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Synovial and systemic pharmacokinetics (PK) of triamcinolone acetonide (TA) following intra-articular (IA) injection of an extended-release microsphere-based formulation (FX006) or standard crystalline suspension in patients with knee osteoarthritis (OA)

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SUMMARY

Objective: Intra-articular (IA) corticosteroids relieve osteoarthritis (OA) pain, but rapid absorption into systemic circulation may limit efficacy and produce untoward effects. We compared the pharmacokinetics (PK) of IA triamcinolone acetonide (TA) delivered as an extended-release, microsphere-based formulation (FX006) vs a crystalline suspension (TAcs) in knee OA patients.
Method: This Phase 2 open-label study sequentially enrolled 81 patients who received a single IA injection of FX006 (5 mL, 32 mg delivered dose, $N = 63$) or TAcs (1 mL, 40 mg, $N = 18$). Synovial fluid (SF) aspiration was attempted in each patient at baseline and one post-IA-injection visit (FX006: Week 1, Week 6, Week 12, Week 16 or Week 20; TAcs: Week 6). Blood was collected at baseline and multiple post-injection times. TA concentrations (validated LC-MS/MS, geometric means (GMs)), PK (non-compartmental analysis models), and adverse events (AEs) were assessed.
Results: SF TA concentrations following FX006 were quantifiable through Week 12 (pg/mL: 231,328.9 at Week 1; 3590.0 at Week 6; 290.6 at Week 12); post-TAcs, only two of eight patients had quantifiable SF TA at Week 6 (7.7 pg/mL). Following FX006, plasma TA gradually increased to peak (836.4 pg/mL) over 24 h and slowly declined to <110 pg/mL over Weeks 12–20; following TAcs, plasma TA peaked at 4 h (9628.8 pg/mL), decreased to 4991.1 pg/mL at 24 h, and was 149.4 pg/mL at Week 6, the last post-treatment time point assessed. AEs were similar between groups.
Conclusion: In knee OA patients, microsphere-based TA delivery via a single IA injection prolonged SF joint residency, diminished peak plasma levels, and thus reduced systemic TA exposure relative to TAcs.

Introduction

Osteoarthritis (OA) is increasingly recognized as a serious condition[1]. Specifically, people with OA have an increased cause-specific mortality risk attributable to cardiovascular and gastrointestinal disorders[2], and those with symptomatic radiographic knee OA have been shown to have 23% higher risk of dying prematurely than individuals without OA[1]. Possible explanations for the excess

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mortality include reduced physical activity; comorbid conditions; and adverse effects of OA medications, particularly non-steroidal anti-inflammatory drugs and opioids. There remains a need for safe therapies that effectively treat the symptoms and functional consequences of OA.

Inflammation plays a key role in OA pathogenesis. Serial magnetic resonance imaging and histopathological analysis of synovial tissues show synovitis is associated with more severe joint pain and cartilage loss, and decreased mobility[3–11]. Thus, synovitis is increasingly recognized as an important treatment target[12–16]. Intra-articular (IA) corticosteroids such as triamcinolone acetonide crystalline suspension (TAcs) are a mainstay in short-term management of OA pain[17–21]. Pain relief associated with IA corticosteroids is consistent with their known anti-inflammatory actions, particularly the regulation of inflammatory transcription factors and post-transcriptional pathways[22–24]. The inflamed synovium is the likely site of IA corticosteroid activity[25–28]. Synovial tissue volume not only shrinks in patients with knee OA post-steroid therapy, but also rebounds when pain relapses[29]. However, pain relief associated with IA corticosteroids diminishes in the weeks following injection[18,30–32], most likely due to efflux of drug from the joint beginning within hours of injection[33]. This rapid systemic absorption from the joint not only limits the duration of analgesic and anti-inflammatory effects of IA corticosteroids, but also can lead to cardiovascular and metabolic systemic effects[34]. Drug development efforts aimed at limiting the rapid efflux and extending the duration of IA corticosteroid presence may enhance the magnitude and duration of analgesic effect and mitigate systemic effects.

FX006 is a novel, extended-release, poly (lactic-co-glycolic acid) (PLGA) microsphere-based TA formulation designed to prolong the drug's residence time in the synovial tissues and fluid following IA injection. *In vitro* experiments show that the crystals of TA from the TAcs formulation completely dissolve in <2 h; in the same time period and under the same conditions, <1% of the embedded TA from the FX006 formulation is released from the PLGA matrix (Sponsor data on file). Previously, a single, 3-mL, IA injection of FX006 substantially increased the duration of TA joint residency, markedly reduced systemic exposure, and demonstrated both prolonged and increased analgesia relative to TAcs 40 mg in patients with knee OA[35–37]. Subsequently, the FX006 diluent reconstitution volume was increased from 3 mL to 5 mL to facilitate microsphere dispersion. The current study was conducted to characterize the synovial fluid (SF) and plasma TA PK profiles of FX006 for the 5-mL injection volume and to compare the SF and plasma TA PK profiles with those of TAcs (1-mL injection volume), one of the most common IA knee OA therapies.

Patients and methods

Ethical considerations

Study procedures complied with ethical standards of the responsible committee on human experimentation (institutional and national) and the Declaration of Helsinki of 1975, as revised in 2000. A central Institutional Review Board was utilized (Schulman Central IRB, Cincinnati, OH, USA). All patients provided written informed consent.

Patient eligibility and study design

In this Phase 2 open-label study (clinicaltrials.gov identifier: NCT02637323), patients with knee OA received a single IA injection of FX006 (5 mL, delivered dose of 32 mg) or TAcs (1 mL, 40 mg). Patients were sequentially enrolled (≥10 patients/cohort) into one of six SF collection cohorts: Week 1, 6, 12, 16, or 20 for FX006 or Week 6 for TAcs. Additional patients were to be assigned to cohorts as needed, with the intent of collecting SF from ≥6 patients/cohort.

Study participants were men and women (≥40 years of age, body mass index (BMI) ≤40 kg/m^2) with knee OA diagnosed according to American College of Rheumatology clinical and radiological criteria[38], with symptoms for ≥6 months prior to screening and patient-reported index-knee pain for ≥15 days of the preceding month. Patients with arthroscopic/open surgery of the index knee (i.e., most painful in patients with bilateral disease) within 12 months of screening or planned/anticipated index-knee surgery during the study were excluded. Additional exclusion criteria included uncontrolled diabetes (hemoglobin A1c >7.5%) and treatment with IA corticosteroids in any joint <6 months; IA hyaluronic acid in the index knee <6 months; intravenous or intramuscular corticosteroids <3 months; oral corticosteroids <1 month; and/or inhaled, intranasal or topical corticosteroids <2 weeks prior to screening. These same treatments, and any IA intervention (e.g., non-protocol-specified aspiration) of the index knee, were prohibited during study participation. Because this study did not evaluate efficacy, there was no minimum pain-intensity criterion at entry, and analgesic medications (e.g., non-steroidal anti-inflammatory drugs and opiates) were allowed.

Study interventions

FX006 powder was reconstituted in diluent containing an isotonic, sterile, aqueous sodium chloride solution (0.9% weight/weight [w/w]), carboxymethylcellulose (0.5% w/w) plus polysorbate-80 (0.1% w/w) and administered as a 5-mL injection. FX006 dose-delivery studies determined that this resulted in administration of 32 mg of TA. Commercially available TAcs 40 mg (Kenalog®-40; Bristol-Myers Squibb Company, Princeton, NJ, USA) was administered as a 1-mL injection, allowing comparisons between FX006 and a current standard of care. Patients were to avoid strenuous activities or prolonged weight-bearing activities for 24–48 h post-IA injection and maintain a stable level of physical activity throughout the study.

Study assessments

To optimize needle placement and volume of SF collected, index knee aspirations were performed with ultrasound guidance at baseline and Weeks 1, 6, 12, 16, or 20 for FX006 cohorts or at baseline and Week 6 for the TAcs cohort (mean [standard deviation (SD)] actual volume [mL]: 2.03 [3.45]; range: 0–27). Blood samples were collected at baseline and Hours 1, 2, 4, 6, 8, 10, 12, and 24 for all patients; Weeks 1, 6, 12, 16 and 20 for FX006-treated patients; and Week 6 for TAcs-treated patients. Laboratory assessments included non-fasting blood glucose at screening, Day 1 (prior to IA injection), and Week 6 (TAcs cohort) or Weeks 6, 12, 16, and 20 (FX006 cohorts).

SF and plasma TA concentrations were both measured using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays. For SF, frozen samples were thawed at room temperature, followed by addition of internal standard working/phosphate-buffered solutions. Following mixing and a liquid–liquid extraction (methyl tert-butyl ether and hexanes), the samples were centrifuged. Using the Janus liquid handling system (PerkinElmer, Shelton, CT, USA), a portion of each sample's organic phase was transferred into a 96-well plate. The organic phase was evaporated, and samples were reconstituted using a methanol/water mixture prior to injection into a LC-MS/MS (ACE C18, 30 mm × 4.6 mm, 3-μm particle size column, water/methanol mobile phase, ionization in positive mode with a heated

nebulizer). Masses monitored for the analyte in SF were 435/415 and 435/397 and for the internal standard were 442/422 and 422/404. The TA quantification process employed a 2.5× overall dilution factor.

The lower limits of quantification (LLOQs) differed between the two matrices, i.e., 50.0 pg/mL for SF and 10.0 pg/mL for plasma, with the SF matrix exhibiting less assay sensitivity due to more interference from the SF components detected at the analyte's retention time relative to that observed in plasma. The LLOQ of 50 pg/mL represents 1–2% of the expected maximum concentration (C_{max}) in SF and is judged sufficient to characterize the PK profile of the analyte in SF.

Safety was assessed through monitoring of adverse events (AEs). Index knees were assessed for tenderness, heat/redness, swelling, effusion, and Baker's cyst. Clinically significant findings during screening/baseline were reported as part of the patient's medical history, while new clinically significant findings or worsened post-baseline findings were recorded as AEs.

Data analysis

The SF and plasma TA concentrations were assessed using non-compartmental analysis (Phoenix 64®: WinNonlin® [Version 6.4]) models on the SF and Plasma Drug Concentration Populations, respectively (Fig. 1). Sparse sampling methodology following Day 1 was implemented across the plasma concentration profile to capture population-based data at the end of the sampling profile. All plasma concentration values were set to 0 for pre-dose (time 0) and post-baseline samples that were assayed as below the LLOQ (BLOQ); geometric mean (GM) data are presented. Due to the small number of patients contributing SF samples at each post-dose time point, we also employed sparse sampling modeling, with each patient contributing only a pre-dose sample and one post-dose sample. For SF samples, pre-dose and post-baseline concentrations BLOQ were set to 0. To obtain a reasonable estimate of the GM SF values, a scaler value of 1 was added to each observed concentration value; thus, values BLOQ for the computation of GM appear in the descriptive SF summaries as a value of 1. TA concentration data were presented by time point pooled across FX006 cohorts and separately for the TAcs cohort.

The PK non-compartmental analysis models were completed using Phoenix® 64 WinNonlin® (Version 6.4) using the PK Population (Fig. 1), and graphical displays of PK analyses and concentration information were completed with Sigma Plot (Version 13.0). PK parameters derived from plasma TA concentrations were either observed or estimated by WinNonlin® (Version 6.4). A linear trapezoidal rule was applied to compute observed area under the plasma concentration–time curve (AUC). Partial AUC parameters were computed for the intervals $AUC_{0-24\ h}$ and $AUC_{0-6\ weeks}$. Patients contributing samples at Week 1, 12, 16, or 20 provided information to compute partial AUCs for these intervals. When sufficient information was available to estimate a terminal rate (λz) constant, the extrapolated parameters (half-life [$t_{½}$], $AUC_{0-\infty}$, clearance, mean residence time [$MRT_{0-\infty}$]) were estimated.

AEs were coded using the Medical Dictionary for Regulatory Activities (Version 18.0). Concomitant medications were coded using the World Health Organization Drug Dictionary (March 2014 edition).

No formal sample size computations were completed for this descriptive study. A minimum of 10 patients/cohort was planned to provide ≥6 patients/cohort providing SF TA concentration data for estimation of GM values to within approximately 3-fold with 95% confidence. The sample size of 10 patients/cohort also was considered sufficient to characterize the FX006 PK profile using a 5-mL injection volume.

Results

Patient disposition

The study was conducted from 11/30/2015 through 9/8/2016 at four United States study centers. Eighty-one enrolled patients received a single IA injection of FX006 ($N = 63$) or TAcs ($N = 18$) on Day 1. Patients sequentially enrolled into FX006 SF collection cohorts included: 13 for the Week 1; 20 for the Week 6; and 10 each for the Weeks 12, 16, and 20 cohorts (Table I). Three patients discontinued the study early (two FX006 lost to follow-up (LTFU), one TAcs protocol non-compliant).

Sixty of 63 (95.2%) FX006 and 18/18 (100%) TAcs patients were included in the Plasma Drug Concentration and PK Populations. Twenty-nine of 63 (46.0%) FX006 and eight of 18 (44.4%) TAcs patients were included in the SF Drug Concentration Population. Not all cohorts achieved ≥6 patients providing SF samples at later visits, primarily due to <0.1 mL of SF being aspirated at the final sampling time point, yielding 2–9 patients/cohort and 37/81 (45.7%) patients across all cohorts providing SF samples. Details of the Safety, Plasma Drug Concentration, SF Drug Concentration, and PK populations are provided in Fig. 1.

Baseline characteristics

Among patients treated across study cohorts (Safety Population), the 36 (44.4%) men and 45 (55.6%) women had a mean age of 60 years, mean BMI ranging from 30 to 32 kg/m^2 across cohorts, mean time since primary OA diagnosis of almost 8 years, and a mean of 29 days of index-knee pain within the month preceding screening. As expected, based on lack of randomization and relatively small cohort sample sizes, baseline characteristics varied across cohorts (Table I).

FX006 SF and plasma TA concentrations

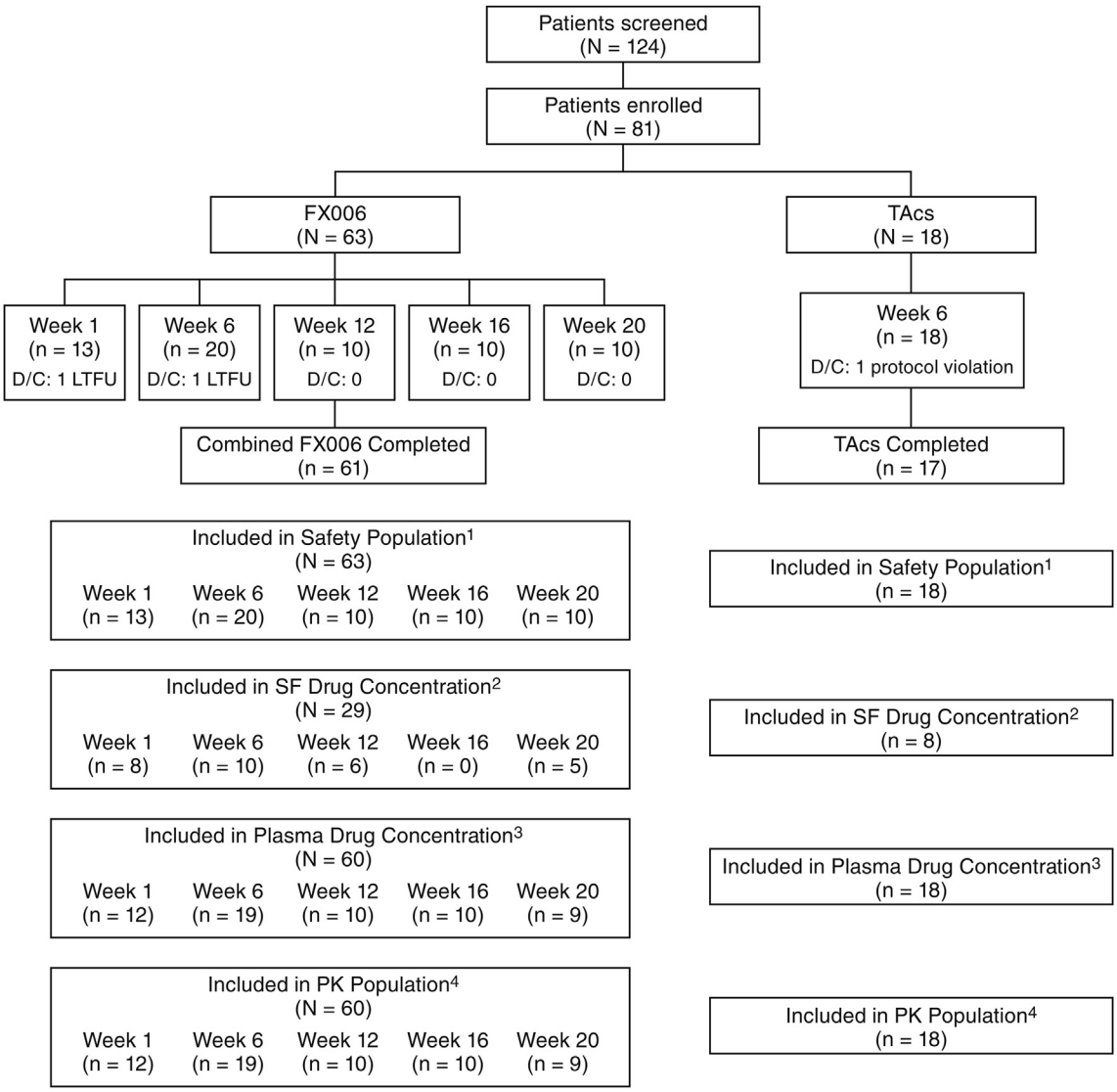
Following a single IA injection of FX006, the GM SF TA C_{max} of 231,321.3 pg/mL was reached at a median time to maximum concentration (t_{max}) of 168.0 h (i.e., Week 1). Thereafter, SF TA concentrations declined, with GMs of 3590.0 pg/mL at Week 6 and 290.6 pg/mL at Week 12. After Week 12, TA was BLOQ in the small number of SF samples obtained (Fig. 2).

FX006-TA demonstrated some systemic absorption, with a plateau in plasma TA concentrations through Hour 24 followed by slow elimination from the systemic circulation (Fig. 3). The GM C_{max} (966.7 pg/mL) was reached at a median t_{max} of 7.0 h. The median $t_{½}$ was 347.0 h, GM MRT was 453.7 h, and GM clearance was slow (0.0001 mL/h/kg). The GM last measurable concentration (106.4 pg/mL) was observed at a median time to last measurable concentration (t_{last}) of 1008.0 h (approximately Week 6) (Table II).

TAcs SF and plasma TA concentrations

Following a single IA injection of TAcs, TA SF concentrations were BLOQ at Week 6 (Fig. 2). Of the eight patients who contributed samples at Week 6, only two had measurable SF TA concentrations at this time point, yielding a GM SF TA concentration of 7.7 pg/mL, notably lower than that seen with FX006 at Week 6 (3590.0 pg/mL).

Systemic TA absorption was relatively rapid post-TAcs injection, with GM plasma TA concentrations determined for each sampling time point peaking at 4 h (9628.8 pg/mL) and declining to 4991.1 pg/mL at Hour 24 post-injection (Fig. 2). The observed GM C_{max} of 11,064.7 pg/mL was reached at a median t_{max} of 6 h. The median $t_{½}$ (72.5 h) was shorter than that seen with FX006. GM MRT



1 Treated patients.

2 The SF Drug Concentration Population was defined as all patients who received study drug and had SF obtained at the final visit and assayed for drug concentration levels.

3 The Plasma Drug Concentration Population was defined as all patients who received study drug and had ≥1 plasma sample obtained beyond 24 hours post-injection and assayed for drug concentration levels.

4 The PK Population was defined as all patients who received study drug and had sufficient non-missing post-baseline plasma samples obtained and assayed for drug concentration levels to allow for computation of PK parameters, i.e., a minimum of four samples following C_{max} to allow for calculation of a terminal rate constant.

Fig. 1. Patient disposition. D/C – discontinuation, LTFU – lost to follow-up.

observed was 60.9 h, and GM $AUC_{(0–6\ weeks)}$ was 3,082,834.8 h * pg/mL, which was 6× higher than that observed with FX006 (Table II).

Safety

AEs were reported for 11.1% (7/63) of FX006-and 11.1% (2/18) of TAcs-treated patients. The only AEs reported by >1 patient were hypertension in two FX006-treated patients (both with a history of hypertension) and flushing in two TAcs-treated patients (considered to be related to the injection procedure in both patients). The vast majority of AEs were Grade-1 or 2 and assessed by the investigator to be unrelated to study drug. One Grade-4 pulmonary embolism occurred in an FX006-treated patient with a history of pulmonary embolism (serious AE considered unrelated to study drug, injection procedure, or index knee). The AEs considered related to study drug were a Grade-1 arthralgia and a Grade-2 patellofemoral pain syndrome in FX006-treated patients and the Grade-1 flushing noted above in two TAcs-treated patients. The incidence of index-knee-related AEs in the patients treated with FX006 was 3.2% (2/63). No patient died or discontinued due to an AE.

Discussion

Results of this Phase 2 open-label study indicate that microsphere-based extended-release delivery of TA via a single 5-mL IA injection of FX006 prolonged TA IA residency time,

Table I
Baseline demographic, patient, and disease characteristics by cohorts (safety population)

Parameter	FX006 cohorts					All FX006 ($N = 63$)	TAcs cohort
	Week 1 ($N = 13$)	Week 6 ($N = 20$)	Week 12 ($N = 10$)	Week 16 ($N = 10$)	Week 20 ($N = 10$)		Week 6 ($N = 18$)
Male – *n* (%)	7 (53.8)	6 (30.0)	5 (50.0)	6 (60.0)	7 (70.0)	31 (49.2)	5 (27.8)
Age (years)	55.5 (8.61)	58.8 (7.02)	57.2 (6.61)	67.3 (6.04)	63.0 (8.08)	59.9 (8.16)	61.9 (7.68)
BMI (kg/m^2)	30.7 (4.66)	30.3 (4.16)	31.9 (6.17)	29.4 (4.76)	32.3 (4.38)	30.8 (4.69)	32.0 (4.32)
Years since primary diagnosis	3.8 (2.68)	8.3 (7.66)	8.1 (5.97)	14.8 (5.31)	4.7 (2.67)	7.8 (6.53)	7.92 (6.46)
Kellgren–Lawrence grade – *n* (%)							
1	1 (7.7)	3 (15.0)	1 (10.0)	0	0	5 (7.9)	4 (22.2)
2	7 (53.8)	10 (50.0)	3 (30.0)	7 (70.0)	2 (20.0)	29 (46.0)	7 (38.9)
3	5 (38.5)	7 (35.0)	4 (40.0)	3 (30.0)	6 (60.0)	25 (39.7)	5 (27.8)
4	0	0	1 (10.0)	0	2 (20.0)	3 (4.8)	2 (11.1)
Unknown	0	0	1 (10.0)	0	0	1 (1.6)	0
Days with index-knee pain*	28.5 (3.26)	27.9 (3.02)	29.8 (1.40)	28.8 (3.79)	28.7 (4.11)	28.6 (3.18)	29.1 (3.02)

Data presented are mean (SD) unless noted otherwise.
* Within the month prior to screening.

diminished peak TA plasma levels, and substantially reduced the systemic TA exposure relative to a single IA injection of a standard crystalline suspension of TA. Over the first week following IA FX006 injection, TA concentrations in the systemic circulation plateaued at low levels relative to values produced by TAcs, suggesting that TA remains in the SF and synovial tissues following injection, with slow absorption into systemic circulation. Prolonged joint residency is further supported by assessments of local TA concentrations in SF showing variable but quantifiable levels out to Week 12 post-injection, whereas those post-TAcs were either very low (two patients) or not quantifiable (six patients) at Week 6.

Synovial concentrations of TA – clinical implications

The differences in SF TA concentrations observed in this study—GMs of 3590.0 and 290.6 pg/mL at Week 6 and Week 12, respectively, for FX006 and 7.7 pg/mL at Week 6 for TAcs—likely constitute the basis for pharmacologic differentiation and provide a rational hypothesis for between-treatment efficacy differences observed in Phase 2/3 trials[37,39,47]. For example, exploratory analyses from a Phase 3, multicenter, double-blind, 24-week study showed that the same dose of FX006 demonstrated statistical improvements in pain, stiffness, and physical function, as assessed by the Western Ontario & McMaster Universities Osteoarthritis Index (WOMAC), at Weeks 4, 8, and 12 vs TAcs in patients with knee OA[39]. These effects are clinically important; post-hoc analyses demonstrated that the improvements in all WOMAC subscale scores afforded by FX006 exceeded the minimum clinically important improvement (MCII) thresholds utilized by the American Academy of Orthopedic Surgeons (AAOS)[39–42]. Specifically, FX006 effects achieved the AAOS definition of 'clinically significant' at Weeks 4 and 8 and 'possibly clinically significant' at Week 12 for all WOMAC subscale scores[39,40]. Conversely, TAcs never achieved the AAOS definition of 'clinically significant' at any time point for any WOMAC subscale[39,40]. These results represent the first time an injectable OA therapy has surpassed the AAOS-defined threshold for 'clinical significance,' and are likely attributable to sustained TA occupancy of the corticosteroid receptor afforded by the prolonged IA residency of TA associated with FX006 relative to TAcs reported herein. Similarly, animal studies have shown the anti-inflammatory

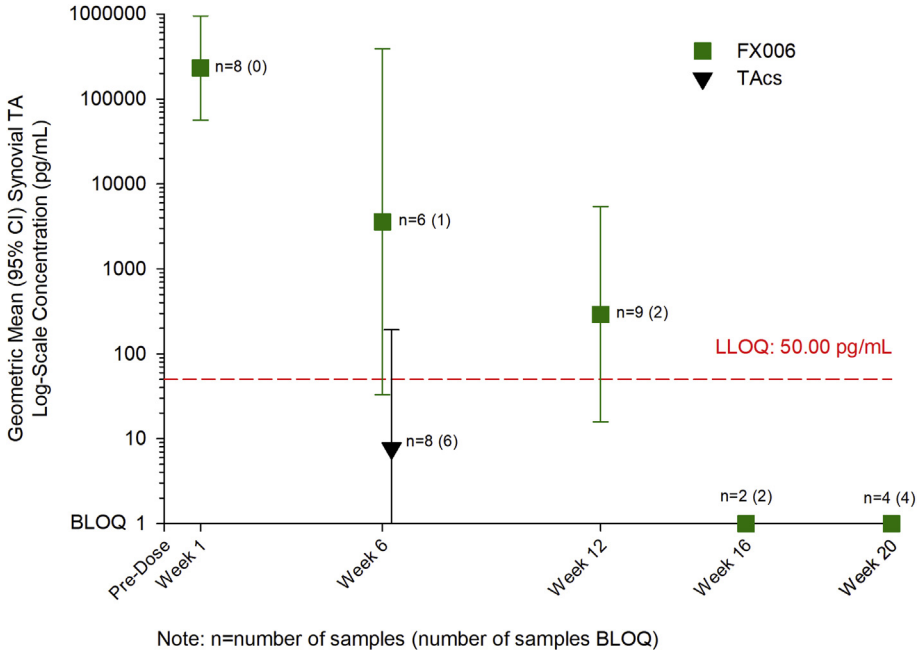


Fig. 2. SF TA concentrations (log-linear scale) over time following a single IA injection of FX006 or TAcs. To obtain a reasonable estimate of the GM SF values, a scaler value of 1 was added to each observed concentration value. CI – confidence interval.

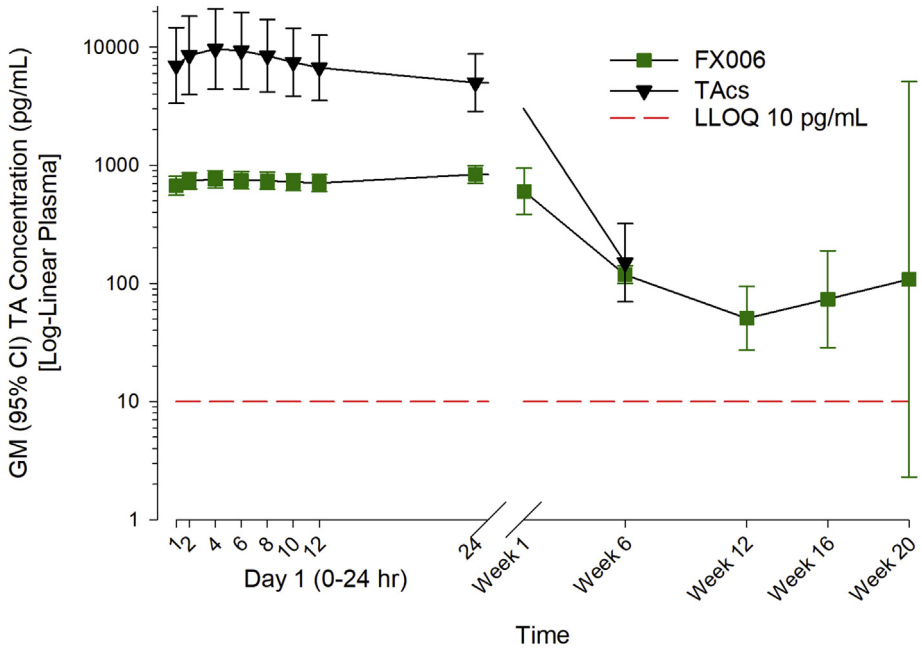


Fig. 3. Blood plasma TA concentrations (log-linear scale) over time following a single IA injection of FX006 or TAcs.

activity of corticosteroids is increased with prolonged delivery or targeting to inflamed synovial tissues as assessed histologically.[43,44]

Systemic concentrations of TA — clinical implications

The plasma TA concentration profile associated with FX006 in this study—a GM of 759.5 pg/mL at 4 h post-injection that plateaued over the first 24 h, followed by a slow decline to ~100 pg/mL through Week 20—indicates TA remains in the SF and tissues following IA injection, with corresponding slow elimination into systemic circulation (Fig. 3). These data are consistent with the results from two prior studies in patients with knee OA that included plasma PK sampling after a single injection of the same dose of FX006; plasma TA concentration curves over time were similar across studies[36,37]. Conversely, systemic TA absorption was rapid following IA injection of TAcs: plasma TA concentrations reached a GM C_{max} of 11,064.7 pg/mL at a median t_{max} of 6 h, and declined to 4991.1 pg/mL by 24 h. A similar plasma TA concentration profile following TAcs also was reported in a previous study in patients with knee OA, in which levels peaked at approximately 13,000 pg/mL on Day 1 and decreased in a log-linear manner to a value of approximately 100 pg/mL on Day 18[37]. The difference in systemic TA profiles resulting from IA FX006 compared with IA TAcs is underscored by the results from a post-hoc analysis used to demonstrate bioequivalence[45], which confirmed significantly lower systemic TA exposure following FX006 relative to TAcs (Online Supplement, Table S1).

While the total systemic exposure to TA from FX006 is substantially lower than that of TAcs, the duration of measureable levels of TA associated with FX006 is longer due to the extended-release mechanism of the PLGA microsphere. In a prior study, persistent low levels of systemic TA resulting from the same dose of FX006 were examined to determine whether or not they affected the function of the hypothalamic-pituitary-adrenal axis as measured by serum cortisol in patients with knee OA[35]. Through Week 6 post-injection, the cumulative cortisol suppression associated with FX006 was similar to that associated with TAcs[35]. As such, it is unlikely that IA administration of FX006 will compromise cortisol production or responses to stress throughout its duration of

Table II
Plasma PK parameters for pooled FX006 and TAcs cohorts (plasma drug concentration population)

PK Parameter	FX006 pooled cohorts (5-mL injection volume)		TAcs cohort (1-mL injection volume)	
C_{max} (pg/mL), GM	$N = 60$	966.7	$N = 18$	11,064.7
$AUC_{0-24\ h}$ (h * pg/mL), GM	$N = 60$	17,908.8	$N = 18$	173,690.8
$AUC_{0-6\ weeks}$ (h * pg/mL), GM	$N = 60$	508,939.6	$N = 17$	3,082,834.8
$AUC_{0-12\ weeks}$ (h * pg/mL), GM	$N = 14$	512,607.9*	Not assessed*	Not assessed*
AUC_{0-t} (h * pg/mL), GM	$N = 60$	440,718.6*	$N = 18$	594,276.8*
AUC_{0-inf} (Observed) (h * pg/mL), GM	$N = 33$	543,115.0	$N = 14$	1,248,903.3
t_{max} (h), median	$N = 60$	7.0	$N = 18$	6.0
$t_{1/2}$ (h), median	$N = 33$	347.0	$N = 14$	72.5
t_{last} (h), median	$N = 60$	1008.0	$N = 18$	24.0
C_{last} (pg/mL), GM	$N = 60$	106.4	$N = 18$	1508.6
$V_{(ss)}$ (Observed) (mL/kg)	$N = 33$	0.0497	$N = 14$	0.0029
CL ([mL/h]/kg), GM	$N = 33$	0.00012	$N = 14$	0.0000
MRT (h), GM	$N = 33$	453.7	$N = 14$	60.9

AUC (0—inf = time 0 extrapolated to infinity; 0—t = time 0 to the last quantifiable concentration; 0—24 h, 6 weeks, 12 weeks = time 0—24 h, 6 weeks, 12 weeks, respectively), CL — clearance, C_{last} — last measurable concentration, $V_{(ss)}$ — steady state volume of distribution.

* Measurements for TAcs extended through Week 6; those for FX006, depending on the cohort, extended to Week 6, Week 12, Week 16 or Week 20. $AUC_{0-12\ weeks}$ aligns with the time point at which synovial TA concentrations remained quantifiable.

exposure in patients with intact hypothalamic-pituitary-adrenal axis function.

Differences in systemic TA absorption following FX006 or TAcs IA injection may have clinical significance. Results from a recent study of patients with knee OA and type-2 diabetes mellitus assessing the effects of FX006 and TAcs on blood glucose over 72 h post-injection showed that FX006 was associated with a statistically significant ($P < 0.05$, 2-sided) and clinically relevant reduction in the rise of blood glucose[46]. As such, the overall reduced systemic TA exposure associated with FX006, particularly the low peak concentrations, suggests that FX006 has the potential to confer an improved safety profile relative to TAcs; additional studies are needed.

Safety data – clinical implications

Safety data from the 63 FX006-treated and 18 TAcs-treated patients identified no new or unexpected safety concerns for either agent. These findings are consistent with those observed in larger Phase 2/3 clinical trials of FX006, in which a single IA FX006 injection demonstrated systemic and local safety profiles generally similar to placebo and TAcs, and no post-injection flares were observed.[37,39,47]

Despite prolonged TA joint residence associated with microsphere delivery, index-knee related AEs were limited to two FX006-treated patients (Grade-1 arthralgia, Grade-2 patellofemoral pain syndrome). This lack of adverse local effect is not unexpected given that the FX006 PLGA matrix degrades to oligomeric poly-acid units, then to lactic and glycolic acids[48], followed by elimination as carbon dioxide and water.

Study limitations

Results are limited by small sample sizes and acquisition of fewer-than-planned SF samples. Patients were not randomized, but were sequentially enrolled starting with FX006 and followed by TAcs treatment groups. Nonetheless, findings are generally consistent with SF and plasma PK evaluations conducted for the earlier FX006 3-mL formulation, which also showed that FX006 substantially reduced systemic exposure to, and prolonged IA residence of, TA relative to TAcs[35–37]. Specifically, in a previous assessment of SF TA concentrations, variable but quantifiable TA levels were observed through 16 weeks post-injection[37]. Additionally, FX006 (3-mL formulation) produced substantially lower peak plasma TA concentrations than TAcs when given at matched doses in a larger ($N = 228$), double-blind, randomized, multicenter, Phase 2 trial in knee OA.[37]

Conclusions

These SF and plasma PK observations are consistent with slow release of TA into SF and tissues and blunted absorption into systemic circulation following a single IA injection of FX006 microspheres, yielding prolonged IA residency of TA relative to TAcs in patients with knee OA.

Author contributions

All authors made substantial contributions to study conception/design (JL, JJ, JH, NB), data acquisition (HAA, PM, AJK, JL, JH, NB), PK modeling (JJ), or data analysis/interpretation (VBK, HAA, PM, AJK, JL, JJ, SK, NB); drafted the article or revised it critically for important intellectual content (VBK, HAA, PM, AJK, JL, JJ, SK, NB); and approved the final submitted version (VBK, HAA, PM, AJK, JL, JH, JJ, SK, NB). VBK (kraus004@duke.edu) and NB (nbodick@flexiontherapeutics.com) take responsibility for the integrity of the work as a whole, from inception to finished article.

Competing interests statement

VB Kraus received consulting fees from Flexion Therapeutics, Inc.

PG Conaghan has received compensation for consultancies from AbbVie, Bioiberica, Flexion Therapeutics, Inc., Infirst, and Merck-Serono.

HA Aazami has reported no conflicts of interest.

P Mehra has reported no conflicts of interest.

AJ Kivitz has received consulting fees from AbbVie, Genentech, Janssen, Pfizer, and UCB.

J Lufkin, J Hauben, JR Johnson, and N Bodick are/were employed by, and own stock/stock options in, Flexion Therapeutics, Inc.

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Flexion Therapeutics, Inc., who funded this clinical trial, was involved in the study conception/design, data acquisition, and data analysis/interpretation. Authors currently or formerly employed by Flexion Therapeutics, Inc. (JL, JJ, JH, NB) drafted the article or revised it critically for important intellectual content; and approved the final submitted version.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.joca.2017.10.003.

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