of Pooling Data

Not applicable to the present study.

4.2.4. Adjustments for Covariates

No formal statistical analysis that adjusts for possible covariate effects is planned.

4.2.5. Multiple Comparisons/Multiplicity

Not applicable.

4.2.6. Subpopulations

Not applicable to the present study.

4.2.7. Discontinuations and Loss to Follow-up

Each treated patient from this study receives study medication as two single IA injections. Therefore, discontinuation from treatment is not applicable. Whether or not patients may receive the entire content of study drug injection will be considered in the study drug exposure analyses.

Each patient may only discontinue from the study for further assessments and study visits. Data collected from discontinued patients will be included in the CSR. Patients who discontinue from the study may be replaced at the discretion of the sponsor.

4.2.8. Missing, Unused, and Spurious Data

Missing values will not be imputed and data will be analyzed "as observed".

4.2.9. Visit Windows

Visit windows will be calculated based on the schedule of assessments.

All endpoints will be summarized and presented according to the nominal visit (and hour, if applicable) as recorded on the Case Report Form (CRF).

4.2.10. Baseline definitions

For all endpoints, for exposed patients, baseline is the Baseline/Day 1 assessment prior to study drug administration date and time, in the first knee. If baseline result is missing, the first non-missing result prior to study drug administration may be used from the Screening period. For randomized and not exposed patients the latest assessment during the screening period will be used.

4.3. Interim Analyses

An interim analysis is not planned for this study.

4.4. Patient Disposition

All patients who are enrolled will be accounted for in this study.

Patient disposition will be tabulated and include the number randomized, treated and completed; the number in each patient population for analysis; and the number who discontinued prior to completing the study and reason(s) for discontinuation.

Summary data will be presented by treatment arm and overall.

A by-patient data listing of study completion information including the reason for early study discontinuation, if applicable, will be presented.

A listing of patients by analysis population will also be provided, including reason for exclusion from an analysis population.

4.5. Protocol Deviations

All protocol deviations will be presented in a data listing.

4.6. Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized on the safety population and will be presented by treatment group.

No formal statistical comparisons will be performed.

All collected demographic, baseline characteristic, bilateral knee assessment and medical history data will be provided on the safety population.

All collected demographic, baseline characteristic, and medical history data will be provided in data listings.

4.6.1. Demographic characteristics

The following baseline parameters will be described:

- Age (year) at consent - Age will be calculated as the years between date of birth and date of informed consent, and will be rounded down to the nearest year.
- Weight (kg);
- Height (cm);
- Gender (Male/Female);
- Body mass index (BMI) (kg/m²);
- BMI category:
    - Underweight: <18.0 kg/m²
    - Normal: 18.0 to <25.0 kg/m²
    - Overweight: 25.0 to <30.0 kg/m^2
    - Obesity Class I: 30.0 to <35.0 kg/m^2
    - Obesity Class II: 35.0 to <40.0 kg/m^2
    - Obesity Class III: >=40.0 kg/m^2
- Ethnicity (Hispanic or Latino / Not Hispanic or Latino);
- Race (White, Asian / Black or African American / American Indian or Alaska Native / Native Hawaiian or Other Pacific Islander);
- Country.

Age, height, weight, and body mass index (BMI) will be summarized using descriptive statistics (number of patients, mean, standard deviation, median, minimum, and maximum). The number and percentage of patients in each gender, ethnicity, race and BMI category will also be presented.

4.6.2. Osteoarthritis (OA) Medical History

General OA and knee OA medical history data as collected on the CRF will also be tabulated for the Safety Population.

For each knee, time from primary diagnosis of OA in the knee to Day 1 of the study in days (first dose date – date of diagnosis + 1), will be computed and presented descriptively. If only month and year of initial diagnosis is available, day will be imputed as 1 for calculations. If month and day are missing, the time from primary diagnosis will be computed as year of first dose minus year of diagnosis. If year is missing, time from diagnosis will not be computed.

Knee OA history data will be summarized by knee injected (first injection and second injection separately).

4.6.3. Prior medication

All prior medications will be presented in the concomitant medication data listing with a flag identifying which medications are prior medications (refer to Section 4.9.4 for details on defining prior and concomitant medications).

4.7. Pharmacodynamics Evaluation

The actual value and change from baseline (Day 1 or Screening if Day 1 is missing) will be summarized for serum cortisol levels by actual treatment arm. In the event of repeat values, the last non-missing value per study day/time will be used.

All serum cortisol data will be provided in tables and listings. Results falling outside of the expected collection time of 7:00am to 9:00 am will be flagged”

4.8. Study Drug Exposure and Pharmacokinetics Evaluation

4.8.1. Study Drug Exposure

Details of study drug administration will be tabulated and presented by actual treatment arm and knee injected (first injection and second injection separately) for the Safety Population. Specifically, the position of the knee during injection, the approach during injection, the numbing agent to be used, whether or not the entire contents of the syringe was injected, and whether a 21 gauge or larger needle was used will be presented.

The time between the first knee IA injection and second knee IA injection will be included in listings.

All dosing data will be presented in a data listing.

4.8.2. Plasma drug concentration analysis

Tabular summaries of mean TA plasma concentrations, by treatment for each nominal sampling time will be presented for the PK population.

Visit date and times are calculated relative to the Baseline/Day 1 visit. The reference time point is Day 1 time of 1st study drug administration in the first knee (Time 0). Of note, the pre-dose sample is only relevant if a quantifiable concentration of TA is present.

All data collected will be summarized and presented in individual plots. However, all data will be presented in data listings with those results falling outside of the visit window flagged. Both nominal (planned) and actual sampling time will be presented.

Descriptive statistics (n, mean, SD, geometric mean, log-scale SD, 95% CI, median, minimum, maximum) will be calculated by time point for plasma drug concentration levels. Frequencies of patients with values identified as BLOQ will be presented by time point.

4.8.3. Handling of Concentration Data below the Limit of Quantification (BLOQ)

The bioanalytical assay sensitivity for TA will be noted in the table and listings. Values assayed below these limits will be identified as BLOQ. Concentrations that are not detected will be identified as “Not Detected” and recorded as BLOQ.

For the PK analysis and individual concentration vs. time plots, a concentration that is BLOQ is assigned a value of zero if it occurs in a profile before the first measurable concentration. If a BLOQ value occurs after a measurable concentration in a profile and is followed by a value above the lower limit of quantification, then the BLOQ is treated as missing data. If a BLOQ value occurs at the end of the collection interval (after the last quantifiable concentration) it is set to zero. If two BLOQ values occur in succession after Cmax, the profile is deemed to have

terminated at the first BLOQ value and any subsequent concentrations are set to zero for PK calculations.

4.8.4. Pharmacokinetic analysis

PK analysis will be performed on the PK population. PK parameter estimates will be completed by the study pharmacokineticist and results provided for incorporation into study datasets.

PK parameters will be derived using model-independent methods (non-compartmental analysis (NCA)) as implemented in Phoenix 7, WinNonlin® (version 7) and will be based on TA plasma concentrations from patients in the PK Population. Mean concentration profiles will be computed for each treatment arm: FX006 64 mg and TAcs 80 mg.

Actual sampling times will be used for the estimation of the PK parameters if significant deviations from the nominal sampling time are noted in data review by the study pharmacokineticist. All descriptive summary tables and figures will be presented with the nominal (planned) sampling times.

All blood samples will be assayed for concentration levels of TA using a validated High Performance Liquid Chromatographic Method with Tandem Mass Spectrometry Detection method (LC/MS-MS), each matrix with a validated assay range in pg/mL. Bioanalytical methods for the assay of TA will be provided in a separate bioanalytical report that will include information on calibration standards for the assay.

Each PK parameter will be estimated for each patient with NCA methodology.

The PK endpoints to be computed on plasma TA concentration measurements will be either observed from those concentration measurements or estimated by WinNonlin® (version 7) using the trapezoidal rule for calculation of the AUC estimates to the last measureable concentration. Additional estimates of the half-life clearance, residence time, volume of distribution and the extrapolated area for the $AUC_{0\text{-}inf}$ and related parameters will be estimated using the slope estimate for the terminal elimination rate constant for each patient with WinNonlin® (version 7).

PK variables consist of PK parameters identified in Table 2.

Table 2 PK Parameters

PK Parameter	**Description (Computation Method)**
C_{max}	Peak exposure, Maximum plasma concentration observed.
t_{max}	Time from dosing to peak exposure, time to maximum plasma concentration observed.
C_{last}	Last quantifiable plasma concentration (last value observed above assay BLOQ)
t_{last}	Time of last quantifiable plasma concentration
λ_z	Terminal elimination rate constant (lambda_z)
$t_{1/2}$	Terminal half-life: Computed as $t_{1/2}= \frac{Ln(2)}{\lambda_z}$
$AUC_{(0\text{-}t)}$	Exposure: Area Under the Plasma Curve from time 0 to the last quantifiable concentration (t). Calculated using the linear trapezoidal rule.
$AUC_{(0\text{-}24)}$	Exposure: Area Under the Plasma Curve from time 0 to 24 hours post IA injection. Calculated using the linear trapezoidal rule
$AUC_{(0\text{-}\infty)}$	Exposure: Area Under the Plasma Curve from time 0 extrapolated to infinity. Calculated as follows: $AUC_{inf} = AUC_{(0-t)} + \frac{C_{last}}{\lambda_z}$ where C_{last} is the last quantifiable concentration.
CL	Total Body Clearance, computed as Dose/ $AUC_{(0\text{-}\infty)}$
MRT	Mean residence time extrapolated to infinity, computed as $AUMC_{(0-\infty)}/AUC_{(0-\infty)}$
Vz_F	Volume of Distribution, based on terminal phase, computed as : $\frac{Dose}{\lambda_z . AUC_{(0-\infty)}}$

Descriptive summaries of the individual patient PK parameters and population summaries over all patients will be presented. Individual linear-linear and log-linear concentration profiles will be completed for each patient. Graphical display (plasma drug concentration curves) will be used to present geometric mean with 95% CIs for plasma drug concentrations (linear-linear and log-linear plots) for each treatment arm.

All PK parameters and data will be presented in a data listing. Results from the PK analysis will be included in the CSR. Along with the bioanalytical report from the assay laboratory, model output from the NCA analysis will be provided in a separate document.

A linear model will be used to compare the PK parameters from extended-release FX006 and immediate-release TAcs for each C_{max}, T_{max}, C_{last}, MRT, and AUC parameters. The model will include treatment as a single fixed effect. The least square mean difference (LSMD) between the two treatments will be explored to examine the magnitude of difference in exposure PK parameters between FX006 and TAcs. All p-values will be considered informative.

4.8.5. Exploration of Bioequivalence between FX006 and TAcs

Bioequivalence (BE) ratios between extended-release FX006 (Test) and immediate-release TAcs (Reference) for C_{max} and AUC parameters will be explored. Bioequivalence between Test and Reference will be evaluated using the average BE method for the mean ratio between test and reference products ($\mu T / \mu R$) as described in FDA guidance for the assessment of bioequivalence (FDA, 2013).

The FDA Guidance on the assessment of Bioequivalence recommends that bioequivalence measures of AUC and C_{max} be log-transformed. In this study, the natural log will be used to transform the values for the model, and the C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$. PK parameters will be exponentiated from a mixed effects model to examine bioequivalence.

In this study bioequivalence is not expected for the ratio of FX006 / TAcs. The properties of FX006 suggest that a reduced C_{max} (reduced maximum exposure) is evident due to slower release of TA into systemic circulation. The potential shift in T_{max} and potential decay in C_{max}, both of which would be associated with corresponding differences in AUC will be explored.

A linear mixed effects model may be used for this study to average bioequivalence with PK parameters of log-transformed AUC_{0-24}, $AUC_{0-\infty}$, and $C_{max.}$ In the initial model fixed effects for treatment and site will be included, with a random effect for patient.

GM with 95% CIs will be presented for each treatment group. BE ratio with standard error (SE) and 90% CIs will be presented for C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$.

4.9. Safety Analyses

Safety analyses will be conducted using the Safety Population.

4.9.1. Adverse Events

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and displayed in tables and listings using System Organ Class (SOC) and Preferred Term.

Analyses of adverse events will be performed for those events that are considered treatment emergent, where treatment emergent is defined as any adverse event with onset after the administration of study medication in the first knee through the end of the study or any event that was present at baseline but worsened in intensity through the end of the study.

If the start date/time of an AE is partially or completely missing, the date/time will be compared as far as possible with the date/time of the start of administration of study drug. The AE will be assumed to be treatment emergent if it cannot be definitively shown that the AE did not occur or worsen during the treatment-emergent period (worst case approach). The following general rules will be used:

- If the start time of an AE is missing but the start date is complete, an AE will only be excluded as being treatment emergent if the start date is before the date of study drug administration or if the stop date/time is before study drug administration.
- If the start time and day are missing but the start month and year are complete, an AE will only be excluded as being treatment emergent if the start month/year is before the

month/year of study drug administration or if the stop date/time is before study drug administration.

- If the start day and month are missing but the start year is complete, an AE will only be excluded as being treatment emergent if start year is before the year of study drug administration or if the stop date/time is before study drug administration.
- If the start date is completely missing, an AE will be considered treatment-emergent unless the stop date/time is before study drug administration.

Adverse events are summarized by patient incidence rates; therefore, in any tabulation, a patient contributes only once to the count for a given SOC or preferred term.

Adverse events tables and listings will be presented by actual treatment arm.

The number and percentage of patients with any treatment-emergent AE (TEAE), with any serious TEAE, with any TEAE leading to study discontinuation (related or serious), with any TEAE by maximum severity and with any TEAE by relationship will be tabulated.

Similar data presentations will be made for knee related TEAEs. In these tabulations, related is defined as any TEAE deemed possibly, probably or definitely related to study drug by the investigator.

Tabulations presented in SOC internal agreed order and PT in alphabetical order within each SOC will also be produced for:

- all treatment-emergent AEs
- related TEAEs
- TEAEs by maximum severity
- for serious TEAEs

Knee related TEAEs will be tabulated in a similar manner. Knee related TEAEs that occur in both knees will be provided in a separate listing.

No formal hypothesis-testing analysis of AE incidence rates will be performed.

All AEs occurring on-study will be provided in data listings.

By-patient listings will also be provided for the following: patient deaths, Serious Adverse Events (SAE), and AEs leading to discontinuation.

4.9.2. Laboratory Data

Clinical laboratory values will be expressed in SI units.

The actual value and change from baseline (Day 1 or Screening if Day 1 is missing) will be summarized for each hematology and clinical chemistry laboratory parameter by actual treatment arm. In the event of repeat values, the last non-missing value per study day/time will be used.

All laboratory data will be provided in data listings.

4.9.3. Vital Signs and Physical Examinations and Knee Assessment

The actual value and change from baseline (Day 1) at each time point will be summarized for vital signs by actual treatment arm. Vital sign measurements will be presented for each patient in a data listing.

All physical examination abnormalities will be presented in a data listing.

The incidence of inflammation, as determined from the knee assessment, will be tabulated for each visit. For those patients experiencing inflammation, the details of the inflammation will also be tabulated. In these tabulations, percentages will be based on those patients who have a non-missing knee assessment at a given visit. In addition, a shift table displaying shifts in knee assessments from baseline over time will be presented. Knee assessment as well as knee aspiration data will be presented in a data listing. Inflammation data will be summarized separately for each knee with the first and second knee defined as stated previously.

4.9.4. Electrocardiogram

ECG data will be provided in patient data listing.

4.9.5. Concomitant Medications

Concomitant medications will be defined as those medications that were initiated after first dose of treatment or those that were ongoing at the time of first study drug administration. If the start date or stop date of a medication is partially missing, the date will be compared as far as possible with the date of the start of administration of study drug. The medication will be assumed to be prior medication if it cannot be definitively shown that the medication did not start or continue during the treatment period. The following approach will be taken:

- If the start date of medication is complete and occurs on or after the day of the first dose, the medication will be assumed concomitant. If the start date occurs prior to the first dose date but the end date is on or after the first dose date or the medication is recorded as ongoing, the medication will be considered concomitant.
- If the start day is missing but the start month and year are complete, a medication will only be excluded as being concomitant if the start month/year is before the month/year of study drug administration and if the stop date (full date, month and year if missing day, or year if missing month and day) is before study drug administration.
- If the start day and month are missing but the start year is complete, a medication will only be excluded as concomitant if the start year is before the year of study drug administration and if the stop date (full date, month and year if missing day, or year if missing month and day) is before study drug administration.
- If the start date is completely missing and the stop date is prior to first dose or completely missing, the medication will be assumed to be a prior medication.

The use of concomitant medications and pain/restricted medications will be included in a by-patient data listing. Pain/restricted medications are specifically identified in the CRF and will be noted in the listing.

5. CHANGES TO PLANNED ANALYSES

Although the protocol states that listings will be provided by study site and patient, per this SAP, by-patient listings will be sorted by treatment arm and then patient number.

6. REFERENCES

Ayral X, Pickering EH, Woodworth TG, Mackillop N, Dougados M. Synovitis: a potential predictive factor of structural progression of medial tibiofemoral knee osteoarthritis -- results of a 1 year longitudinal arthroscopic study in 422 patients. Osteoarthritis Cartilage 2005;13:361-7.

Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B. Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis 2005;64:1263-1267.

Bodick N, Lufkin J, Willwerth C, Blanks R, Inderjeeth C, Kumar A, Clayman M. FX006 prolongs the residency of triamcinolone acetonide in the synovial tissues of patients with knee osteoarthritis. Osteoarthritis Cartilage. 2013;21(Suppl):S144-5.

Conaghan, P. G., Cohen, S. B., Berenbaum, F., Lufkin, J., Johnson, J. R. and Bodick, N. Phase 2b trial of a novel extended-release microsphere formulation of triamcinolone acetonide for intra-articular injection in knee osteoarthritis. Arthritis Rheumatol. Accepted Author Manuscript. doi:10.1002/art.40364

Creamer P, Hochberg MC. Osteoarthritis. Lancet 1997;350(906):503–508.

EMA (2010) – *Guidance on investigation bioequivalence*, CPMP/EWP/QWP/1404/98 Rev. 1/ Corr, Committee for medicinal products for Human Use.

FDA (2001) – *Guidance for industry – Statistical approaches to Establish Bioequivalence*, U.S. Department of Health and Human Services, Food and Drug administration, Center for drug Evaluation and Research, BP.

FDA (2013) – *Guidance for industry – Bioequivalence study with Pharmacokinetic Endpoints for drug Submitted Under ANDA*, U.S., Department of Health and Human Services, Food and Drug Administration, Center for drug evaluation and Research, Biopharmaceutics

Goldring SR, Goldring MB. Clinical aspects, pathology and pathophysiology of osteoarthritis. J Musculoskelet Neuronal Interact 2006;6(4):376–378.

Hochberg MC, Altman RD, April KT, Benkhalti M, Guyatt G, McGowan J, Towheed T, Welch V, Wells G, Tugwell P. American College of Rheumatology 2012 Recommendations for the Use of Nonpharmacologic and Pharmacologic Therapies in Osteoarthritis of the Hand, Hip, and Knee. Arthritis Care & Research 2012;64:465-474.

Jordan KM, Arden NK, Doherty M, Bannwarth B, Bijlsma JW, Dieppe P, Gunther K, Hauselmann H, Herrero-Beaumont, Kaklamanis P, Lohmander S, Leeb B, Lequesne M, Mazieres B, Martin-Mola E, Pavelka K, Pendleton A, Punzi L, Serni U, Swoboda B, Verbruggen G, Zimmerman-Gorska I, Dougados M. EULAR Recommendations 2003: an evidence based approach to the management of knee osteoarthritis: Report of a Task Force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). Ann Rheum Dis 2003;62(12):1145–1155.

Sellam J and Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nature Reviews Rheumatology 2010;6:625-635.

Wenham CYJ and Conaghan PG. The role of synovitis in osteoarthritis. Ther Adv Musculoskel Dis 2010;2:349-359.

7. CLINICAL STUDY REPORT APPENDICES

7.1. Statistical Tables to be Generated

Table 14.1.1	Patient Enrollment and Disposition
Table 14.1.2	Demographic and Baseline Characteristics (Safety Population)
Table 14.1.3	Osteoarthritis History and Bilateral Knee Characteristics (Safety Population)
Table 14.1.4	Summary of Protocol Deviations (Safety Population)
Table 14.1.5	Study Drug Exposure Summary (Safety Population)
Table 14.1.6	Summary and Change from Baseline for Serum Cortisol (nmol/L) by Visit (Safety Population)
Table 14.1.7	Plasma Drug Concentrations (pg/mL) by Time Point (PK Population)
Table 14.2.1	Plasma Pharmacokinetic Parameters (PK Population)
Table 14.2.2	Summary of Bioequivalence Geometric Mean Ratios (PK Population)
Table 14.3.1.1	Summary of Treatment-Emergent Adverse Events (Safety Population)
Table 14.3.1.2	Treatment-Emergent Adverse Events by System Organ Class and Preferred Term (Safety Population)
Table 14.3.1.3	Treatment-Emergent Adverse Events by System Organ Class and Preferred Term - Related to Study Drug (Safety Population)
Table 14.3.1.4	Treatment-Emergent Adverse Events by System Organ Class and Preferred Term by Maximum Severity (Safety Population)
Table 14.3.1.5	Knee-Related Treatment-Emergent Adverse Events by System Organ Class and Preferred Term (Safety Population)
Table 14.3.1.6	Knee Related Treatment-Emergent Adverse Events by System Organ Class and Preferred Term- Related to Study Drug (Safety Population)
Table 14.3.1.7	Knee Related Treatment-Emergent Adverse Events by System Organ Class and Preferred Term by Maximum Severity (Safety Population)
Table 14.3.1.8	Serious Adverse Events by System Organ Class and Preferred Term (Safety Population)
Table 14.3.2.1	Patient Listing of Deaths
Table 14.3.2.2	Patient Listing of Serious Adverse Events
Table 14.3.2.3	Patient Listing of Discontinuations Due to Adverse Events
Table 14.4.1.1	Summary and Change from Baseline for Hematology Parameters by Visit (Safety Population)
Table 14.4.1.2	Summary and Change from Baseline for Chemistry Parameters by Visit (Safety Population)
Table 14.4.2	Summary and Change from Baseline for Vital Signs by Visit (Safety Population)
Table 14.4.3	Summary of Bilateral Knee Assessments (Safety Population)

7.2. Statistical Figures to be Generated

Figure 14.1.7	GM with 95% CI for Plasma Drug Concentration Curve (Plasma Drug Concentration)
Figure 14.2.1	Individual Patient Linear-Linear and Log-Linear Plasma Drug Concentration Profiles (PK Population)

7.3. Data Listings to be Generated

Listing 16.2.1	Patient Disposition
Listing 16.2.2.1	Inclusion/Exclusion Criteria Not Met
Listing 16.2.2.3	Protocol Deviations
Listing 16.2.3.1	Analysis Populations
Listing 16.2.4.1	Demographics and Baseline Characteristics
Listing 16.2.4.2	Medical History
Listing 16.2.4.3	Medical History - Knee Osteoarthritis
Listing 16.2.4.4	Medical History - Prior Treatment of Knee Osteoarthritis
Listing 16.2.4.5	Medical History - Other Osteoarthritis
Listing 16.2.4.6	Medical History - Other Osteoarthritis Procedures or Surgeries
Listing 16.2.4.7	Knee X-ray/Kellgren-Lawrence Grade
Listing 16.2.5.1	Study Drug Exposure
Listing 16.2.5.2	Plasma Sampling Times and Plasma Drug Concentration Measurements
Listing 16.2.5.3	Plasma PK Parameters
Listing 16.2.6	Serum Cortisol (nmol/L)
Listing 16.2.7.1	Adverse Events – Sorted By Patient and Onset Date
Listing 16.2.7.2	Adverse Events Occurring in Both Knees – Sorted By Patient and Onset Date
Listing 16.2.7.3	Adverse Events – Sorted by System Organ Class, Preferred Term, Treatment, Patient, and Onset Date
Listing 16.2.7.4	Adverse Events – Glossary by MedDRA SOC, Preferred Term and Verbatim
Listing 16.2.8.1	Laboratory Assessment – Hematology
Listing 16.2.8.2	Laboratory Assessment – Chemistry
Listing 16.2.8.3	Screening Laboratory Assessments and Urine/Serum Pregnancy Test Results
Listing 16.2.8.4	Bilateral Knee Assessments
Listing 16.2.9.1	Vital Signs
Listing 16.2.9.2	Physical Examination Findings
Listing 16.2.9.3	Electrocardiograms Results
Listing 16.2.9.4	Prior and Concomitant Medications
Listing 16.2.9.5	Knee Aspiration
Listing 16.2.9.6	Surgical Procedures