CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208845Orig1s000

NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 208845
Supporting document/s:	SDN 1, SDN 8, SDN 10, and SDN 22
Applicant's letter date:	12/08/2016, 3/17/2017, 4/4/2017, and 7/20/2017
CDER stamp date:	12/08/2016, 3/17/2017, 4/4/2017, and 7/20/2017
Product:	ZILRETTA (triamcinolone acetonide ER) injection for intra-articular use
Indication:	Management of osteoarthritis (OA) pain
Applicant:	Flexion Therapeutics, Inc.
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products (DAAAP)
Reviewer:	Misol Ahn, PhD
Team Leader:	Jay H. Chang, PhD
Supervisor:	R. Daniel Mellon, PhD
Division Director:	Sharon Hertz, MD
Project Manager:	Kimberly Compton, PharmD

Disclaimer
Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208845 are owned by Flexion or are data for which Flexion has obtained a written right of reference.
Any information or data necessary for approval of NDA 208845 that Flexion does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208845.

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Flexion Therapeutics submitted NDA 208845 seeking marketing approval of Zilretta™ (triamcinolone acetonide (TCA or TAC) USP, extended release injectable suspension) for intra-articular (IA) injection for osteoarthritis (OA) pain management. The NDA 208845 is a 505(b)(2) application referencing the listed drug, Kenalog-40 (TCA, injectable IR suspension, USP, NDA 14901) and literature.

OA is a common chronic condition of the joints, which is characterized by intra-articular inflammation, deterioration of articular cartilage, and degenerative changes to periarticular and subchondral bone. OA occurs most often in large weight-bearing joints such as the knees and hips, but also occurs in the shoulders, lower back, neck, small joints of the fingers, and the bases of the thumb and big toe.

TCA is a corticosteroid approved in the U.S. for administration through multiple routes (inhalation, intramuscular, intravitreal, and topical) for various disease conditions including OA. The originally proposed maximum recommended human dose (MRHD) of TCA, the active ingredient, in Zilretta was 40 mg; however, the MRHD has been changed to 32 mg based on the actual delivered dose in clinical studies (refer to CMC and clinical reviews for details about the actual delivered dose). A comparative bioavailability study demonstrated that the systemic exposure levels (AUC and C_{max}) to TCA following IA administration of Zilretta at the MRHD in human subjects was within the approved reference product (Kenalog-40, injectable IR suspension) for the same route (refer to clinical pharmacology review for detailed information); therefore the systemic safety of TCA can be bridged to the reference product.

1.2 Brief Discussion of Nonclinical Findings

Zilretta is formulated in 75:25 poly(lactic-co-glycolic acid) (PLGA) microspheres to confer extended release of TCA to the synovial tissues with the intention of providing more persistent pain relief than currently available IR formulations of TCA. PLGA has not been previously employed for any FDA-approved IA drug and therefore is new for the route. The safety of PLGA was appropriately evaluated through the conduct of IA toxicity studies. All other excipients in Zilretta are considered qualified for safety as they are within levels found in FDA-approved products for the IA route. The specifications for the drug substance impurities and drug product degradants are acceptable as they were adequately justified. Moreover, the container closure system of the drug product has been adequately qualified through appropriate extractables and leachables data.

Single-dose and repeat-dose GLP IA toxicity studies were conducted in dogs to justify the safety of the PLGA excipient and the product formulation. Notably, NDA 208845 is seeking approval for a single-use indication at this time so the single-dose IA studies are considered pivotal. Based on single-dose IA dog study data, Zilretta appears to cause slightly higher local toxicities than the active comparator, Kenalog-40 IR (aka IR TCA). Microscopic changes observed in Zilretta-treated animals included increased

multinucleated macrophages, lymphocyte infiltration, plasma cell infiltration, and hyperplasia, most of which are expected foreign body reactions. They were generally of only slight severity and appear attributed to the PLGA component though there were comparable incidences of infiltrates and hyperplasia observed in animals treated with the active IR TCA comparator albeit for shorter durations with the IR. Nevertheless, this suggests that the API may contribute to these changes. In addition, a detailed joint evaluation, which included Safranin O staining, showed that Zilretta caused a dose-dependent loss of cartilage with peak effects at 3 months following administration. At 3 months following administration, up to severe cartilage reduction was noted in the patellar region at the highest Zilretta dose tested compared to a slight to moderate reduction observed with IR TCA. Changes at the distal femur and tibial plateau were more comparable between the two groups. At 4 months and longer following the single-dose administration, the effects at the joint were comparable between the Zilretta and IR TCA groups with the cartilage effects resolved by 9 months in the IR TCA group and nearly fully resolved with Zilretta. Microscopic evidence of residual polymer in the IA space was apparent for up to 3 months but not at 4 months and longer. This may account for the more severe effects with Zilretta at the 3-month time point. However, interestingly, there was no reduction in cartilage with the microspheres alone, which suggests that the adverse changes were related to both PLGA microspheres and the longer exposure of TCA in the injection site, the knees. At most time points and for most knee regions, the differences were subtle, and therefore it is not clear how clinically relevant they are. In contrast, the local toxicity was more evident in repeat-dose toxicity studies. Animals treated with Zilretta showed clear increases in microscopic changes to the local tissue and more severe cartilage loss relative to control and IR TCA, and these local responses were not fully reversed by the end of the 9-month recovery period. Therefore, the data may not adequately support the safety of Zilretta if the Applicant pursues a repeat use or chronic indication in the future from a nonclinical perspective. We will have to revisit the data and evaluate the findings in the context of the risk:benefit for the proposed patient population at that time.

The Applicant is referencing the label of the listed drug product Kenalog-40 IR for reproductive toxicology, genetic toxicity, and carcinogenicity information for the proposed Zilretta label. Therefore, no new studies were conducted to address these endpoints. In addition, the Applicant submitted a literature review of published studies in animals to address the effects of TCA on reproduction and embryonic development in accordance with the Pregnancy Labeling and Lactation Rule (PLLR). Based on a review of the literature submitted, this reviewer has recommended that some of this information should be included in the proposed Zilretta label. The recommended changes are detailed in the labeling section of this review and have been incorporated in the Division's recommended labeling changes to be sent to the Applicant.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, this NDA application may be approved. There are adequate data, including single-dose toxicity data in dogs, that support the relative safety of Zilretta for the proposed single-use indication.

1.3.2 Additional Non Clinical Recommendations

N/A

1.3.3 Labeling

The Applicant submitted a proposed label in the PLLR format and performed a literature search entitled "literature review in support of the PLLR for FX006" for potential reproductive and developmental toxicity efforts of TCA.

The Applicant's proposed label language, this reviewer's recommended changes, and the rationale for the recommended changes/comments are listed in the table below. Note that the final label will be found on the Action letter after further internal discussion and negotiations with the Applicant.

Table 1. Labeling Recommendation

Applicant's Proposed Labeling	Reviewer's Recommended Changes in Labeling	Rationale for Changes/Comments
Highlights of Prescribing Information		
Indications and Usage		
(b) (4)	ZILRETTA (b) (4) indicated as an intra-articular injection for the management of osteoarthritis pain of the knee. (1)	This section must include an appropriate established pharmacologic class for the drug substance(s), if available, per 21 CFR 201.57. The Applicant included the pharmacologic class of Zilretta (b) (4) (b) (4) Refer to the clinical review.
8 Use in Specific Populations		
8.1 Pregnancy		
Risk Summary In animal reproductive studies (b) (4)	Risk Summary In animal reproduction studies	The draft label from the Applicant is submitted in a

pregnant mice, rats, rabbits, or primates, (b) (4) (b) (4) Data *Animal Data* (b) (4)	from published literature, pregnant mice, rats, rabbits, or primates administered triamcinolone acetonide during the period of organogenesis at doses less than the maximum recommended human dose (MRHD) caused resorptions, decreased fetal body weight, craniofacial and/or other developmental abnormalities such as omphalocele, (b) (4) *[see Data].* Data *Animal Data* The exposure margins listed below are based on body surface area comparisons (mg/m^2) to the MRHD of 32 mg triamcinolone acetonide via ZILRETTA (b) (4) Pregnant mice dosed with triamcinolone acetonide via intramuscular or subcutaneous injection at doses equivalent to 0.8 times the MRHD or higher during organogenesis caused cleft palate and a higher rate of resorption. In pregnant rats dosed with triamcinolone acetonide via intramuscular or subcutaneous injection at doses equivalent to 0.3 times the MRHD or higher during organogenesis caused developmental abnormality (cleft palate, omphalocele, late resorption, and growth retardataion) and fetal mortality. No notable maternal toxicity was observed in rodents. Pregnant rabbits dosed with triamcinolone via intramuscular injection for 4 days during organogenesis at doses equivalent to 0.15 times the MRHD or higher caused resorption and cleft palate. No notable maternal toxicity was observed. Pregnant primates dosed with	PLLR format. The risk statement of nonclinical data must include (§ 201.57(c)(9)(i) (B)(2)): • The number and type(s) of species affected • Timing of exposure • Animal doses expressed in terms of human dose or exposure equivalents • Outcomes for pregnant animals and offspring The NDA did not include (b) (4) this reviewer recommends deleting it from labeling. Animal data section of the labeling must describe the following (§ 201.57(c)(9)(i)(D)(4)): • Types of studies • Animal species • Animal doses or exposures described in terms of human dose or exposure equivalents and the basis for those calculations • Duration and timing of exposure • Study findings • Presence or absence of maternal toxicity • Limitations of the data Descriptions of maternal and offspring findings must include dose-response and severity of adverse developmental outcomes (§ 201.57(c)(9)(i)(D)(4)). Human equivalent dose (HED) calculations were based on the following:

(b) (4) No peri- and post-natal development studies of triamcinolone acetonide in animals have been conducted.	triamcinolone via intramuscular injection for 4 days during organogenesis at doses equivalent to 3 times the MRHD or higher caused severe craniofacial CNS and skeletal/visceral malformation and a higher prenatal death. No notable maternal toxicity was observed. No peri- and post-natal development studies of triamcinolone acetonide in animals have been conducted.	(b) (4)
12 CLINICAL PHARMACOLOGY		
12.1 Mechanism of Action		
(b) (4)	TCA is a corticosteroid with anti-inflammatory and immunomodulating properties. It binds to and activates the glucocorticoid receptor, leading to activation of anti-inflammatory transcription factors such as lipocortins and inhibition of inflammatory transduction pathways by blocking the release of arachidonic acid and preventing the synthesis of prostaglandins and leukotrienes.	This section is revised to provide a brief but informative description of the mechanism of action of TCA.

(b) (4)		
13 NONCLINICAL TOXICOLOGY		
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility		
(b) (4)	Carcinogenesis Long-term animal studies to evaluate the carcinogenic potential of Zilretta have not been conducted. Mutagenesis Adequate mutagenicity studies have not been conducted with ZILRETTA. Impairment of Fertility Studies in animals to evaluate the impairment of fertility of Zilretta have not been conducted.	The format of this section is updated according to the PLR (Physician Labeling Rule). (b) (4)
(b) (4)		

(b) (4)		

2 Drug Information

2.1 Drug

CAS Registry Number (Optional):
76-25-5

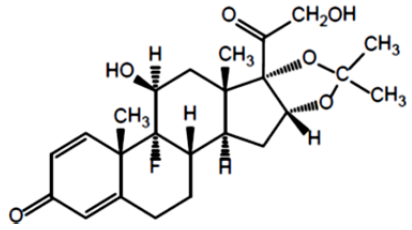
Generic Name:
Triamcinolone acetonide (TCA)

Code Name:
FX006

Chemical Name:
9α-Fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione 16,17-acetonide

Molecular Formula/Molecular Weight:
$C_{24}H_{31}FO_6$/434.50

Structure:



Pharmacologic Class:
Corticosteroid (FDA Established Pharmacological Class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 208845 were developed under IND 111325 and referencing an approved product, Kenalog (NDA 14901). Additionally, the Applicant referenced DMFs to support this application (See the table below).

Table 2. Relevant IND and NDA

Drug name	API	Dosage/Route	Strength	Indication	Company	Status
IND 111325	FX006	(b) (4)	40 mg	(b) (4) OA	Flexion	Active
Kenalog (NDA # 014901)	TCA	IR suspension	40 mg	Acute, OA	Apothecon Inc Div Bristol Myers Squibb	Approved in 1965

Table 3. List of Relevant DMFs

Authorized Reference DMF Numbers	Name of Item	Company	Date
(b) (4)	Triamcinolone Acetonide	(b) (4)	Feb, 2000
	(b) (4)		Dec, 2015
			Sep, 1995
			Oct, 2007
			May, 2011
			Feb, 2006
			June, 1991
			Oct, 1997
			Feb, 1993

2.3 Drug Formulation

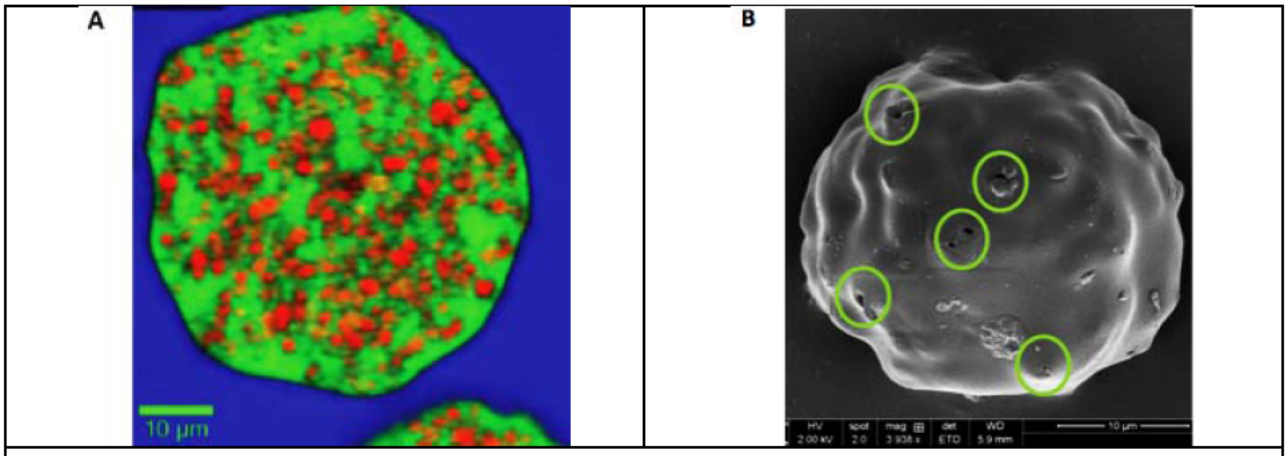
Zilretta is an extended-release injectable suspension of TCA. The to-be-marketed product will be packaged with two separate components: 1) a sterile (b) (4) drug powder containing 40 mg of TCA and (b) (4) mg of PLGA 75:25 microspheres, and 2) 5 mL of sterile diluent containing 0.9% sodium chloride, 0.5% sodium carboxymethyl-cellulose, and 0.1% polysorbate-80 (b) (4) TCA incorporated in the PLGA microspheres confers extended release of TCA. All pivotal GLP nonclinical studies used the to-be-marketed clinical formulation.

Table 4. Composition of Zilretta™, Injection, Powder for Suspension, Extended Release, 40 mg

Component	Reference to Quality Standards	Content per Dose (mg) [a]	Weight %	Function
Triamcinolone acetonide, (b) (4)	USP/Ph. Eur[b]	40	(b) (4)	Active Pharmaceutical Ingredient
75:25[c] Poly(D,L (b) (4) co-glycolide) (b) (4) (b) (4)	In-house specification 3.2.P.4.1	(b) (4)	(b) (4)	(b) (4)
Total	NA			NA

[a] As described in 3.2.P.2.2.2 Overages, the vial fill includes a (b) (4) % overfill to account for losses due to extractable volume limitations.
[b] Additional specifications for the drug substance are described in 3.2.S.4.1
[c] The 75:25 ratio reflects the proportion of lactic to glycolic acid in the PLGA microspheres (b) (4)
NA = not applicable

Small crystals of TCA are actually embedded in the PLGA microspheres, which are approximately 45 mcm in diameter. TCA is slowly released as the polymer breaks down. The polymer breakdown is staged to form poly-acid units which eventually form lactic acid and glycolic acid. Images of the microspheres are reproduced from the Flexion Therapeutics website[1] below (accessed 9/1/2017):



A. Depiction of TCA (red) crystals embedded in the PLGA polymer (green); **B.** According to the website below, "In the initial phase of release, small channels (green circles) of approximately 500 nanometers in diameter appear on the smooth, largely intact, surface of the microsphere, slow the release of Triamcinolone from the microsphere."

2.4 Comments on Novel Excipients

The use of PLGA 75:25 via the IA route of administration is novel. The remaining excipients in this formulation, which are a part of the diluent, are within the maximum potencies listed in the FDA Inactive Ingredient Database (IID) for the IA route (See the table below). PLGA 75:25 is listed for at a higher concentration for chronic intramuscular use but not for the IA route. Therefore, PLGA 75:25 was qualified for

[1] https://flexiontherapeutics.com/our-science/fx006-zilretta/

the intended route through its inclusion in the IA toxicology studies submitted to the NDA. See table below for a summary of the information submitted and available to justify the safety of the excipients in Zilretta from a local and systemic perspective.

Table 5. Systemic and Local Safety Evaluation of Excipients used in Zilretta

Excipient Name	Quantity in Zilretta per the MRHD at 32 mg	Adequacy for Systemic Safety	Adequacy for Local Safety
Drug Product			
Poly(lactic-co-glycolic acid) 75:25 (PLGA 75:25)	(b) (4)	**Adequate** • Justified through single-dose IA tox study in dogs. At LOAEL of study, dogs were administered 75 mg PLGA, which translates to an HED of 250 mg based on a dog weight of 10 kg and human weight of 60 kg. This exceeds the (b) (4) mg that will be exposed to patients at the MRHD of 32 mg. Moreover, the PLGA is not likely to be systemically exposed as it degrades locally in the joint.	**Adequate** • Qualified via toxicology studies: a single dose IA toxicity study in dogs using clinical formulation. The concentration exposed to dogs at the LOAEL dose was (b) (4) %, which exceeds the % that will be exposed to patients.
Diluent			
Sodium Chloride (NaCl)	(b) (4) (0.9%)	**Adequate** • Within the maximum potency listed in the IID (oral tablet ER), (b) (4) mg	**Adequate** • Within the maximum potency listed in the IID for the IA route (0.9%) • Also qualified through a single-dose IA tox study in dogs
Carboxymethyl Cellulose Sodium (CMCS)	(b) (4) (0.5%)	**Adequate** • Within the maximum potency listed in the IID (oral tablet ER), (b) (4) mg	**Adequate** • Within the maximum potency listed in the IID (IA) route (b) (4) %) • qualified through a single-dose IA tox study in dogs
Polysorbate 80 (PS-80)	(b) (4) (0.1%)	**Adequate** • Within the maximum potency listed in the IID (oral capsule), (b) (4) mg	**Adequate** • Within the maximum potency listed in the IID (IA) route (b) (4) %) • o qualified through a single-dose IA tox study in dogs

2.5 Comments on Impurities/Degradants of Concern

The Applicant originally proposed the dosage of Zilretta to be 40 mg per injection for single use. However, due to the viscosity of the drug and inability to deliver the entire 40 mg through the syringe, it was discovered that the actual dose of TCA delivered in pivotal clinical studies was 32 mg (b) (4)% (See the CMC review for more detail).

Drug Substance (DS)

The DS, (b) (4)TCA, is provided by (b) (4) and reference is made to Type II DMF (b) (4) This DMF is also referenced by an FDA-approved generic drug product application. The drug substance specifications for impurities and residual solvent, which comply with the USP and Ph. Eur. monographs, are shown in the Applicant's table below. Based on a MRHD of 32 mg, the appropriate qualification threshold for specified impurities in a DS is NMT (b) (4)% (or (b) (4)mg TDI) and identification threshold for unspecified impurities is NMT (b) (4)% (or (b) (4)mg TDI). Therefore, the proposed DS impurity specifications are acceptable. The proposed specifications for (b) (4) are all within the permissible daily exposures (PDEs) of (b) (4)ppm, respectively, as listed in ICH guidance for industry *Q3C Tables and Lists*.

Table 6. Specification for TCA (b) (4)

Test	Analytical Method	Acceptance Criteria
(b) (4)		(b) (4)
Single unspecified impurities		
Total (known and unknown)		
Residual Solvents	In-house (HS-GLC)	
(b) (4)		(b) (4) ppm
		(b) (4) ppm
		(b) (4) ppm

Drug Product (DP)

The proposed DP specifications are shown in the table below. Based on the MRHD of 32 mg, the appropriate thresholds for qualification and identification are NMT (b) (4)% (or (b) (4) mg TDI) and NMT (b) (4)% (or (b) (4)mg TDI) (b) (4) Therefore, the proposed DP specifications of NMT (b) (4)% for individual unspecified degradants and NMT (b) (4)% for three individual

specified degradants (b) (4) (See the table below) exceed these thresholds.

Table 7. Quality Control Specifications of FX006 (TCA ER powder, suspension for injection), 40 mg

Test	Method	Acceptance Criteria
Appearance	Visual	White to off-white powder
Identity[a]		
A – HPLC	HPLC	Retention time of sample peak matches retention time of reference standard
B –LC-MS	LC-MS	Protonated sample mass matches that of reference standard within 1.0 Da.
Drug Load	HPLC	(b) (4)
Assay	HPLC	
Related Substances:		
(b) (4)	HPLC	(b) (4)
Unspecified[b]		
Total		
Free Drug (Immediately available TCA)	HPLC	
In-Vitro Release:	USP 2 Apparatus, HPLC	Staged testing is performed in accordance with USP <711>
4 hours		(b) (4)
24 hours		
120 hours		

Uniformity of Dosage Units[a]	HPLC, ICH Q4B	Meets requirements
Particle Size[c] D_{10} D_{50} D_{90}	Laser diffraction (Volumetric Distribution)	
Particulate Matter	ICH Q4B	
Bacterial Endotoxins	ICH Q4B	
Sterility	ICH Q4B	Sterile, no growth
Tests Performed on Reconstituted Suspension of the Drug Product		
Reconstitution Time	Visual	
Syringeability	Visual	
pH	USP<791> / Ph.Eur. 2.2.3	
Viscosity[a]	USP <912> Method 2	

The Applicant proposed that the specifications should be based on a maximum daily dose (MDD) of less than the dose administered since the product is intended to provide prolonged TCA release facilitated by the PLGA polymer. The Applicant's rationale to base the specifications on a MDD of , which is % of the dose per vial, is excerpted below. Note that based on a MDD of , the appropriate thresholds for identification and qualification would be NMT % and NMT %, respectively, which would justify the Applicant's proposed specifications.

> The maximum FX006 dose currently being developed is a single 40 mg dose. Impurity limits are founded on ICH guidance thresholds based on a Maximum Daily Dose (MDD) calculated from FX006 human PK studies. FX006 is an extended release product, so both TCA and any impurities are released into the joint and the systemic circulation over a 90 day period. The maximum daily exposure of TCA from FX006 in humans is on the first day after dosing, based on the median plasma T_{max} of 7 hr (Clinical study FX006-2015-009). In the same study, the exposure on day 1 (AUC_{0-24}) is less than % of the total drug exposure (based on both AUC_{0-t} and $AUC_{0-\infty}$), so the MDD can be considered to be

Table 8. Plasma PK Parameters Pooled across FX006 Cohorts (Plasma Drug Concentration Population)

Parameter	N	Median	95% CI	Min; Max
T_{max} (h)	60	(b) (4)		
AUC_{0-t} (h*pg/mL)	60			
$AUC_{0-\infty}$ (h*pg/mL)	33			
AUC_{0-24} (h*pg/mL)	60			
AUC_{0-6wk} (h*pg/mL)	60			
AUC_{0-12wk} (h*pg/mL)	14			

Values were taken from Table 20 in the Clinical Study Report: FX006-2015-009.

Based on the T_{max} and AUC data (See the table above), the plasma levels of TCA are less than (b) (4)% of the total drug exposure within the first 24 hours by a conservative way of calculation (the maximum AUC_{0-24} in the 95% CI (b) (4)) / minimum $AUC_{0-\infty}$ in the 95% CI (b) (4)) * 100% = (b) (4)%). Although there appear to be large variations (minimum and maximum values), the median values and the range of 95% CI appear to be reasonable to determine the systemic exposure through IA injection. Taken together, it is reasonable to consider the MDD of TCA to be less than (b) (4)% of the total administered dose, or in other words (b) (4) mg of TCA. However, unless the individual degradants release at a similar rate as TCA, lowering the thresholds for these degradants based on the MDD is not fully justified.

In the 74-day filing letter, we informed that, without further justifications, the Applicant must assume that the total amount of individual impurities may be exposed on the first day post-injection and specifications must be based on the total administered dose. On July 20, 2017 (SDN 22), the Applicant provided in vitro release (IVR) data demonstrating (b) (4) Additionally, similar findings were observed for other unspecified impurities except one (RRT (b) (4) group, see the figure below, (b) (4).

Figure 1. Mean % Area Ratio of Dominant Peaks versus TCA

(b) (4)

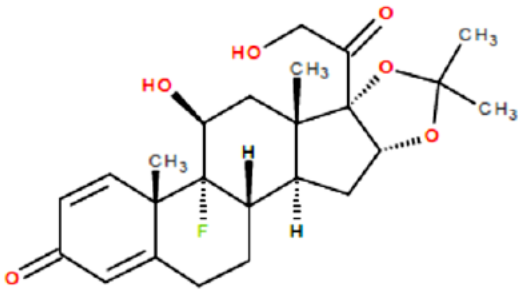
Therefore, given these data it is reasonable to consider that (b) (4) Even if the rest of (b) (4) is released at once on Day 6, theoretically, it will be released at (b) (4) % of 32 mg, which is within the qualification threshold per the ICH Q3B(R) (See the calculation below), on Day 6.

(b) (4)

Therefore, the proposed specification for (b) (4) appears to be acceptable.

The Applicant did not provide IVR data for other two impurities (b) (4) Taken together, it is reasonable to conclude that the proposed specifications for the three impurities listed above are acceptable.

Figure 2. Chemical Structure of TCA and its Impurities in the Drug Product



TCA

(b) (4)

(b) (4)

Figure 3. Chromatograms Representative of QL for TCA at 4, 24, and 120 h

IVR Time Point	Chromatogram
	(b) (4)

One of drug product degradants, (b) (4) has a potential structural alert for mutagenicity, which possibly comes from (b) (4) The Applicant conducted QSAR prediction studies (DEREK 3.0.1 Nexus 1.5. and Leadscope) for potential mutagenicity of TCA and several degradants of the drug product (See the section, **7. Genetic Toxicity** for more details). (b) (4) The Applicant's overall assessment predicted that (b) (4) would be negative in the bacterial reverse mutation test.

To confirm the Applicant's prediction, (b) (4) was submitted for evaluation to CDER/OTS/OCP/DARS for bacterial mutagenicity using (Q)SAR models (DEREK Nexus 5.0.2 (DX), Leadscope Model Applier 2.2.1-1 (LMA), and CASE Ultra 1.6.2.1 (CU). Consistent with the Applicant's result, (b) (4) was predicted to be positive in Salmonella and *E. coli* TA100 and TA102 mutagenicity via DEREK analysis due to the presence of the (b) (4) However, the CDER report noted that the (b) (4) therefore, it can be dismissed if there are adequate data to conclude that TCA is not mutagenic itself. However, the Applicant did not provide data to that effect, therefore, the structural alert cannot be dismissed.

Bacterial Mutagenicity (Q)SAR Predictions[1]

(b) (4)

Software	*Salmonella* Mutagenicity	*E. coli*/ TA102 Mutagenicity
Derek Nexus	+	+
Leadscope Model Applier	-	-
CASE Ultra	-	-
Overall Software Prediction	+	+
Overall Expert Prediction	-	-

Positive Predictivity of Structural Alerts

Alert	Positive Predictivity
(b) (4)	

However, the level of (b) (4) (See a calculation below) is lower than (b) (4) mcg per day, based on the MDD of Zilretta ((b) (4) % of the total exposure) and a similar rate of drug release, which is an acceptable daily intake for 1 to 12 months per ICH M7 guidance on genotoxic impurities in therapeutic products. As noted, this NDA is seeking an approval of Zilretta for a single use. Therefore, the proposed specification for this impurity is acceptable.

Maximum Daily Intake of (b) (4)

2.7 Regulatory Background

- Pre-IND meeting, DPARP (June 15, 2011): Plan to develop via 505(b)(2) pathway
  - DPARP agreed that, for this drug product, data from one GLP, single-dose toxicology study in dogs using the clinical route of administration (IA) was

appropriate to support initial single-dose clinical study (refer to meeting minutes July 15, 2011)[2].

- IND 111325 submission (November 16, 2011)
- Full clinical hold issued due to nonclinical deficiencies (December 14, 2011): Granulomatous inflammation in the knees and no NOAEL for local toxicity in a single IA injection study in dog
- Removal of full clinical hold (December 14, 2012) by submitting a complete response to clinical hold that included additional data demonstrating that the local responses in the knees were consistent with a foreign body response and were reversed over the course of a 9-month recovery period.
- Type B End-of-Phase 2, DPARP (September 24, 2013)
  - DPARP agreed that toxicity studies performed in dogs appeared adequate to support Phase 3 clinical program and NDA filing pending full review of studies (refer to meeting minutes dated October 24, 2013).
- Application Transfer from DPARP to DAAAP (November 22, 2013)
- Type C meetings (June 2014, June 2015, and August 2015): Full clinical hold due to SAE of septic right knee (October 2014) - removed in November 2014 (SAE not related to the test article)
- Pre-NDA Meeting (May 2016)
  - Honoring the previous agreements, DAAAP stated "the toxicology studies described in your meeting package appear appropriate to support an NDA submission for a single-use indication."
- NDA submission (December 8, 2016)

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is patients with OA of the knee(s). Dosing regimen is 32 mg of TCA in 5 mL via intra-articular injection for a single use.

3 Studies Submitted

3.1 Studies Reviewed

Study Name	Study Number
A Single Intra Articular Dose Toxicity Study with FX006 (Triamcinolone Acetonide in 75:25 PLGA Microspheres) in Beagle Dogs with a 3, 4, 6, and 9 Month Recovery	FX006-TOX-2011-003
A Repeat Intra Articular Dose Toxicity Study with FX006 (Triamcinolone Acetonide in 75:25 PLGA Microspheres) in Beagle Dogs	FX006TOX-2012-001
Extractable Study Report for Components of FX006 – A Dry Powder for Injection Drug Product	RPT49852.02 AB4890

[2] In general, DAAAP recommends that two species be tested for a new IA drug substance or excipient, unless adequately justified otherwise.

Leachables Simulation Study Report for Vial Adapter Used with the FX006 Product	RPT 60848.00 05120-001
Assessment of Leachables for FX006 – A Dry Powder for Injection Drug Product	PT53289.01 S-AB7882
Leachable Study Report for FX006 Dry Powder for Injection Drug Product	RPT61564.01 05321-001
DEREK Evaluation of FX006 Structures	FX006-TOX-2013-001
Computational Toxicity Assessment Using the Leadscope Model Applier	FX006-TOX-2015-001
DEREK and Leadscope Evaluation of FX006 Unspecified Impurities	FX006-TOX-2016-001
PK/PD Modeling and Simulations to Support IND Submission of FX006	FX006PHARM-2011-003

3.2 Studies Not Reviewed

NA

3.3 Previous Reviews Referenced

Studies listed below were reviewed in the IND 111325 nonclinical reviews by Dr. Leshin (12/15/2011 and 12/12/2012).

Study Name	Study Number
Dose Responsive Effects of Intra-articular Immediate Release Triamcinolone Acetonide (TCA IR) or FX006 in a Peptidoglycan-polysaccharide (PGPS)-Induced Knee Arthritis Model in Rats	FX006PHARM-2011-001
Effects of Intra-articular Administration of FX006 (25% Triamcinolone Acetonide (TCA) in 75:25 PLGA Microspheres, Placebo Microspheres and TCA IR in Male Lewis Rats with emphasis on Joint Morphology	FX006TOX-2011-001
A Single Intra Articular Dose Toxicity Study with FX006 (Triamcinolone Acetonide in 75:25 PLGA Microspheres) in Beagle Dogs	FX006TOX-2011-002

4 Pharmacology

4.1 Primary Pharmacology

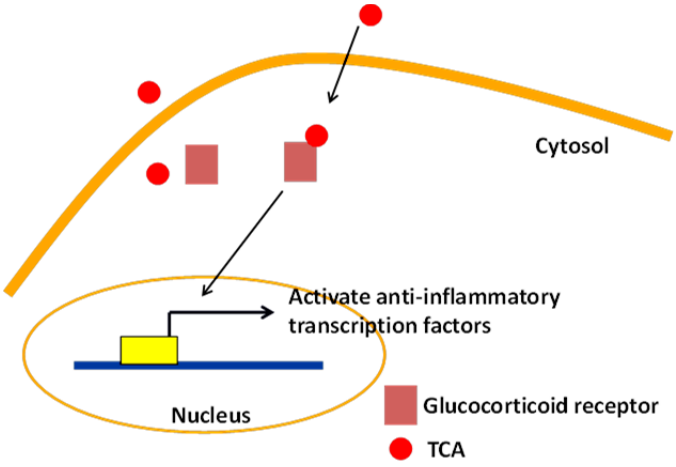
No primary pharmacology studies were submitted with this NDA.

Mechanism of action

TCA is a corticosteroid with anti-inflammatory and immunomodulating properties. It binds to and activates the glucocorticoid receptor, leading to activation of anti-inflammatory transcription factors such as lipocortins and inhibition of inflammatory transduction pathways by blocking the release of arachidonic acid and preventing the synthesis of prostaglandins and leukotrienes. It also inhibits pro-inflammatory cytokine production such as interleukin (IL)-1 and IL-6 and activation of cytotoxic T-lymphocytes. It is also known to decrease the number of circulating lymphocytes, induce cell

differentiation, and stimulate apoptosis through increasing Iκ-B expression and curtailing activation of NFκ-B.

Figure 4. Schematic Mechanism of Action of TCA



Study Title: Dose Responsive Effects of Intra-articular Immediate Release Triamcinolone Acetonide (TCA IR) or FX006 in a Peptidoglycan-polysaccharide (PGPS)-Induced Knee Arthritis Model in Rats

This study was reviewed for IND 111325 by Dr. Leshin and reproduced below from his review.

Report FX006PHARM-2011-001

The pharmacology study (Report FX006PHARM-2011-001) used a localized streptococcal peptidoglycan-polysaccharide (PGPS)-induced osteoarthritis/arthritis model in rats in which repeated flares of synovitis and local right knee pain are induced. In this paradigm, rats are first sensitized with PGPS injected into the knee. Following the passing of the acute phase of the disease, knee inflammation was reactivated by a tail vein injection of PGPS two weeks later with intravenous injections. With each challenge signs of osteoarthritis develop and rats were monitored quantitatively for limb weight bearing. Doses were 0.28, 0.12 or 0.03 mg TCA of FX006, or 0.03 or 0.06 mg TCA IR, Kenalog®-40 (n=10/group). The dose of 0.06 mg TCA IR was chosen to provide overall TCA exposure comparable to that with FX006 at 0.28 mg (AUC~ 350 ng.hr/mL). The dose of 0.06 mg TCA IR also approximates the clinical concentrations associated with a 40 mg IA injection of TCA IR in humans (Cmax ~12 ng/ml). The dose of 0.03 mg of TCA IR was chosen to match the TCA in the 0.28 mg FX006 that was expected to be released in the first 24 hr (less than 10%). Differences in weight-bearing and gait (as a measure of joint pain experienced by the animals), plasma pharmacokinetics and pharmacodynamics (HPA axis effect) of TCA were characterized.

Administration of FX006 prior to PGPS challenge prolonged the analgesic efficacy (gait improvement) relative to TCA-IR formulation of commercially-available Kenalog®-40 (TCA-IR) on 2 subsequent reactivations, 2 weeks apart (3 reactivations of flares of synovitis/arhritis in total) on days 14 and 28. There was no functional inhibition of the HPA axis as assessed by blood corticosterone concentrations. Histopathological evaluation of the knees taken from all animals at the end of the PGPS study demonstrate a significant improvement (reduction) in histological scores of joint tissues (nflammation, panus formation, cartilage damage and bone resorption) with scores approximating normal tissue architecture in animals treated with FX006. TCA has no deleterious effect on cartilage, and reduced cartilage damage in an inflammatory milieu.

4.2 Secondary Pharmacology

No secondary pharmacology studies were submitted with this NDA submission.

4.3 Safety Pharmacology

No safety pharmacology studies were submitted with this NDA submission.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study Title: Effects of Intra-articular Administration of FX006 (25% Triamcinolone Acetonide (TCA) in 75:25 PLGA Microspheres, Placebo Microspheres and TCA IR in Male Lewis Rats with emphasis on Joint Morphology

This study was reviewed for IND 111325. The summary review is excerpted below.

Report RTOX/FN-2

A non-GLP exploratory study of the safety of FX006 in rats evaluated the effects of IA-injected FX006 microspheres, TCA IR, placebo microspheres, and diluent on articular cartilage, body and organ weights, TCA toxicokinetics and serum levels of corticosterone. Male Lewis rats were administered a single IA dose of TCA IR (0.18 and 1.125 mg), FX006 TCA microspheres (0.28, 0.56 and 1.125 mg TCA), or placebo microspheres into the right knee joint, or diluent into the left knee joint (n=8/dose group, 4 rats for histology and 6 rats for TK for each necropsy days 28 and 42). The FX006 dose of 1.125 mg TCA was the maximum feasible dose based on joint space, drug loading and syringeability considerations. These TCA doses corresponded to 4.5, 2.2 and 1.1 mg of microspheres, respectively. Reductions in body weight during the first 2 weeks, and reductions in thymus, spleen and adrenal weights also occurred, but were not the focus of the review of this non-GLP study.

Histological observations were as follows:

- Injected joints from placebo (blank PLGA microspheres)-treated anmals had minimal multifocal macrophage infltration in association with approximately 20-130 flm diameter microspheres (as measured microscopically on histology slides where microsphere presence was judged by round spaces) whereas none of the active FX006-injected joints showed the presence of any microspheres at Day 28.

- Placebo-treated rat joints had no cartilage or joint changes save for the presence of spontaneous cartilage cysts in a few joints (1 at Day 28, 2 at Day 42) in the right (injected) knees. Left knees were normal.
- In comparison, both knees in the high dose TCA IR and the high and mid-dose FX006 -groups showed some mild bone marrow hypocellularity and growth plate atrophy (dose-dependent for FX006) suggesting a systemic effect of TCA. Both knees in the low doseTCA IR and FX006 anmals were normal.
- Spontaneous carilage cysts observed in placebo animals were also observed in all groups dosed with FX006 with no increase in incidence or severity. High dose TCA IR increased cartilage cysts at Day 42 but not at Day 28.

Overall results indicate that a single dose of 0.28 mg of TCA in FX006 resulted in minimal, reversible changes in the knee of rats. In general, FX006-treated animals had normal articular carilage despite the presence of lesions on other joint structures. This non-GLP report refered to as a joint "safety" study, does not provide support for the FX006 local safety since the blank microsphere group had macrophage infiltrates in the synovium of minimal severity in 7 or 8 animals, and mild severity in 1 of 8 animals, and bone cysts occurred in most groups in the injected knee, a greater incidence than occured in the uninjected control knee. There were 2 of 8 animals in the low dose (75 mg FX006, 0.28 mg TCA) and 2 of 8 animals in the mid dose (75 mg FX006 0.56 mg TCA) that carried their leg on the injected knee side, ie., not weight bearing. In two of these cases the cause was attributed to infections (the injections were not performed sterilily).

Study Title: PK/PD Modeling and Simulations to Support IND Submission of FX006

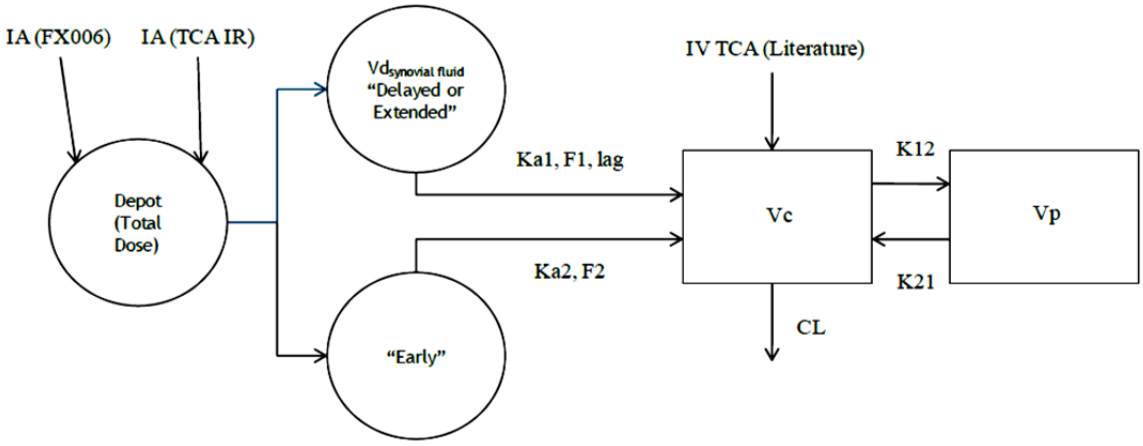
Study Number: FX006PHARM-2011-003
The object of the study: To assess the PK of FX006 and TCA IR in plasma following IA administration in rats and dogs to determine the residence time of each product at the site of injection

This study is briefly reviewed and summarized because it was a modeling and simulation study predicting the safety of clinical doses used during the development.

In Vivo Preclinical Datasets Used in the Allometric Analyses

Species	Source
Rat	FX006TOX-2011-001 (b) (4) Study No.: RTOX-FN-2 (b) (4) project no.: 110009ACJH)
Dog	FX006TOX-2011-002 (b) (4) Study No.: 0406DF21.001 (b) (4) project no.: 110177ADEP)

Conceptual Structural PK Model for TCA



Summary:

- Noncompartmental analysis (NCA): The absorption of TCA with FX006 was slower than the one of TCA IR at similar dose level (1.125 mg) and the overall mean residence time (MRT) was much longer with FX006 compared to the one for TCA IR formulation in rats. Overall, the absolute bioavailability of TCA in the systemic circulation was 3-fold lower with FX006 compared to TCA IR. Similar findings, slower absorption and longer MRT, lower absolute bioavailability of TCA in the systemic circulation, were predicted in dogs.

Table 6.1.1 NCA PK Parameters of TCA in Rats

Parameters	Mean (CV%)				
	FX006 (0.28 mg)	FX006 (0.56 mg)	FX006 (1.125 mg)	TCA IR (0.18 mg)	TCA IR (1.125 mg)
AUC_{0-24} (ng•h/mL)	31.0 (76.0)	33.0 (19.1)	136 (6.0)	297 (21.5)	1403 (13.2)
AUC_{0-t} (ng•h/mL)	335 (66.5)	532 (23.8)	2142 (14.4)	456 (31.3)	6013 (3.4)
$AUC_{0-\infty}$ (ng•h/mL)	356 (62.0)	572 (21.5)	2856 (17.2)	479 (32.6)	6065 (3.7)
CL/F (mL/h)	1308 (96.6)	1014 (24.4)	403 (19.1)	387 (27.3)	186 (3.6)
C_{max} (ng/mL)	1.82 (66.2)	1.91(10.2)	8.15 (12.5)	41.6 (25.1)	125 (5.3)
$MRT_{0-\infty}$ (h)	181 (34.9)	321 (12.2)	600 (9.8)	32.1 (55.7)	130 (38.3)
$T_{1/2}$ (h)	99.5 (38.9)	180 (27.0)	451 (20.8)	35.6 (63.5)	107 (56.7)
T_{max} (h)	17.7 (148.9)	16.7 (162.8)	3.33 (69.3)	2.00 (0.0)	1.00 (0.0)
V_{ss}/F (mL)	274215 (117.0)	326966 (29.9)	240481 (17.7)	12069 (53.4)	23829 (34.4)
F_{abs} (%)[a]	17.9	23.1	58.1	60.5	~100

[a] PK parameters in rats after IV administration from literature[3] was used to derived F_{abs}.

MRT (mean residence time extrapolated to infinity), Fab (absolute bioavailability)

Table 6.1.2 Relative Bioavailability of TCA in Rat Plasma in the Early vs. Delayed Release

Parameters	Treatment				
	FX006 (0.28 mg)	FX006 (0.56 mg)	FX006 (1.125 mg)	TCA IR (0.18 mg)	TCA IR (1.125 mg)
AUC_{0-24} (ng.h/mL)	31.0	33.0	136	297	1403
$AUC_{0-\infty}$ (ng.h/mL)	356	572	2856	479	6065
$AUC_{24-\infty}$ (ng.h/mL)	325	539	2720	182	4662
% Early	8.69	5.76	4.76	62.1	23.1

Table 6.1.3 NCA PK Parameters of TCA in Dogs

Parameters	Mean (CV%)			
	FX006 (2.1 mg)	FX006 (6.25 mg)	FX006 (18.75 mg)	TCA IR (18.75 mg)
AUC_{0-24} (ng•h/mL)	4.74 (29.7)	20.0 (19.4)	61.2 (20.5)	658 (13.6)
AUC_{0-t} (ng•h/mL)	21.6 (71.8)	163 (24.1)	674 (27.3)	1614 (17.0)
$AUC_{0-\infty}$ (ng•h/mL)	146 (NC)	215 (7.9)	834 (20.0)	1592 (13.0)
CL/F (mL/h)	14384 (NC)	29210 (7.6)	23135 (17.8)	11924 (12.8)
C_{max} (ng/mL)	0.281 (45.9)	1.05 (21.6)	3.09 (24.8)	44.2 (9.92)
$MRT_{0-\infty}$ (h)	836 (NC)	266 (45.1)	399 (21.9)	36.3 (21.4)
$T_{1/2}$ (h)	581 (NC)	198 (23.6)	335 (18.5)	33.2 (47.6)
T_{max}[a] (h)	11.0 (93.2)	13.0 (72.7)	18.0 (98.1)	4.0 (0.0)
V_{ss}/F (mL)	12023501 (NC)	7627133 (22.3)	9333110 (35.2)	433128 (23.0)

*Vss/F (volume of distribution at steady-state, calculated as MRT0-∞*CL)*

- Compartmental Analysis: Plasma TCA concentrations following a single IA injection in the knee were best modeled using a 2-compartment structural model with two first-order absorption processes. The MRT for the early phase was similar between the IR and FX006, the MRT during the delayed release phase were noticeably longer for FX006. Also, in rats, there was a linear relationship between the fraction absorbed in vivo and the fraction dissolved in vitro with time, indicating that the in vivo absorption from the joint is directly related to the release of TCA from the microspheres.

Table 6.1.4 Compartmental PK Model Buildup of TCA in Rats

Structural Model	Absorption	-2LL	AIC	VAF
2-Compartment	Two zero-order (K0) absorption processes	-162	-148	√
	A zero-order (K0) and a first-order (Ka) absorption processes	-175	-161	√√
	Two first-order (Ka) absorption processes	**-179**	**-165**	**√√√**

-2LL (Log-likelihood); AIC (akaike criterion); VAF (visual assessments of fit)

Compartmental Plasma PK Parameters of TCA in Rats Following FX006 IA Administration

Parameters	Treatment		
	FX006 (0.28 mg)	FX006 (0.56 mg)	FX006 (1.125 mg)
Ka1 (delayed) (h^{-1})	0.00930	0.00283	0.00216
Ka2 (early) (h^{-1})	0.0556	0.0643	0.0589
F1 (delayed)	25%	22%	50%
F2 (early)	4.1%	2.1%	4.4%
MRT(delayed) (h)[a]	107	353	463
MRT(early) (h)[a]	18.0	15.6	17.0
Lag (delayed) (h)	47.2	44.5	42.5

[a] MRT was calculated as 1/Ka

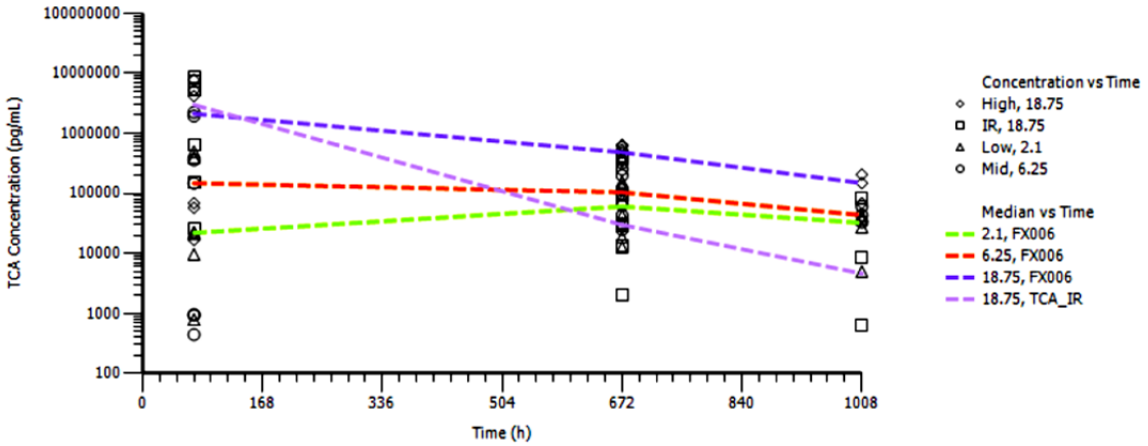
Compartmental Plasma PK Parameter of TCA in Dogs Following FX006 IA Administration

Parameters	Treatment		
	FX006 (2.1 mg)	FX006 (6.25 mg)	FX006 (18.75 mg)
Ka1 (delayed) (h^{-1})	0.0122	0.000264	0.00181
Ka2 (early) (h^{-1})	0.0763	0.0251	0.0248
F1 (delayed)	4.57%	88.2%	19.0%
F2 (early)	1.48%	4.85%	4.97%
MRT(delayed) (h)[a]	82.0	3788	552
MRT(early) (h)[a]	13.1	39.8	40.3
Lag (delayed) (h)	11.7	20.8	21.1

[a] MRT was calculated as 1/Ka

- TCA concentrations in the knee of FX006-treated dogs were maintained higher over the period time than the one of the IR formulation-treated dog knee, which supports a slower systemic release of TCA by FX006 formulation. As note, synovial TCA concentrations measured from the treated knee of dogs showed large variation (See the figure below).

Synovial Fluid Concentration in the Treated Knee of Dog



- Allometric scaling and simulation of TCA in human: TCA PK analysis was performed on predicted plasma TCA concentrations and PK parameters (See the tables below) and FX006 dose levels up to 60 mg was predicted to cause clinically insignificant or no measurable effect on endogenous cortisol production.

Table 6.2.1 Allometrically-Scaled TCA parameters for FX006 formulation

Parameters	Allometrically-Scaled Parameters			
	Scaling	Rat (0.25 kg)	Dog (10 kg)	Humans (70 kg)
F (delayed)	BW^0	25%	25%	25%
F (early)	BW^0	4%	4%	4%
MRT(delayed) (h)	$BW^{0.25}$	244	614	999
MRT(early) (h)	$BW^{0.25}$	14.3	36.0	58.6
lag(slow) (h)	$BW^{-0.25}$	44.2	17.6	10.8

Table 6.2.2 Predicted PK parameters of TCA in human plasma following IA FX006 administration

Parameters	FX006		
	10 mg	40 mg	60 mg
$AUC_{0-\infty}$ (ng.h/mL)	72.4	290	434
C_{max} (ng/mL)	0.239	0.956	1.44
AUC_{0-24} (ng.h/mL)	4.84	19.4	29.0
AUC_{0-168} (ng.h/mL)	22.5	90.0	135
$T_{1/2}$ (h)	540	540	539
Time above the BLQ over the dosing interval (3 months) (day)	~ 4.58	~ 43.3	~ 60.1

6 General Toxicology

6.1 Single-Dose Toxicity

Study Title: A Single Intra Articular Dose Toxicity Study with FX006 (Triamcinolone Acetonide in 75:25 PLGA Microspheres) in Beagle Dogs

This study was reviewed in a nonclinical review for IND 111325 by Dr. Leshin (12/15/2011, See below) as a draft report. The review is excerpted verbatim below. Note that the final study report for the pivotal single dose IA toxicity study (Study Number FX006TOX-2011-003) was fully reviewed further below in this review.

Report FX006TOX2011-002

In the GLP dog toxicity study, animals (10/sex/dose group) received a single injection into the right femorotibial joint of either vehicle control diluent (consisting of 0.9% sodium chloride solution containing 0.5% carboxymethylcellulose sodium, and 0.1 % polysorbate-80), a TCA IR positive control (Kenalog®-40, 18.75 mg TCA), blank PLGA microspheres (placebo), or FX006 at TCA doses of 2.1, 6.25, or 18,75 mg corresponding to 8.33, 25, and 75 mg of FX006, respectively. The high FX006 dose was the maximum feasible dose that could be administered into the dog's knee joint. The left knee was used as an untreated control for all dose groups. Animals were followed for up to 43 days and

sacrificed at 3 timepoints (day 4, n=4/sex/group; day 29, n=4/sex/group; and day 43, n=2/sex/group). Standard toxicological assessments were conducted (body weight, food consumption, clinical observations, clinical pathology, and anatomic pathology). Extensive histopathological analysis and synovial fluid analysis was conducted for the treated and untreated knee of each animal, described in the histopathology section below.

Systemic findings were similar among the TCA IR and the FX006 groups. However, the incidence and/or intensity of systemic findings were slightly higher for high dose FX006 than for TCA IR at the same dose level of TCA (18.75 mg/mL/joint) at the latter time points. Joints injected with TCA IR suspension and FX006 microspheres (all doses) showed a consistent decrease in Safranin O staining due to loss of extracellular matrix (glycosaminoglycans) with negligible effects on articular carilage structure, cellularity, and tidemark integrity.

There were no clinical observations suggesting an effect of treatment. Body weight loss and reduced food consumption were observed in all groups, sporadic, with no clear dose-dependent effects. Most changes in clinical pathology parameters could be attributable to corticosteroid effects and appeared to mostly reverse by day 29. The effects evident on day 4 included reduced lymphocytes (TCA-IR: males 39%, females 44%; FX006 high dose males and females 55%), increased liver weight (TCA-IR: males 137%, and females 149%; FX006 high dose males 130%, females 143%; day 29 FX006 high dose males 135%, females 170%), associated with cellular swelling in histopath on all necropsy days 4, 29, 43, increase in alkaline phosphatase (males), and alanine aminotransferase (females TCR-IR 197%, FX006 high dose 144%), decreased creatinine (males and females, 78% to 93%), increased cholesterol (males, up to 147%) and increased total protein (males 110%-111%), albumin (males 110%-111%) and globulin (males 110%-117%) without a change in the A/G ratio (males). The effects were most pronounced for the TCA-IR formulation, with smaller magnitude changes but lasting longer (still evident on day 29 in a few cases, e.g., creatine). There were no gross macroscopic findings observed at the scheduled sacrifice on days 4, 29, or 43.

Microspheres were not detected in tissues distal to the intra-articular administration.There were no systemic alterations and no local toxicity in articular cartilage related to the administration of blank microspheres. Also there was no local drainage reaction in the regional popliteal lymph nodes which would have been expected with microspheres traveling through the lymphatics. There were no effects on articular cartilage structure, cellularity and tidemark integrity (the interface between the articular and calcified cartilage). There was a reduction in articular cartilage extracellular matrix of glycosaminoglycans in TCA treated animals with or without microspheres, as detected by Safranin O staining. There was a low incidence in the day 4 animals, but became more prominant in articular cartilage of the patella and distal femur than the tibia by day 29 and 43, virtually every treated joint demonstrated a reduction in extracellular matrix, but this was not dose-dependent for the FX006 microsphere treated groups.

The report stated the data for synovial fluid analysis was not yet corrected for dilution. Therefore this was not reviewed. Nevertheless the Sponsor indicated that blank microspheres injected IA resulted in small increase in synovial fluid WBC cells suggestive of inflammation. Samples from the injected knee and contralateral control were used for urea nitrogen analysis and bioanalytical evaluation of triamcinolone concentrations. The urea nitrogen was used to establish dilution factors for each sample.

A NOAEL could not be determined in this study, as blank microspheres resulted in inflammatin and all doses of TCA reduced articular extracellular matrix of glycosaminoglycans.

Histopathology

Adequate Battery: Yes
Peer Review: No

The femorotibial articulation (stifle or knee joint) was examined beyond standard histopathological procedures for all animals in all groups. Histologic sections were obtained in a cranial to caudal orientation (longitudinal section) through the femorotibial articulation of each knee joint to examine the patella, distal femoral articular cartilage (condyle), and proximal tibial articular cartilage (tibial plateau). Besides hematoxylin and eosin stain, additional sections were stained with Safranin O stain, to detect extracellular matrix glycosaminoglycans. Synovial tissue was also examined. The synovium and articular surfaces were examined. Each knee joint was also examined for the presence or absence of microspheres.

Joint Evaluation: Evaluation of articular cartilage and synovium employed a semi-quantitative, modified Mankin scoring system to evaluate for degeneration of articular cartilage and inflammatory changes of synovium. The modified Mankin scoring system is presented below.

Category	Score
Structure	
Normal	0
Surface irregularities	1
Pannus and surface irregularities	2
Clefts to transitional zone	3
Clefts to tidemark	4
Clefts to subchondral bone	5
Complete disorganization	6
Cells	
Normal	0
Diffuse hypercellularity	1
Cloning	2
Hypocellularity (necrosis)	3
Safranin O staining	
Normal	0
Slight reduction, tangential zone	1
Moderate reduction (to tidemark)	2
Severe reduction (to subchondral bone in at least 1 area)	3
No dye observed	4
Tidemark Integrity	
Intact	0
Crossed by blood vessels	1
Total	0-14

The Mankin joint scores were obtained from the average of the sum of the scores for the distal femur, proximal tibia, and patella are presented in Table 10 for the treated right knee femorotibial joint and untreated left knee. The scoring system ranges from 0 to 14, with a score of 0 indicative of no articular damage. The scores were relatively low and were attributed to the results of reduced Safranin O staining, since there were neglible effects on articular cartilage structure, cellularity and tidemark integrity. Only 1 male in group 5 on day 43 had a single focus of clefts to the tidemark with chondrocyte cloning (proliferation) and severe reduction in Sarafin) staining. There was one untreated knee from a group 6 male that had a focal cleft in the transitional zone accompanied by a slight reduction in Safranin O staining.

Adverse Joint Scores

Group	1		2		3		4		5		6	
Treatment	vehicle Diluent		Blank Microsphere		TCA-IR Kenalog-40		FX006 TCA microspheres					
Dose of TCA	0		0		18.75		2.1		6.25		18.75	
Gender	M	F	M	F	M	F	M	F	M	F	M	F
Joint Scores												
Day 4 (n=4/sex/dose)												
right	0.08	0	0.08	0	0.50	0.08	0.42	0.08	0.08	0.25	0.08	0.08
left	0	0.08	0	0.33	0	0	0	0	0	0	0.42	0
Day 29 (n=4/sex/dose)												
right	0	0	0	0	1.64	1.58	1.50	1.42	1.83	1.50	1.67	1.75
left	0	0	0	0	0.08	0	0	0	0.09	0	0.08	0.09
Day 43 (n=2/sex/dose)												
right	0	0	0	0.17	1.67	1.33	1.83	1.67	2.83	1.50	1.50	1.83
left	0	0	0	0	0	0	0	0	0	0	0	0.33

Histological Findings

Systemic histopathological findings:
Systemic microscopic observations related to intra-articular administration of FX006 were present in adrenal glands (Days 29 and 43), lymphoid tissues (spleen, thymus, mandibular, mesenteric, and popliteal lymph nodes (all three time points), liver (all three time points), and bone marrow (Day 4 females only). The findings in the TCA treatment groups were similar for both TCA-IR and FX006 high dose (18.75 mg/ml/joint). The histopathological findings included adrenocortical atrophy, lymphoid depletion most notable in the thymus, hepatocellular swelling, and bone marrow hypocellularity (FX006 microspheres high dose females only). Within the FX006 treatment groups, there were dose-dependent trends relative to the incidence and/or intensity of these findings. On Day 43 thymic lymphoid depletion was still present at all dose levels, adrenocortical atrophy was present at the high dose level. The other findings were absent or reduced in incidence and severity by Day 43. These findings of effects distant to the site of administration are known effects of hypercorticoidism, therefore data tables were not presented.

There were no systemic alterations related to the administration of blank microspheres and microspheres were not detected in tissues distal to the intra-articular administration site. The pathology report notes, as might be expected, "that most microsphere were lost during tissue processing, but spaces remain."

There were no systemic alterations and no local toxicity in articular cartilage related to the administration of blank microspheres (Group 2). There was no local drainage reaction to the regional, popliteal lymph nodes in response to blank microspheres or FX006 microspheres.

Local intra-articular histopathological findings:
Alterations in articular cartilage in FX006-treated joints were due to decreased Safranin 0 staining with negligible effects on articular cartilage structure, cellularity, and tidemark. The reduction in Safranin O staining is indicative of a loss of the glycosaminoglycan containing extracellular matrix. In general, this decrease in Safranin O staining in Groups 3, 4, 5, and 6 was of low incidence at Day 4, was more readily detectable in articular cartilage of the patella and distal femur than the tibia,and affected virtually every treated joint at Day 29 and Day 43. Safranin O staining in the majority of cases for days 29 and 43 were due to a moderate reduction in staining extending to the tidemark and without indications of dose-dependency.

There was no local toxicity in articular cartilage related to the administration of blank microspheres.

Granulomatous infiltration involving the synovium occurred in all groups with microsphere treamtments, including blank microspheres (Groups 2, 4, 5, and 6). These granulomatous infiltrates were due to the presence of macrophages with fewer multinucleated cells on the synovial surface often in association with variable-sized round spaces consistent with microspheres. These infiltrates were present on day 43, the last study day. An increased degree of infiltration appeared to be related to increased quantity of microspheres injected into each right femorotibial joint.

Table 5: Summary of Histopathology of the Knee Joint Synovium*

Group	1		2		3		4		5		6	
Treatment	vehicle Diluent		Blank Microsphere		TCA-IR Kenalog-40		FX006 TCA microspheres					
Dose of TCA	0		0		18.75		2.1		6.25		18.75	
Gender	M	F	M	F	M	F	M	F	M	F	M	F
Left Knee Joint Synovium												
Day 4 (n=4/sex/dose)												
mononucl infiltr												
grade 1	0	0	0	0	0	0	0	0	0	1	0	0
hyperplasia												
grade 1	0	1	0	1	1	0	3	0	0	0	0	0
grade 2	1	0	0	0	0	0	0	0	0	0	0	0
Day 29 (n=4/sex/dose)												
mononucl infiltr												
grade 1	0	0	0	1	0	0	0	0	0	0	0	0
hyperplasia												
grade 1	0	0	1	0	0	0	0	1	0	1	1	0
fibroplasia												
grade 2	0	0	0	0	0	0	0	0	0	0	0	1
Day 43 (n=2/sex/dose)												
mononucl infiltr												
grade 1	0	1	1	0	0	0	0	0	0	0	0	0
hyperplasia												
grade 1	0	0	0	0	0	0	0	0	0	0	0	1

Right Knee Joint Synovium												
Day 4 (n=4/sex/dose)												
granulomatous infiltr												
grade 1	0	0	1	2	0	0	0	0	1	0	1	2
grade 2	0	0	1	1	0	0	0	0	0	0	0	2
mononuclear infiltr												
grade 1	1	0	0	0	0	0	0	0	1	0	0	0
hyperplasia												
grade 1	1	0	1	2	1	1	0	2	1	2	0	1
grade 2	0	1	0	2	0	3	0	0	0	1	0	3
cellular debris												
grade 1	0	0	0	0	0	0	0	0	0	1	0	2
grade 2	0	0	0	1	0	0	0	0	0	0	0	1
fibroplasia												
grade 1	0	0	0	2	0	0	0	0	0	0	0	1
Day 29 (n=4/sex/dose)												
granulomatous infiltr												
grade 1	0	0	1	2	0	0	2	3	1	2	0	1
grade 2	0	0	1	2	0	0	1	0	1	2	3	1
grade 3	0	0	2	0	0	0	0	0	0	0	1	1
mononuclear infiltr												
grade 1	0	0	3	1	0	0	0	0	0	0	1	0
grade 2	0	0	0	1	0	0	0	0	0	0	0	0
hyperplasia												
grade 1	0	0	3	0	2	0	4	0	1	0	1	1
grade 2	0	0	0	2	0	0	0	0	1	1	0	0
cellular debris												
grade 1	0	0	0	0	0	0	0	0	1	1	4	1
grade 2	0	0	0	0	0	0	0	0	1	0	0	2
fibroplasia												
grade 1	0	0	0	0	0	0	0	0	0	0	1	1
Day 43 (n=2/sex/dose)												
granulomatous infiltr												
grade 1	0	0	0	2	0	0	0	1	0	1	0	0
grade 2	0	0	0	0	0	0	0	0	0	0	0	2
mononuclear infiltr												
grade 1	0	0	0	0	0	0	0	0	0	0	0	1
hyperplasia												
grade 1	0	0	0	0	0	1	0	0	0	0	0	0
cellular debris												
grade 1	0	0	0	0	0	0	0	0	0	0	0	1
grade 2	0	0	0	0	0	0	0	0	0	0	0	1

Study Title: A Single Intra Articular Dose Toxicity Study with FX006 (Triamcinolone Acetonide in 75:25 PLGA Microspheres) in Beagle Dogs with a 3, 4, 6, and 9 Month Recovery

This study (a draft report) was briefly reviewed in a nonclinical review for IND 111325 by Dr. Leshin (12/12/2012). However, at the time of submission, the 9-month recovery data and TK data were not included; therefore, the final report is reviewed here. The final conclusion is the same as for the IND 111325 review of the draft report by Dr. Leshin, as reproduced below.

Summary

In this second study of the effects of FX006 on local knee joint toxicity following IA injection, microspheres (blank microspheres or FX006) were observed in the synovium at month 3, but not at months 4 or 6. There was an increase in white blood cells due mainly to an increase in small monocytes in the synovial fluid, but this was mild and not dose or time dependent. Histopathology of the synovium indicated the presence of mutinucleated giant macrophages present at month 3 and 4, but not by month 6. This response occurred if microspheres were present, but the severity was not dependent on the mass of microsphere injected, and did attenuate with time. These responses are consistent with a foreign body response.

There were no structural alterations of the joint. There was extracellular matrix loss, a known effect of corticosteroid administration that also occurs in humans. Its significance is unknown. In this study there was no effect on the extracellular matrix attributed to the microspheres.

Although toxicokinetics were not submitted yet for this study, the initial study found that joint TCA concentration were 2 to 3 orders of magnitude greater than blood concentrations at day 42 after IA injection (day 42, high dose: pg/mL in serum and ng/mL in synovial fluid). For the extended study, detectable concentrations of TCA in the joint would be expected at least at the 3 month timepoint since microspheres (i.e., partially degraded drug product) was present.

Safety margins based on estimated human synovial fluid volumes of 1 to 3 mL and dog synovial fluid volumes of ~0.25 to 1 mL provide approximately a 1 to 3-fold safety margin for the highest proposed clinical dose of 60 mg TCA.

	Max Dose FX006	**Conc FX006 in Joint**	**Safety ratio**
Human Dose/ Injection human synovial fluid volume 1-3 mL	**60 mg**	20 mg/mL to 60 mg/mL	
Dog Dose (maximum feasible) synovial fluid volume of dog knee ~0.25 to 1 mL,	**18.75 mg**	18.75 mg/mL to 75 mg/mL	**~1X - 3X**

Study no.:	FX006TOX-2011-003
Study report location:	ECTD 4.2.3.1, SDN 1 12/8/2016
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 28, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TCA (Lot L0G322, purity 99.8%) in 75:25 PLGA microspheres at 25% loading (w/w), Lot FLX FLX 11102011-Low (Group 4 and 5); Lot FLX FLX 11102011-Mid (Group 5), purity: total 0.37% and range: 0.05-0.19% for FLX 11102011-Low and total 0.38% and range 0.05-0.21% for FLX 11102011-Mid

Key Study Findings

- No mortality/morbidity and no test article-related clinical signs were observed.
- No test article-related hematology, clinical chemistry, and urinalysis parameter changes were observed
- No test article-related organ weight changes were observed.
- No test article-related macroscopic findings were observed while there were sporadic and incidental observations such as discoloration of right articular fat pad, abdominal fat red nodule, intestine, and adrenal gland and small/asymmetrical testes.
- Systemic microscopic findings: Common hypercortisolism such as slight atrophy of adrenal gland and lymphoid depletion were observed in animals treated with 18.75 mg/mL TCA from the IR and FX groups (Groups 3 and 6, respectively). Microscopic findings in other organs appear to be incidental because there were no clear dose-dependent relationships. Systemic findings observed in the FX006 test article groups were comparable to the active comparator (Kenalog-40 IR).
- Local toxicity findings:

  Microscopic changes:
  - Left knee (no injection): Microscopic findings in the left knee appear to be sporadic and not related to the test article because there were no dose-dependent relationships.
  - Right knee (injection site): Microscopic findings including minimal to mild multinucleated macrophage, lymphocyte infiltration, plasma cell infiltration, and fibrosis were observed and it appears to be related to both microspheres and the API. These changes were slightly worse in animals treated with the test article (Group 6) compared to animals treated with the active comparator (Kenalog-40 IR) at Day 92 but these changes were reversible over the course of a 9-month recovery period.

Joint changes based on Modified Mankin Score based on structure, cellularity, Safranin O staining, and Tidemark integrity:

- Using these scoring systems, dose-dependent cartilage changes were observed in animals treated with the test article with effects at the HD (Group 6) being slightly worse or comparable to animals treated with the active comparator, Kenalog-40 IR (Group 3) at all time points. These changes were most severe at the Day 92 sacrifice and full recovery was observed in almost all groups by Day 274 except in HD males, which still showed slight changes.

- TK data are listed in the table below.

	Group 1 Diluent	Group 2 Blank M	Group 3 Kenalog IR	Group 4 LD (2.1 mg)	Group 5 MD (6.25 mg)	Group 6 HD (18.75 mg)
C_{max} (ng/mL)	-	-	41.7 ± 3.61	0.81 ± 0.51	1.15 ± 0.65	4.32 ± 2.62
$AUC_{0\text{-}1\ day}$ (ng*day/mL)	-	-	22.19 ± 5.41	0.56 ± 0.40	0.78 ± 0.47	3.09 ± 1.76
$AUC_{0\text{-}\infty}$ (ng*day/mL)	-	-	69.34 ± 10.65	3.46 ± 1.03	7.57 ± 2.38	38.32 ± 12.58
$T_{1/2,e}$ (day)	-	-	18.3	5.61	11.2	32.3 ± 20.57

- The acceptable LOAEL for both systemic and local toxicity is 18.75 mg.

Methods

Doses:	0, 2.1, 6.25, and 18.75 mg/mL of TCA
Frequency of dosing:	Once
Route of administration:	Aseptic intra-articular injection into the right knee joint
Dose volume:	1.0 mL/knee
Formulation/Vehicle:	Diluent (sterile isotonic aqueous solution) containing 0.9% sodium chloride, 0.5% carboxymethylcellulose, and 0.1% polysorbate 80), Lot RX501125.003
Positive Control:	Kenalog-40, 40 mg/mL (TCA IR) with sodium chloride for isotonicity, 0.99% (w/v) benzyl alcohol, 0.75% carboxymethylcellulose sodium and 0.04% polysorbate 80, Lot 1G66249
Species/Strain:	Beagle dog
Number/Sex/Group:	8 males and 8 females per group
Age:	10-13 months old
Weight:	7.8-10.8 kg (males) and 5.7-8.8 kg (females) at the outset (Day 1) of the study
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	There were no deviations affecting the integrity of the study.

Group	Concentration (mg/ml)	Dose Volume (ml/knee)	Number of Animals*	
			Male	Female
1. Vehicle control (Diluent)	0	1	8	8
2. Blank microspheres	75 (0 TCA)	1	8	8
3. TCA IR suspension	18.75 (TCA)	1	8	8
4. FX006 microspheres Low Dose	8.33 (2.1 TCA)	1	8	8
5. FX006 microspheres Mid Dose	25 (6.25 TCA)	1	8	8
6. FX006 microspheres High Dose	75 (18.75 TCA)	1	8	8

* Animals were dosed via the intra articular route on study Day 1. A subset of two animals/sex/group were sacrificed on study Days 92/93 (3 month recovery), on study Days 120/121 (4 month recovery), on study Days 176/177 (6 month recovery), and on study Days 274/275 (9 month recovery).

Justification for dose selection:

The maximum dose (18.75 mg of TCA) was selected from the maximal workable concentration of microspheres in suspension for injection, 75 mg/mL, where homogeneity can be maintained and the injection delivered through a needle of reasonable gauge for IA administration. The maximum dose in the single-dose toxicity study translates on a weight adjusted basis to about 1.9 mg/kg assuming 10 kg body weight for dogs. As noted, a single definitive literature study of the LD_{50} of daily oral administration of TCA for 3 weeks in dogs is <3.5 mg/kg (1). Lastly, the maximum dose (18.75 mg TCA) is equivalent to 37.5 mg/m^2 based on a body surface area. FX006 is developed to provide an extended release (b) (4) of the active ingredient, TCA, and maintain local (intra-articular) concentration.

Dose Preparation

The FX006 microspheres and blank microspheres test article formulations were prepared on the day of dosing for each animal by reconstituting the individual test article vials with the appropriate volume of diluent to form a suspension for injection containing the appropriate dose.

The positive control (Kenalog-40) dosing suspension was prepared by diluting the TCA (Triamcinolone Acetonide) suspension in saline to obtain a concentration of 18.75 mg/mL.

The vehicle (diluent) was dosed as received.

Observation and Results

Mortality

Animals were observed for mortality/morbidity twice daily (a.m. and p.m.) and once prior to the scheduled sacrifices. No mortality/morbidity was observed during the study. All

animals survived until scheduled euthanization (Days 92/93, 120/121, 176/177, and 274/275).

Clinical Signs

Clinical observations were recorded prior to dose administration, at approximately 1-2 h post-dose, and additionally as needed. No test article-related clinical signs were observed. Incidental clinical signs of soft/loose feces, mucus in the feces, or emesis were observed in all dose groups.

Body Weights

No statistically significant test article-related body weight changes were observed.

Food Consumption

Food consumption of animals of Group 6 was reduced during D26-41 compared to other groups and there were fluctuation in food consumption. However, it does not appear to be dose-dependent or test article-related.

Ophthalmoscopy

No ophthalmoscopy parameters were monitored in this study.

ECG

No ECG parameters were evaluated in this study.

Hematology

Parameters Analyzed:

Hematology Parameters	
Red Blood Cell Count (RBC) and Morphology	Platelet count (PLT)
White Blood Cell Count (WBC)*	Hematocrit (HCT)
Mean Corpuscular Hemoglobin (MCH)	Hemoglobin (HGB)
Mean Corpuscular Hemoglobin Concentration (MCHC)	Reticulocyte Count (Retic)
Mean Corpuscular Volume (MCV)	

*Total and differential white blood cell counts, including neutrophils, basophils, eosinophils, monocytes, lymphocytes and large unstained cells.

No clear test article-related/dose dependent hematological parameter changes were observed. Levels of eosinophil were lower in males of Group 6 at Day 92 and 120 compared to the control Group 1. However, considering a large standard deviation in

the control group and lower levels at predose Day 6, these changes may not be test article-related and toxicologically relevant.

Male		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Eosinophils ($x10^3$/mcL)	Predose Day 6	0.356 ± 0.312	0.195 ± 0.093	0.359 ± 0.154	0.225 ± 0.144	0.191 ± 0.106	0.223 ± 0.115
	Day 92	0.326 ± 0.277	0.211 ± 0.096	0.213 ± 0.099	0.379 ± 0.183	0.281 ± 0.113	**0.149* ± 0.089**
	Day 120	0.437 ± 0.291	0.177 ± 0.062	0.328 ± 0.167	0.270 ± 0.125	0.240 ± 0.055	0.197 ± 0.090
	Day 176	0.523 ± 0.504	0.223 ± 0.107	0.330 ± 0.097	0.483 ± 0.312	0.333 ± 0.067	0.215 ± 0.057
	Day 274	0.285 ± 0.007	0.170 ± 0.071	0.270 ± 0.014	0.250 ± 0.141	0.360 ± 0.042	0.270 ± 0.085

**Mann-Whitney U Test Significant at 0.05 levels.*

Parameters Analyzed:

Coagulation Parameters	
Activated Partial Thromboplastin Time (APTT)	Prothrombin Time (PT)

There were no biologically relevant changes in group mean coagulation parameters and all values were within normal historical control range (PT: 6.8-8.4 (male) and 6.8-8.4 (female); APTT 9.6-11.8 (male) and 9.8-12.0 (female)).

Clinical Chemistry

Parameters Analyzed:

Clinical Chemistry Parameters	
Alanine Aminotransferase (ALT)	Globulin (calculated)(GLOB)
Albumin (ALB)	Glucose (GLU)
Albumin/Globulin ratio (calculated)(A/G)	Phosphorus (PHOS)
Alkaline Phosphatase (ALP)	Potassium (K)
Aspartate Aminotransferase (AST)	Sodium (NA)
Calcium (CA)	Total Bilirubin (T-BIL)
Chloride (CL)	Total Protein (TP)
Cholesterol (CHOL)	Triglycerides (TRIG)
Creatinine (CREAT)	Urea Nitrogen (BUN)

No clear test article-related changes were observed. TBIL levels in Group 3 males were statistically significantly lower at Day 92 and partially recovered over the course. Although it was not statistically significant, its level in Group 3 males remained lower than other groups.

Urinalysis

Parameters Analyzed:

Urinalysis Parameters	
Specific gravity	Appearance/Color
pH	Bilirubin
Protein	Blood
Glucose	Leukocytes
Ketone	Microscopic examination of spun deposit
Urobilinogen	

No test article-related changes in urinalysis parameters were observed.

Synovial Fluid

There were changes in BUN and WBC levels observed in synovial fluid, but there were no clear dose-dependent relationships or test article-related changes. As note, the sample size was only 2; therefore, it is difficult to determine whether these changes are toxicologically meaningful. Additionally, no significant hematological changes in the liver and kidney functions were observed.

Male (n=2)			Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Right Knee	BUN (mg/dl)	Day 92	13.0 ± 1.41	14.5 ± 2.12	12.0 ± 1.41	12.0 ± 2.83	14.0 ± 0.00	9.0 ± 0.00*
		Day 120	10.5 ± 2.12	12.0 ± 2.83	5.0 ± 2.83	14.5 ± 3.54	8.0 ± 0.00*	-
		Day 176	10.5 ± 2.12	12.0 ± 0.00	11.5 ± 3.54	14.5 ± 0.71	9.5 ± 0.71	11.0 ± 0.00*
		Day 274	10.5 ± 0.71	9.0 ± 4.24	14.0 ± 1.41	12.0 ± 1.41	10.0 ± 1.41	9.5 ± 3.54
	WBC ($x10^3$/mcL)	Day 92	0.063 ± 0.018	0.125 ± 0.071	0.100 ± 0.00	0.200 ± 0.14	0.125 ± 0.071	0.063 ± 0.018
		Day 120	0.100 ± 0.106	0.200 ± 0.141	0.025 ± 0.00	0.113 ± 0.018	0.050 ± 0.00	0.050 ± 0.00*
		Day 176	0.100 ± 0.106	0.400 ± 0.035	0.313 ± 0.407	0.200 ± 0.141	0.425 ± 0.212	0.075 ± 0.00
		Day 274	0.138 ± 0.018	0.175 ± 0.106	0.150 ± 0.141	0.038 ± 0.018	0.113 ± 0.053	0.100 ± 0.106
Left Knee	BUN (mg/dl)	Day 92	12.5 ± 2.12	10.5 ± 3.54	10.0 ± 4.24	11.5 ± 0.71	9.5 ± 0.71	7.5 ± 2.12
		Day 120	8.0 ± 0.00	11.0 ± 1.41	9.5 ± 2.12	10.5 ± 6.36	9.0 ± 0.00	6.5 ± 2.12
		Day 176	11.0 ± 5.66	8.0 ± 2.83	10.0 ± 1.41	8.0 ± 0.00	6.0 ± 2.83	10.0 ± 0.00
		Day 274	11.0 ± 1.41	11.0 ± 1.41	8.0 ± 0.00	7.5 ± 2.12	9.5 ± 0.71	10.0 ± 1.41

*N=1

Female (n=2)			Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Right Knee	BUN (mg/dl)	Day 93	11.0 ± 1.41	13.0 ± 0.00	7.0 ± 0.00*	14.0 ± 0.00*	13.5 ± 3.54	12.0 ± 0.00*
		Day 121	9.0 ± 2.83	12.5 ± 0.71	10.5 ± 2.12	14.0 ± 0.00	10.5 ± 2.12	8.5 ± 6.36
		Day 177	12.5 ± 6.36	10.0 ± 4.24	9.0 ± 4.24	12.0 ± 1.41	10.0 ± 2.83	12.0 ± 0.00
		Day 275	8.5 ± 0.71	11.5 ± 3.54	11.0 ± 0.00*	13.0 ± 5.66	11.5 ± 0.71	12.5 ± 0.71
	WBC ($x10^3$/mcL)	Day 93	0.175 ± 0.106	0.038 ± 0.018	0.051 ± 0.070	0.113 ± 0.053	0.125 ± 0.071	0.138 ± 0.124
		Day 121	0.038 ± 0.053	0.363 ± 0.442	0.013 ± 0.018	0.325 ± 0.424	0.013 ± 0.018	0.063 ± 0.053
		Day 177	0.063 ± 0.018	0.188 ± 0.018	0.100 ± 0.0354	0.263 ± 0.230	0.250 ± 0.177	0.563 ± 0.548
		Day 275	0.075 ± 0.00	0.138 ± 0.088	0.050 ± 0.00*	0.188 ± 0.053	0.263 ± 0.195	0.075 ± 0.035
Left Knee	BUN (mg/dl)	Day 93	10.5 ± 3.54	11.5 ± 2.12	13.5 ± 0.71	12.0 ± 1.41	14.0 ± 4.24	10.0 ± 4.24
		Day 121	11.5 ± 2.12	9.5 ± 0.71	10.0 ± 0.00	12.0 ± 2.83	9.5 ± 0.71	11.0 ± 1.41
		Day 177	16.5 ± 3.54	11.0 ± 1.41	5.5 ± 2.12	9.0 ± 1.41	12.5 ± 0.71	9.0 ± 5.66
		Day 275	9.5 ± 3.54	12.5 ± 3.54	10.0 ± 0.00	11.5 ± 4.95	9.0 ± 1.41	12.0 ± 0.00

N=1

Gross Pathology

No test article-related gross findings were observed.

Organ Weights

Organs Weighed	
Adrenals	Testes
Brain	Ovaries
Heart	Spleen
Kidneys	Thyroids/Parathyroids
Liver	Popliteal Lymph Node
Thymus	

No test article-related organ weight changes were observed. Spleen weight in Group 3 females (Kenalog-40 IR) were reduced greater than 50% up to Day 177. However, there were no associated microscopic findings observed in the spleen.

Female (% body weight)		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Spleen	Day 93	0.91 ± 0.251	1.22 ± 0.205	**0.47 ± 0.150**	0.93 ± 0.127	0.70 ± 0.434	0.77 ± 0.124
	Day 120	0.90 ± 0.437	0.82 ± 0.541	**0.30 ± 0.037**	1.20 ± 0.470	0.83 ± 0.738	0.65 ± 0.047
	Day 177	0.79 ± 0.162	0.69 ± 0.398	**0.32 ± 0.018**	1.25 ± 0.261	0.99 ± 0.213	0.82 ± 0.327
	Day 275	0.75 ± 0.422	0.54 ± 0.068	1.00 ± 0.205	1.12 ± 0.158	0.57 ± 0.424	1.33 ± 0.058

Histopathology

Adequate Battery

As note, for bone/cartilage from each knee joint underwent decalcification, one section was stained with hematoxylin and eosin (H&E) and a second section was stained with Safranin O. Additionally, knee joint synovial tissues collected from the femoropatellar, left femorotibial, and right femorotibial sacs were sectioned and stained with H&E.

The severity scale employed for the implant site evaluation in accordance with ISO 10993-6 was on a scale of 0-4 as follows:

0 = Not present
1 = Minimal/Slight - 1 to 25 % of the implant site is involved or 1 - 5 cells per high power field (400x)
2 = Mild - 26 to 50% of the implant site is involved or 5 - 10 cells per high power field (400x)
3 = Moderate - 51 to 75% of the implant site is involved or a heavy infiltrate of cells per high power field (400x)
4 = Marked /Severe - 76 to 100% of the implant site is involved or the cells are packed per high power field (400x)

Adrenals*
Aorta
Brain*
Cecum
Cervix
Colon
Duodenum
Epididymides
Esophagus
Eye with optic nerve
Femur (distal) with articular surface* a)
Gallbladder
Gross findings*
Heart
Ileum
Jejunum
Knee joint synovium from each synovial sac (suprapatellar synovium from the femoropatellar sac, the left femorotibial sac, and the right femorotibial sac)*
Kidneys*
Lacrimal glands
Larynx
Liver*
Lymph nodes: inguinal*
Mammary gland
Meniscus b)
Ovaries
Pancreas
Patella*
Pituitary*
Prostate
Proximal tibia*
Rectum
Salivary gland(s)
Sciatic nerve
Skeletal muscle (thigh)
Skin
Spinal cord – cervical
Spinal cord – lumbar
Spinal cord – midthoracic
Spleen*
Sternum with bone marrow*
Stomach
Testes
Thymus*
Thyroid/parathyroid*
Tongue

Lung with mainstem bronchus	Trachea
Lymph nodes: mandibular*	Urinary bladder
Lymph nodes: mesenteric*	Uterus
Lymph nodes: popliteal*	Vagina

* Tissues evaluated microscopically

a) Following collection of the synovial fluid, both the right and left knee joint(s) of each animal were opened by initially removing the skin overlying the joint then incising through the joint capsule, patellar tendon, and medial and lateral collateral ligaments. Once the knee joint space was entered, the cruciate ligaments were transected and the femur was separated from the tibia (disarticulation). Subsequently, the menisci were removed from the tibial articular surface. Throughout the dissection, care was taken to prevent damage to articular surfaces. The synovium and articular surfaces were carefully examined and any abnormalities were documented. Additionally, each knee joint was examined for the presence or absence of residual test material, as appropriate. The distal femur, proximal tibia, patella, and a representative sample of the knee joint synovium from each synovial sac (suprapatellar synovium from the femoropatellar sac, the left femorotibial sac, and the right femorotibial sac) were saved in 10% neutral buffered formalin.

b) Collected but not evaluated.

Macroscopic Evaluation

No macroscopic observations related to the test article were observed. There were sporadic and incidental observations as following:

Day	Sex	Animal Number (Group Number)	Necropsy Findings	Corresponding Microscopic Findings
92/93	M	11 (5)	Right articular fat pad brown discoloration, lateral aspect	Steatitis (inflammation of fat, Grade 3)
	F	53 (6)	**Rectum discoloration, diffuse, dark red, mucosa**	**Congestion of the mucosa and submucosa (Grade 2)**
120/121	M	1 (2)	Testes small, bilaterally symmetrical	Necrosis (Grade 4), granulation tissue (Grade 3)
		25 (3)	Abdominal fat red nodule	Fat necrosis (Grade 3), fibrosis (Grade 3), hemorrhage (Grade 3)
		41 (6)	**Ileum, colon, and duodenum, red mucosal surface**	**Congestion of the mucosa and submucosa (Grade 2 or 3)**
176/177	M	13 (5)	Adrenal gland, discoloration, red	No microscopic observation

Microscopic Evaluation

Systemic Organ/Tissue Findings

Microscopic findings were observed in lymphoid tissues for MD and in adrenal glands and lymphoid tissues for HD and these findings were attributable to hypercortisolism from TCA. These findings in animals treated with the test article were not worse than the ones in animals treated with the active comparator, Kenalog. There were no systemic microscopic findings in blank microsphere-treated or LD groups.

- Adrenal Glands: Slight atrophy of the zona fasciculate of the adrenal cortex was observed in females of Group 3 and 6 at Day 92/93. Adrenal atrophy was

observed in Group 6 at Day 120/121 and 176/177. No adrenocortical atrophy was observed at Day 274/275.

- Lymphoid Tissues: Lymphoid depletion was observed in Group 3 at Day 92/93 and Group 6 at Day 120/121. At Day 176/177, one female of Group 5 showed lymphoid depletion. No lymphoid depletion was observed at Day 274/275. Erythrophagocytosis were observed in lymph nodes of animals in all groups; therefore, it may be related to the IA injection, not to the test article.

Microscopic findings in other organs appear to be incidental or not related to the test article treatment because of the low number of incidence, no dose-dependent relationship, and low severity.

Microscopic Findings of Systemic Organ/Tissue

Days		92/93						120/121						176/177						274/275					
Group (N=2/sex)		1 D	2 B	3 K	4 L	5 M	6 H	1 D	2 B	3 K	4 L	5 M	6 H	1 D	2 B	3 K	4 L	5 M	6 H	1 D	2 B	3 K	4 L	5 M	6 H
Adrenal Glands																									
M	Atrophy:ctx	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 3	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Vacuol:ctx	-	-	-	-	-	-	-	-	1	-	1	2	-	-	1	-	-	1	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	1	-	1	2	-	-	1	-	-	1	-	-	-	-	-	-
F	Atrophy:ctx	-	-	1	-	-	1	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-
	Grade 2	-	-	1	-	-	1	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-
	Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Vacuol:ctx	1	-	1	1	1	-	1	-	1	-	2	1	-	-	-	1	-	-	-	-	-	-	-	-
	Grade 2	1	-	1	1	1	-	1	-	1	-	2	1	-	-	-	1	-	-	-	-	-	-	-	-
	Cyst	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brain																									
M	Mononc infil.	-	-	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	1	1
	Grade 1	-	-	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	1	1
F	Mononc infil.	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-
	Grade 1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-
Kidney																									
M	Mononc infil.	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	1	1	-	-	-	-	1
	Grade 1	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	1	1	-	-	-	-	1
	Fibrosis	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Grade 1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Neutro infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Pigmt:cytop.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2	2	2	1	2
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2	2	2	1	2

F	Mononc infil.	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-
	Grade 1	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Vacuolation	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mineraliz.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-
	Fibrosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	Cyst: tubular	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
	Pigmt: cytop.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	1	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	1	-
Liver																									
M	Mononc infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
F	Mononc infil.	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
	Grade 1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
	Vacuolation	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lymph Node: Inguinal																									
M	Lymp hyperp	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 3	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Erythrophag.	-	1	2	-	-	1	-	-	-	-	1	-	-	-	-	1	2	-	1	1	-	1	-	-
	Grade 1	-	1	2	-	-	-	-	-	-	-	1	-	-	-	-	1	1	-	1	1	-	1	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	Neutro infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	1	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-
	EOS infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
F	Erythrophag.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	EOS infil.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-
	Neutro infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Lymph Node: Left Popliteal																									
M	Erythrophag.	1	1	-	1	1	1	1	-	-	-	-	-	1	1	-	1	2	1	1	-	1	-	-	-
	Grade 1	1	1	-	1	1	1	1	-	-	-	-	-	1	-	-	-	1	1	1	-	1	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-
	Lymphd dep.	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Pigment	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	grade 1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Lymp hyperp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	Grade 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	Fr. bdy granu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-

F	Erythrophag.	-	1	-	1	-	-	2	-	-	-	-	-	1	-	-	1	-	2	-	1	-	-	-	-
	Grade 1	-	1	-	1	-	-	2	-	-	-	-	-	1	-	-	-	-	2	-	1	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	Lymp hyperp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
	Neutro infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-
Lymph Node: Mandibular																									
M	Erythrophag.	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-
	Grade 1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-
	Lymphd dep.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Neutro infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
F	Erythrophag.	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-
	Grade 1	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-
	Lymphd dep.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
	Neutro infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-
Lymph Node: Mesenteric																									
M	Lymp hyperp	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Grade 2	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Erythrophag.	2	1	2	2	1	2	1	2	2	2	2	1	2	2	2	2	1	1	2	2	2	2	2	2
	Grade 1	1	-	-	2	-	1	1	1	-	-	1	-	1	2	-	-	-	1	2	-	-	1	1	2
	Grade 2	1	1	2	-	1	1	-	1	2	2	1	1	1	-	2	2	1	-	-	2	2	1	1	-
	Lymphd dep.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
F	Lymp hyperp	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	Grade 3	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	Erythrophag.	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2
	Grade 1	2	1	1	1	1	1	2	-	1	1	1	-	2	2	2	2	1	2	-	-	1	1	-	1
	Grade 2	-	1	1	1	1	1	-	2	1	1	1	1	-	-	-	-	1	-	2	2	1	1	2	1
Lymph Node: Right Popliteal																									
M	Lymp hyperp	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Erythrophag.	-	2	-	-	1	1	1	-	-	-	-	-	-	2	2	-	2	2	1	-	-	-	-	-
	Grade 1	-	1	-	-	1	1	1	-	-	-	-	-	-	2	2	-	1	1	1	-	-	-	-	-
	Grade 2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-
	Lymphd dep.	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
F	Erythrophag.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	1	2	-	-	-	1	-	1
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	1	2	-	-	-	1	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Neutro infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Parathyroid Glands																									
M	Cyst	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	Ectop. Thym.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-

F	Cyst	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	2	-	1	2	-	2	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1	-	2	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	1	-	-	-
Pituitary Gland																									
M	Cyst	-	1	-	-	-	-	1	-	-	-	1	1	-	1	-	-	1	-	2	1	1	-	1	2
	Grade 1	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	2
	Grade 2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	1	-	1	-	-	2
	Grade 3	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-
	Mononc infil.	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F	Cyst	-	1	-	1	-	1	-	-	-	1	-	-	-	1	2	2	1	-	2	1	1	1	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	1	-	1	-	-
	Grade 2	-	1	-	1	-	1	-	-	-	1	-	-	-	-	2	2	-	-	1	-	-	-	-	-
	Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-
Thymus																									
M	Lymphd dep.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 4	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
F	Lymphd dep.	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thyroid Gland																									
M	Mononc infil.	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Colld miner.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-
F	Cyst	-	-	1	-	-	-	-	-	1	-	-	-	1	2	-	-	1	1	-	-	-	-	1	-
	Grade 1	-	-	-	-	-	-	-	-	1	-	-	-	-	2	-	-	1	-	-	-	-	-	-	-
	Grade 2	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	1	-
	Ectop. Thym.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Mononc infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-
	Colld miner.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-

D (diluent control); B (blank microsphere control, 75 mg/ml); K (Kenalog IR, 18.75 mg/ml TCA); L (low dose, 8.33 mg/ml microsphere and 2.1 mg/ml TCA); M (mid dose, 25 mg/ml microsphere and 6.25 mg/ml TCA); H (high dose, 75 mg/ml microsphere and 18.75 mg/ml); Atrophy:ctx (atrophy: cortex zona fasciculate); Vacuol:ctx (vacuolation: cortex zona fasciculate); Myld Hyperp. (myeloid hyperplasia); Lymp hyperp (lymphoid hyperplasia); Monon infil. (mononuclear infiltration); Neutro infil. (neutrophil infiltration); Pigmt:cytop. (pigment: cytoplasm proximal convoluted tubule); Mineraliz. (mineralization); Erythrophag. (erythrophagocytosis); EOS infil. (eosinophil infiltration); Fr. bdy granu. (foreign body granuloma); Ectop. Thym. (ectopic thymus); Lymphd dep. (lymphoid depletion); Colld miner. (colloid mineral)

Local Microscopic Findings

- Left knee: There were some microscopic findings in the left knee but it appears to be sporadic and not related to the test article because there was no dose-dependent relationship. No microscopic findings were observed in the animals treated with the test article at Day 274/275.
- Right knee: Microscopic findings at Day 92/93 appear to be related to microspheres and TCA. Microspheres were detected in groups treated with high

microspheres (75 mg, Group 2 and 6) but they were not detectable from Day 120/121. By Day 274/275, these findings appear to be reversible and animals treated with the test article (Group 4 to 6) were no worse than animals treated with the active comparator, Kenalog (Group 3).

Microscopic Findings of Knee Joints

	Days	92/93						120/121						176/177						274/275					
	Group (N=2/sex)	1 D	2 B	3 K	4 L	5 M	6 H	1 D	2 B	3 K	4 L	5 M	6 H	1 D	2 B	3 K	4 L	5 M	6 H	1 D	2 B	3 K	4 L	5 M	6 H
Knee Joint Synovium, Left (no injection)																									
M	Lymph infil.	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
	Grade 1	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
	Hyperplasia	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Grade 2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Jnt Cap infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	PlaCell infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-
F	Lymph infil.	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PlaCell infil.	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Grade 1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Hyperplasia	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	2	-	-	-	-	-	-	-
	Grade 2	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	2	-	-	-	-	-	-	-
	Pigmt: cytop.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Jnt Cap infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Knee Joint Synovium, Right (injection)																									
M	Mcrpg/mltinu	-	2	-	2	1	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 1	-	2	-	2	1	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Lymph infil.	-	-	-	1	1	1	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	-	-
	Grade 1	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-
	Grade 2	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	PlaCell infil.	-	-	-	1	-	1	-	-	-	-	-	1	-	1	-	-	1	-	-	1	1	-	1	1
	Grade 1	-	-	-	1	-	1	-	-	-	-	-	-	-	1	-	-	1	-	-	1	1	-	1	1
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Microsphere	-	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acellur. mat.	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Hyperplasia	1	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	Grade 1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	Grade 2	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Neutro infil.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Fibrosis	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-
	EOS infil.	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Jnt Cap Infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-

	Mcrpg/mltinu	-	2	-	-	-	2	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	1	-
	Grade 1	-	2	-	-	-	2	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	1	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Microsphere	-	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acellur. mat.	-	-	1	-	-	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	Grade 1	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	Fibrosis	-	-	-	1	-	1	-	-	-	-	1	1	-	-	-	-	1	1	-	-	-	-	-
	Grade 1	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	1	1	-	-	-	-	-
F	Hyperplasia	-	-	1	-	-	-	-	-	1	1	1	1	-	-	-	-	1	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	1	1	-	1	-	-	-	-	-	-	-	-	-	-	-
	Grade 2	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-
	Lymph infil.	-	-	-	-	-	-	-	-	-	1	-	1	-	1	-	-	-	1	-	1	1	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	1	-	1	1	-	-
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	PlaCell infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2	-	1	1	-	-	1
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2	-	1	1	-	-	1
	EOS infil.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-

D (diluent control); B (blank microsphere control, 75 mg/ml); K (Kenalog IR, 18.75 mg/ml TCA); L (low dose, 8.33 mg/ml microsphere and 2.1 mg/ml TCA); M (mid dose, 25 mg/ml microsphere and 6.25 mg/ml TCA); H (high dose, 75 mg/ml microsphere and 18.75 mg/ml); Lymph infil. (lymphocyte infiltration); PlaCell infil. (plasma cell infiltration); Acellur. Mat. (Acellular materials); EOS infil. (eosinophil infiltration); Mcrpg/mltinu (macrophage/multinucleated giant cell infiltration)

Modified Mankin Scoring System for Local Toxicity Evaluation

Category	Score
Structure	
Normal	0
Surface irregularities	1
Pannus and surface irregularities	2
Clefts to transitional zone	3
Clefts to tidemark	4
Clefts to subchondral bone	5
Complete disorganization	6
Cells	
Normal	0
Diffuse hypercellularity	1
Cloning	2
Hypocellularity (necrosis)	3
Safranin O staining	
Normal	0
Slight reduction, tangential zone	1
Moderate reduction (to tidemark)	2
Severe reduction (to subchondral bone in at least 1 area)	3
No dye observed	4
Tidemark Integrity	
Intact	0
Crossed by blood vessels	1
Total	0-14

Animals treated with diluent or blank microspheres did not show cellular/structure changes or decrease in Safranin O staining in both joints throughout the study. Mankin scores were higher in a dose-dependent manner of TCA but cellular/structural changes in the injected right joint appear to be reversible over time.

Summary of the Average Joint Scores

Group	Concentration Microspheres/ TCA (mg/ml)	Study Day		Males	Females
1	0/0	Day 92/93	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00
		Day 120/121	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00
		Day 176/177	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00
		Day 274/275	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00
2	75/0	Day 92/93	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00
		Day 120/121	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00
		Day 176/177	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00
		Day 274/275	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00
3	0/18.75	Day 92/93	Right Knee Joint Total Average	1.33	1.67
			Left Knee Joint Total Average	0.00	0.00
		Day 120/121	Right Knee Joint Total Average	1.00	0.33
			Left Knee Joint Total Average	0.00	0.00
		Day 176/177	Right Knee Joint Total Average	1.00	0.50
			Left Knee Joint Total Average	0.00	0.00
		Day 274/275	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00

4	8.33/2.1	Day 92/93	Right Knee Joint Total Average	1.17	1.33
			Left Knee Joint Total Average	0.00	0.00
		Day 120/121	Right Knee Joint Total Average	0.50	1.00
			Left Knee Joint Total Average	0.00	0.00
		Day 176/177	Right Knee Joint Total Average	0.00	1.00
			Left Knee Joint Total Average	0.00	0.00
		Day 274/275	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00
5	25/6.25	Day 92/93	Right Knee Joint Total Average	1.83	1.50
			Left Knee Joint Total Average	0.00	0.00
		Day 120/121	Right Knee Joint Total Average	1.83	1.00
			Left Knee Joint Total Average	0.17	0.00
		Day 176/177	Right Knee Joint Total Average	1.33	0.00
			Left Knee Joint Total Average	0.00	1.33
		Day 274/275	Right Knee Joint Total Average	0.00	0.00
			Left Knee Joint Total Average	0.00	0.00
6	75/18.75	Day 92/93	Right Knee Joint Total Average	2.17	1.67
			Left Knee Joint Total Average	0.00	0.00
		Day 120/121	Right Knee Joint Total Average	1.83	1.67
			Left Knee Joint Total Average	0.00	0.00
		Day 176/177	Right Knee Joint Total Average	1.17	0.50
			Left Knee Joint Total Average	0.00	0.00
		Day 274/275	Right Knee Joint Total Average	2.50	0.00
			Left Knee Joint Total Average	0.00	0.00

N=2 per sex

Safranin O staining appears to be dependent on TCA concentrations and reversible over time. The summary of Safranin O staining scores are following:

Safranin O Staining Score

Day	Tissues (N=2/sex)		Group 1 Diluent	Group 2 Microsphere	Group 3 Kenalog IR	Group 4 LD	Group 5 MD	Group 6 HD
Male								
Day 92/93	Tibial Plateau	R	0.00	0.00	1.00	0.50	0.50	1.50
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Distal Femur	R	0.00	0.00	1.00	1.50	2.00	2.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Patella	R	0.00	0.00	1.50	1.50	3.00	3.00
		L	0.00	0.00	0.00	0.00	0.00	0.00

Day 120/121	Tibial Plateau	R	0.00	0.00	0.50	0.50	1.00	1.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Distal Femur	R	0.00	0.00	1.00	0.50	2.50	2.50
		L	0.00	0.00	0.00	0.00	0.50	0.00
	Patella	R	0.00	0.00	1.50	0.50	2.00	2.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
Day 176/177	Tibial Plateau	R	0.00	0.00	0.50	0.00	0.00	1.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Distal Femur	R	0.00	0.00	1.50	0.00	0.00	1.50
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Patella	R	0.00	0.00	1.00	0.00	1.00	1.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
Day 274/275	Tibial Plateau	R	0.00	0.00	0.00	0.00	0.00	0.50
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Distal Femur	R	0.00	0.00	0.00	0.00	0.00	0.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Patella	R	0.00	0.00	0.00	0.00	0.00	1.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
Female								
Day 92/93	Tibial Plateau	R	0.00	0.00	1.50	0.50	0.50	1.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Distal Femur	R	0.00	0.00	2.00	2.00	2.00	2.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Patella	R	0.00	0.00	2.00	1.50	1.50	2.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
Day 120/121	Tibial Plateau	R	0.00	0.00	0.50	1.00	1.00	1.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Distal Femur	R	0.00	0.00	0.00	1.00	1.00	2.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Patella	R	0.00	0.00	0.00	1.00	1.00	2.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
Day 176/177	Tibial Plateau	R	0.00	0.00	0.50	0.50	0.00	0.50
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Distal Femur	R	0.00	0.00	0.50	0.00	0.00	0.50
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Patella	R	0.00	0.00	0.50	0.00	0.00	0.50
		L	0.00	0.00	0.00	0.00	1.00	0.00
Day 274/275	Tibial Plateau	R	0.00	0.00	0.00	0.00	0.00	0.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Distal Femur	R	0.00	0.00	0.00	0.00	0.00	0.00
		L	0.00	0.00	0.00	0.00	0.00	0.00
	Patella	R	0.00	0.00	0.00	0.00	0.00	0.00
		L	0.00	0.00	0.00	0.00	0.00	0.00

Summary of Combined Group Incidence of Foreign Response (Macrophage/Multinucleated Giant Cell Infiltrates) to Microspheres in the Right Knee Joint Synovium

mg/ml		Group Number	Days Post Injection		Grade of Foreign Body Response*				
Microspheres	TCA		Day	Number of Dogs	0	1	2	3	4
8.33	2.1	4	92/93	4	2	2	0	0	0
			120/121	4	3	1	0	0	0
			176/177	4	4	0	0	0	0
			274/275	4	4	0	0	0	0
25	6.25	5	92/93	4	3	1	0	0	0
			120/121	4	4	0	0	0	0
			176/177	4	4	0	0	0	0
			274/275	4	3	1	0	0	0
75	18.75	6	92/93	4	0	3	1	0	0
			120/121	4	2	2	0	0	0
			176/177	4	4	0	0	0	0
			274/275	4	4	0	0	0	0
75	0	2	92/93	4	0	4	0	0	0
			120/121	4	4	0	0	0	0
			176/177	4	4	0	0	0	0
			274/275	4	4	0	0	0	0

* H&E sections of right synovium were evaluated using a subjective grading scale as follows: grade 0 = no findings; grade 1 = minimal with barely detectable change usually of focal distribution; grade 2 = slight with easily detectable change in tissues but still limited in extent, usually of focal to multifocal distribution; grade 3 = moderate with readily detectable change in tissues of notable extent, usually of multifocal distribution; and grade 4 = marked with very evident to profound change in tissues, usually diffuse in distribution.

Combined Group Incidence and Severity of Changes in the Right Knee Synovium

Day 92/93

Dose Group	1	2	3	4	5	6
Concentration Microspheres/TCA (mg/ml)	0/0	75/0	0/18.75	8.33/2.1	25/6.25	75/18.75
Number/Group	4	4	4	4	4	4
Lymphocyte Infiltration						
Grade 1*	0	0	0	1	1	0
Grade 2*	0	0	0	0	0	1
Plasma Cell Infiltration						
Grade 1*	0	0	0	1	0	1
Grade 2*	0	0	0	0	0	0
Fibrosis						
Grade 1*	0	0	0	1	0	0
Grade 2*	0	0	0	0	0	1

Day 120/121

Dose Group	1	2	3	4	5	6
Concentration Microspheres/TCA (mg/ml)	0/0	75/0	0/18.75	8.33/2.1	25/6.25	75/18.75
Number/Group	4	4	4	4	4	4
Lymphocyte Infiltration						
Grade 1*	0	0	0	1	0	0
Grade 2*	0	0	0	0	0	2
Plasma Cell Infiltration						
Grade 1*	0	0	0	0	0	0
Grade 2*	0	0	0	0	0	2
Fibrosis						
Grade 1*	0	0	0	0	0	2
Grade 2*	0	0	0	0	1	0

Day 176/177

Dose Group	1	2	3	4	5	6
Concentration Microspheres/TCA (mg/ml)	0/0	75/0	0/18.75	8.33/2.1	25/6.25	75/18.75
Number/Group	4	4	4	4	4	4
Lymphocyte Infiltration						
Grade 1*	0	1	0	0	1	1
Grade 2*	0	0	0	0	0	0
Plasma Cell Infiltration						
Grade 1*	0	2	0	0	1	2
Grade 2*	0	0	0	0	0	0
Fibrosis						
Grade 1*	0	0	0	0	0	1
Grade 2*	0	0	0	0	1	1

Day 274/275

Dose Group	1	2	3	4	5	6
Concentration Microspheres/TCA (mg/ml)	0/0	75/0	0/18.75	8.33/2.1	25/6.25	75/18.75
Number/Group	4	4	4	4	4	4
Lymphocyte Infiltration						
Grade 1*	0	2	1	0	0	0
Grade 2*	0	0	0	0	0	0
Plasma Cell Infiltration						
Grade 1*	0	2	2	0	1	2
Grade 2*	0	0	0	0	0	0
Fibrosis						
Grade 1*	0	0	0	0	0	0
Grade 2*	0	0	0	0	0	0

* H&E sections of right synovium were evaluated using a subjective grading scale as follows: grade 0 = no findings; grade 1 = minimal with barely detectable change usually of focal distribution; grade 2 = slight with easily detectable change in tissues but still limited in extent, usually of focal to multifocal distribution; grade 3 = moderate with readily detectable change in tissues of notable extent, usually of multifocal distribution; and grade 4 = marked with very evident to profound change in tissues, usually diffuse in distribution.

For the ISO 10993-6 implant site evaluation, total Irritancy scores were calculated for each implant site for each animal via the following formula: *[(Sum of inflammation scores) x 2] + (sum of tissue response scores)*.

The ranked irritancy score for the test article (Groups 4, 5, and 6) was then calculated by a direct comparison of the average irritancy score of the control group (Group 2) by subtracting the average irritancy score of the control group from the average irritancy score of the test group. (Note: If the ranked irritancy score resulted in a negative number, the irritancy score was determined to be zero.)

The ranked irritancy score for the test article groups was applied an irritancy conclusion as determined to be a non-irritant, slight, moderate, or severe irritant by the following scale present in the ISO 10993 part 6 guidelines:

Non-irritant = 0.0 up to 2.9
Slight irritant = 3.0 up to 8.9
Moderate irritant = 9.0 up to 15.0
Severe Irritant = > 15

Compared to the active comparator control, Kenalog, the test article at an equivalent TCA dose may be slightly more irritating though they both had scores in the slight irritant range. This subtle difference is consistent with the subtle differences in local toxicity findings (Mankin scores and Safranin O staining) between these two groups. It is unclear whether these differences are clinically meaningful.

Irritation Score

Day	Group	Average Irritancy Score	Ranked Irritancy Compared to Control
92/93	2 (Kenalog)	4.5	-
	4 (LD)	-	-
	5 (MD)	-	-
	6 (HD)	7.0	2.5
120/121	2 (Kenalog)	-	-
	4 (LD)	-	-
	5 (MD)	-	-
	6 (HD)	-	-
176/177	2 (Kenalog)	-	-
	4 (LD)	-	-
	5 (MD)	-	-
	6 (HD)	-	-
274/275	2 (Kenalog)	-	-
	4 (LD)	-	-
	5 (MD)	-	-
	6 (HD)	-	-

Synovial Fluid Evaluation

- No clear test article-related changes were observed in levels of WBC, neutrophil, small and large mononuclear cells among groups at all time points.
- Eosinophils were observed in only two animals (1 female ($0.01X10^3$/mcL) at 177 days and 1 female ($0.01X10^3$/mcL) at 275 days).
- Plasma cells and mast cells were not observed in any group.
- Microspheres (small fragments of microspheres, not intact microspheres) were observed in only one male from Group 2 at 120 days. No cells were adhered to

the microspheres. No other animals showed microspheres throughout the recovery periods.

Day	Cells (10^3/mcL)	Sex	Group 1 (Diluent)	Group 2 (Microspheres)	Group 3 (Kenalog)	Group 4 (LD)	Group 5 (MD)	Group 6 (HD)
92/93	WBC	M	0.08	0.16	0.14	0.29	0.18	0.07
		F	0.26	0.05	na	0.11	0.16	0.29
	Neutrophils	M	0	0.04	0	0	0.01	0
		F	0.05	0.01	na	0.02	0	0.03
	Sm Mononuc	M	0.06	0.11	0.07	0.19	0.14	0.04
		F	0.13	0.03	na	0.08	0.15	0.18
	Lg Mononuc	M	0.03	0.02	0.07	0.11	0.04	0.03
		F	0.09	0.01	na	0.01	0.07	0.08
120/ 121	WBC	M	0.18	0.28	0.09	0.14	0.09	na
		F	0.16	0.45	0.04	0.47	0.05	0.23
	Neutrophils	M	0	0.14	0	0.03	0.02	na
		F	0	0	0.01	0.01	0	0
	Sm Mononuc	M	0.24	0.10	0.05	0.09	0.08	na
		F	0.07	0.35	0.03	0.32	0.04	0.15
	Lg Mononuc	M	0.08	0.06	0.04	0.02	0	na
		F	0.09	0.10	0	0.15	0.01	0.09
176/ 177	WBC	M	0.15	0.61	0.44	0.25	0.63	0.12
		F	0.14	0.38	0.15	0.34	0.47	0.68
	Neutrophils	M	0	0.02	0	0	0	0
		F	0	0.01	0.01	0.14	0	0.01
	Sm Mononuc	M	0.11	0.50	0.33	0.19	0.55	0.11
		F	0.09	0.32	0.12	0.17	0.33	0.57
	Lg Mononuc	M	0.05	0.10	0.11	0.07	0.09	0.01
		F	0.06	0.05	0.03	0.03	0.14	0.10
274/ 275	WBC	M	0.18	0.27	0.18	0.06	0.20	0.18
		F	0.15	0.20	0.08	0.31	0.32	0.13
	Neutrophils	M	0	0	0.01	0	0	0
		F	0	0.02	0	0.05	0	0
	Sm Mononuc	M	0.16	0.22	0.15	0.04	0.14	0.15
		F	0.12	0.16	0.08	0.24	0.28	0.11
	Lg Mononuc	M	0.02	0.07	0.03	0.02	0.06	0.03
		F	0.03	0.02	0.01	0.02	0.03	0.02

WBC (white blood cells); sm mononuc (small mononuclear cells); lg mononuc (large mononuclear cells)

Toxicokinetics

Sample Collection

On Day 1, whole blood samples (~1.0 ml each) were collected from 3 animals/sex in Groups 3-6 at the following timepoints post-dose: 0, (pre-dose), 1, 2, 4, 8, 12, 24, and 48 hours. On Day 7 and 14, as well as 4, 6, 8 and 10 weeks after dosing (±2 days), whole blood samples (~1.0 ml each) were collected from three animals in Groups 3-6 (3/sex/group). On Days 92/93 (3 month recovery), Days 120/121 (4 month recovery), Days 176/177 (6 month recovery), and Days 274/275 (9 month recovery), whole blood samples (~1.0 ml each) were collected from 2 animals/sex/group in Groups 3-6 scheduled for sacrifice. In addition, whole blood samples (~1.0 ml each) were collected from 3 animals/sex in Groups 1-2 at 1 and 24 hours post-dose on Day 1. Whole blood was collected from the jugular vein of each animal into tubes containing K_2-EDTA. Derived plasma samples were stored frozen at ~ -70 °C until shipment to [redacted] (b) (4)

Toxicokinetic Parameters for TCA in Plasma Following Repeated Intra-Articular Injections

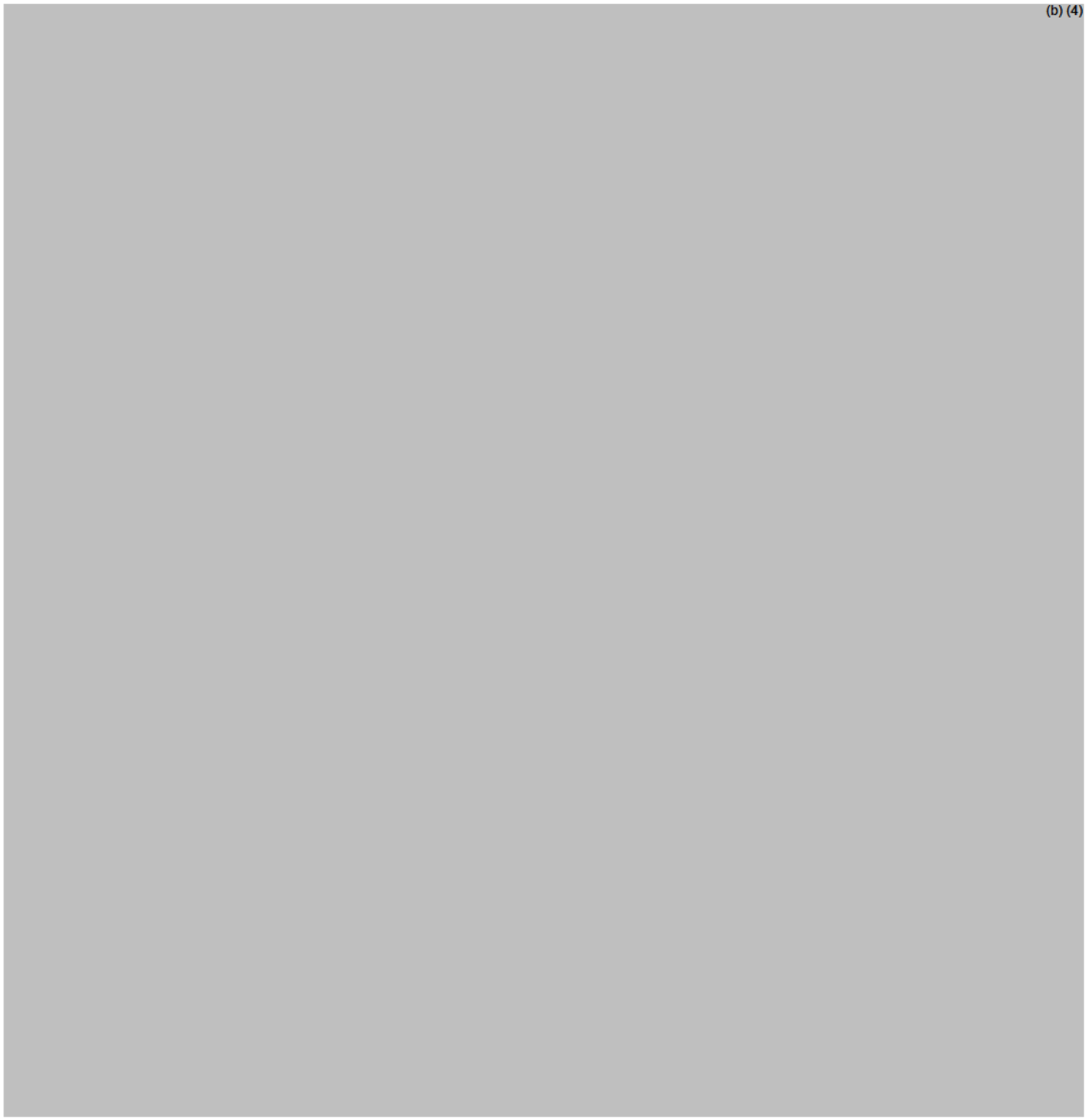
N= 3/sex/group	Group 1 Diluent	Group 2 Blank M	Group 3 Kenalog IR	Group 4 LD (2.1 mg)	Group 5 MD (6.25 mg)	Group 6 HD (18.75 mg)
C_{max} (ng/mL)	-	-	41.7 ± 3.61	0.81 ± 0.51	1.15 ± 0.65	4.32 ± 2.62
$AUC_{0\text{-}1\ day}$ (ng*day/mL)	-	-	22.19 ± 5.41	0.56 ± 0.40	0.78 ± 0.47	3.09 ± 1.76
$AUC_{0\text{-}\infty}$ (ng*day/mL)	-	-	69.34 ± 10.65	3.46 ± 1.03	7.57 ± 2.38	38.32 ± 12.58
$T_{1/2,e}$ (day)	-	-	18.3	5.61	11.2	32.3 ± 20.57

Blank M (blank microspheres)

The Applicant also submitted a synovial TCA evaluation report, but it does not appear to provide a meaningful data because a low number of samples (2 or less samples per sex per group) were evaluated and most values were not reportable value. Therefore, it is not reviewed fully.

Dosing Solution Analysis

Acceptable.


(b) (4)

6.2 Repeat-Dose Toxicity

Study title: A Repeat Intra Articular Dose Toxicity Study with FX006 (Triamcinolone Acetonide in 75:25 PLGA Microspheres) in Beagle Dogs

Study no.:	FX006TOX-2012-001 (or 0476DF21.001)
Study report location:	SDN 1, 4.2.3.2, 12/08/2016
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 11, 2012
GLP compliance:	Yes (exception: Analysis of the synovial fluid for TCA concentrations was not done under GLP. The Sponsor noted that the impact on the study was minimal since the methods used for analysis were qualified.)
QA statement:	Yes
Drug, lot #, and % purity:	FX006 (triamcinolone acetonide (TCA, 99.7%, Lot L0G322)) in 75:25 PLGA microspheres at 25% loading (w/w), Lot 12-083-001-40 (low dose) and 12-083-001-60 (high dose), purity 1.45% total (b) (4) % (range), low dose) and 1.5% total ((b) (4) % (range), high dose)

Key Study Findings

- No mortality/morbidity and no test article-related clinical signs were observed in all groups (both main and subset studies: Group 1 to 8).
- No test article-related body weight changes were observed in all groups (both main and subset studies: Group 1 to 8). There were some changes (~10%) but they do not appear to be test article-related.
- Main Study (three injections every 3 months, Group 1 to 4):
    - TCA-related reductions in lymphocytes were observed in animals (Group 2, 3, and 4) at Day 210/211 but it appears to be reversible by the end of recovery period. TCA-related reductions in eosinophil were observed in females at HD (Group 2 and 4) at Day 210 but it appears to be reversible by the end of recovery period.
    - TCA-related reduction in creatinine levels were observed in animals at HD (Group 2 and 4) at Day 210/211 but these changes appear to be reversible by the end of recovery period (Day 363/364).
    - No test article-related gross findings were observed.
    - TCA-related liver weight increases were observed in animals (Group 2 and 4) but these changes were reversible by the end of recovery period (Day 363/364).
    - Systemic microscopic findings: Dose-dependent/TCA-related common hypercortisolism such as slight to severe atrophy of adrenal gland and lymphoid depletion were observed and it appears to be partially reversible by the end of the recovery period. Minimal to moderate cell swelling in

hepatocytes of the liver were also observed in a TCA-related/dose-dependent manner and it appears to be also partially reversible by the end of recovery period. Minimal to moderate cysts in the pituitary gland were observed in treated animals and it appears to be more prominent in females. Microscopic findings in other organs appear to be incidental because there were no clear dose-dependent relationships. Systemic findings in animals treated with the test article were mostly comparable or slightly worse than the ones treated with the active comparator (Kenalog-40 IR)

- Local microscopic findings: Local findings were observed in the injected knee. Microscopic findings including minimal to mild macrophage, lymphocyte/plasma cell/neutrophil infiltration, neovascularization, granulation tissue, debris, and fibrosis were observed but it appears to be mostly related to microspheres. Note these findings were not observed in animals treated with Kenalog-40 IR, but it appears that TCA attenuates these local adverse effects because animals at HD showed a slightly higher number of incidence and severity than the vehicle control. These changes appear to be partially reversible by the end of 6-month recovery period. Microspheres were observed at Day 210/211 but were not observed at the end of recovery period (Day 363/364). Mild to Moderate hyperplasia, macrophage, mineralization, and degeneration were observed at the end of recovery period but these findings were a slightly worse or comparable with the active comparator, Kenalog-40.
- There were comparable but slightly worse average joint scores (Modified Mankin Score based on surface structure and cellularity, Safranin O staining, and tidemark integrity) in animals treated with the test article compared to the active comparator, Kenalog-40 IR. While the joint scores were not reversible but even got worse at the end of the recovery period compared to Day 210/211, the Safranin O staining levels were partially reversible at the end of the recovery period.
- From the semi-quantitative evaluation per the ISO 10993 Part 6 guidelines, the test article appears to be a slight irritant at Day 210/211.

- Subset Study (three injections every one month: Group 5 to 8):
  - TCA-related reductions in lymphocytes were observed in animals (Group 6, 7, and 8) at Day 92/93 but it appears to be reversible by the end of recovery period. TCA-related reductions in eosinophil were observed in females at HD (Group 6 and 8) at Day 92/93 but it appears to be reversible by the end of recovery period.
  - TCA-related reduction in creatinine levels were observed in animals at HD (Group 6 and 8) at Day 92/93 but these changes appear to be reversible by the end of recovery period (Day 243/244). ALP was increased by about 2-folds in animals at HD (Group 8) at Day 92/93 but it was reversible by the end of recovery period (Day 244).
  - No test article-related gross findings were observed.

- TCA-related liver weight increases were observed in animals (Group 6 and 8) but these changes were reversible by the end of recovery period (Day 243/244).
- Systemic microscopic findings: Similar findings were observed as described in the main study above. Slightly higher incidence and severity of adrenal gland atrophy and lipid depletion which is known to be related to hypercortisolism were observed in this subset group compared to the main study groups.
- Local microscopic findings: Similar microscopic findings observed in the main study, such as multinucleated macrophage, lymphocyte/plasma cell/neutrophil infiltration, neovascularization, debris, and fibrosis were observed.
- Similar to the main study, there were comparable but slightly worse average joint scores (Modified Mankin Score based on surface structure and cellularity, Safranin O staining, and tidemark integrity) in animals treated with the test article compared to the active comparator, Kenalog-40 IR. However, unlike the main study, it appears that the average joint scores appear to be reversible over the recovery period.
- From the semi-quantitative evaluation per the ISO 10993 Part 6 guidelines, the test article appears to be a non-irritant at Day 92/93.

- TK data are listed in the table below.

		Main Study (3-month dosing interval)				Subset Study (1-month dosing interval)			
		1	2	3	4	1	2	3	4
Dose 1	C_{max} (ng/mL)	-	47.2 ± 9.87	1.38 ± 0.532	3.68 ± 2.380	-	49.6 ± 11.21	1.10 ± 0.491	3.64 ± 2.208
	$AUC_{0\text{-}1\ day}$ (ng*day/mL)	-	26.0 ± 5.089	1.0 ± 0.45	2.5 ± 1.57	-	28.3 ± 8.565	0.84 ± 0.393	2.8 ± 1.83
	$AUC_{0\text{-}\infty}$ (ng*day/mL)	-	83.7 ± 14.69	18.0 ± 9.77	38.4 ± 7.75	-	85.4 ± 25.26	11.4 ± 3.51	34.0 ± 15.57
	$T_{1/2,e}$ (day)	-	15.4 ± 22.70	21.9 ± 17.8	29.3 ± 20.15	-	16.5 ± 18.00	15.9 ± 13.86	14.6 ± 9.54
Dose 2	C_{max} (ng/mL)	-	42.2 ± 12.85	1.54 ± 0.686	6.23 ± 3.599	-	48.3 ± 22.71	2.35 ± 0.640	3.61 ± 1.616
	$AUC_{0\text{-}1\ day}$ (ng*day/mL)	-	22.9 ± 7.70	1.2 ± 0.53	4.3 ± 1.52	-	24.3 ± 12.55	2.0 ± 0.57	2.7 ± 1.24
	$AUC_{0\text{-}\infty}$ (ng*day/mL)	-	74.3 ± 9.98	24.4 ± 15.26	53.6 ± 13.47	-	85.5 ± 19.71	19.2 ± 6.35	50.8 ± 12.50
	$T_{1/2,e}$ (day)	-	22.6 ± 19.55	54.1 ± 78.49	25.0 ± 23.70	-	13.7 ± 11.01	6.7 ± 2.71	22.4 ± 15.00
Dose 3	C_{max} (ng/mL)	-	35.3 ± 20.59	1.26 ± 0.895	4.94 ± 1.071	-	35.7 ± 22.81	1.95 ± 1.142	3.81 ± 0.883
	$AUC_{0\text{-}1\ day}$ (ng*day/mL)	-	18.6 ± 9.33	1.0 ± 0.68	3.5 ± 0.81	-	20.6 ± 13.97	1.6 ± 0.95	3.0 ± 0.52
	$AUC_{0\text{-}\infty}$ (ng*day/mL)	-	85.7 ± 29.2	13.6 ± 4.11	46.2 ± 8.70	-	71.5 ± 26.67	16.7 ± 5.56	48.1 ± 6.26
	$T_{1/2,e}$ (day)	-	60.1 ± 84.92	18.5 ± 13.29	20.9 ± 14.88	-	8.6 ± 3.57	12.7 ± 11.67	14.1 ± 3.26

- The acceptable LOAEL for the systemic safety is 18.75 mg and there is no NOAEL/LOAEL for the local safety. The exposure margin is listed below:

	Testing Drugs	Dose	Based on AUC_{0-24}	Based on $AUC_{0-\infty}$
Main Study	**FX006**	6.25 mg	1.2X	0.5X
		18.75 mg	4.1X	1.8X
Subset Study	**FX006**	6.25 mg	1.9X	0.7X
		18.75 mg	3.6X	1.9X

Based on the human PK data (Study Number: FX006-2015-009)

Methods

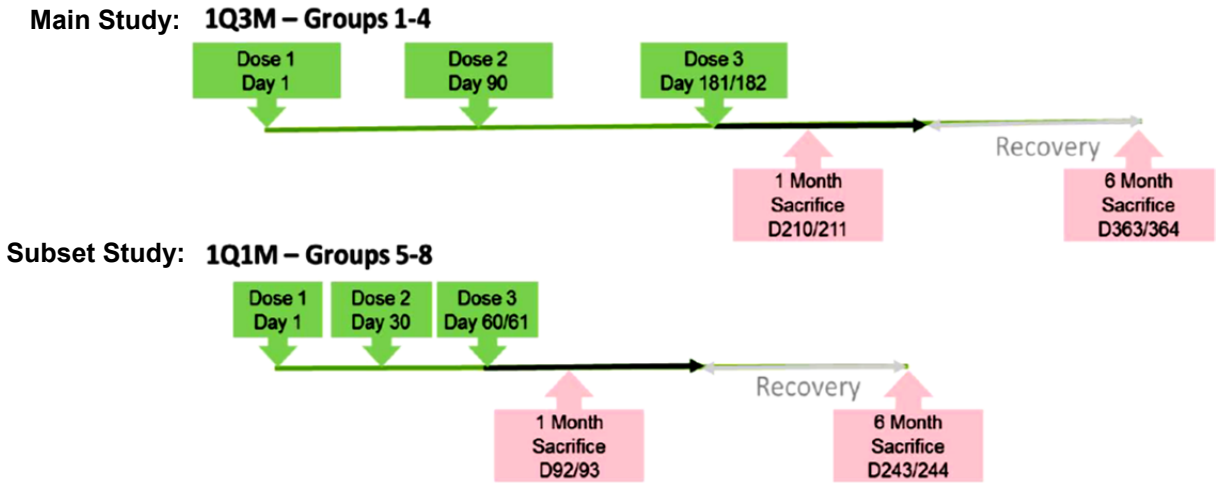
Doses:	0, 6.25, and 18.75 mg/mL of TCA
Frequency of dosing:	Main Study Group 1 to 4 – Day 1, 90, and 181/182 (3 doses, 3 months apart) Subset Study Group 5 to 8 – Day 1, 30, and 60/61 (3 doses, 1 months a part)
Route of administration:	Intra-articular injection into the right and/or left knee joint
Dose volume:	1.0 mL/knee
Formulation/Vehicle:	Diluent (sterile isotonic aqueous solution containing 0.9% sodium chloride, 0.5% carboxymethylcellulose, and 0.1% polysorbate 80)
Species/Strain:	Beagle dog
Number/Sex/Group:	6 males and 6 females per group
Age:	12-14 months old at start of dosing
Weight:	8.1-12.5 kg (males) and 5.7-8.2 kg (females) at the outset (Day 1) of the study
Satellite groups:	Subset study groups of shorter dosing intervals (1 month interval between each dosing of three 40 mg TCA injections) Both main and subset study group animals had a 6-month recovery period. TK evaluations were from the same animals in the main and subset study groups – no additional animals were included for TK.
Unique study design:	The positive control (Kenalog-40; 40 mg/mL) was diluted in saline (0.9% sodium chloride, USP) to obtain a dosing concentration of 18.75 mg/mL.
Deviation from study protocol:	There were no deviations which impact the study results significantly. Some notable deviations are listed below:

Group 1 dogs #0002, #0014 and #0068 were inadvertently dosed on Day 1 in the right knee with 52.3 mg/ml of microspheres and dog #0057 was dosed with 133 mg/ml of microspheres instead of 75 mg/ml of microspheres. This deviation was considered to have insignificant impact on the interpretation of the data, since the Day 90 and Day 180 doses were administered correctly, the affected animals were in the control group and the deviation was considered during the histopathological evaluations.

At necropsy on Day 92 the synovial fluid evaluation slides (200 cell count) for the left knees of Group 5 and 6 animals #0003, 0004, 0010, 0005, 0019 and 0027 were inadvertently not collected. Since the missed slides pertained to only a small number of animals and represent only a small fraction of the total number of parameters and values collected and evaluated over the course of the study, this deviation was considered to have not impacted the interpretation of the overall study outcome.

During Day 60 dose administration animal #0053 (Group 5 female) received a dose of 0.7 ml in the right and left knee and animal #0059 (Group 7 female) received 0.9 ml in the right knee. This deviation had minimal impact on the study, since an attempt was made at the time of dosing to administer the entire 1 ml and the pathology and toxicokinetics PIs were aware of this event.

Justification for dose selection:
The maximum dose (18.75 mg of TCA) was originally selected from the maximal workable concentration of microspheres in suspension for injection, 75 mg/mL, where homogeneity can be maintained and the injection delivered through a needle of reasonable gauge for IA administration. The Group 3 dose (6.25 mg, LD) is equivalent to a 20 mg TCA dose in patient and the Group 4 dose (18.75 mg, HD – 37.5 mg/m^2 based on a body surface area) is equivalent to the maximum proposed FX006 (60 mg – 37 mg/m^2 based on a body surface area) TCA dose in clinical studies. Dosing frequency in the main study mimics the proposed clinical dosing regimen.



Main Study (dosed by intra articular injection on Days 1, 90 and 181/182)

Group	Concentration Microspheres (TCA) (mg/ml)	Dose Volume (ml/knee)	Number of Animals*	
			Male	Female
1. Blank microspheres (right knee) Diluent (left knee)	75 (0 TCA) 0 (0 TCA)	1 1	6	6
2. TCA IR suspension (right knee)	0 (18.75 TCA)	1	6	6
3. FX006 microspheres Low Dose (right knee)	25 (6.25 TCA)	1	6	6
4. FX006 microspheres High Dose (right knee)	75 (18.75 TCA)	1	6	6

* Animals were dosed via the intra articular route into the right and/or left knee joint on Days 1, 90 (3 months) and 181/182 (6 months) – 3 doses 3 months apart. A subset of 3 animals/sex/group were sacrificed I month after 3rd and final dose (Day 210 males/Day 211 females) and after 6 months recovery from 3rd and final dose (Day 363 males/Day 364 females).

Subset Study (dosed by intra articular injection on Days 1, 30 and 60/61)

Group	Concentration Microspheres (TCA) (mg/ml)	Dose Volume (ml/knee)	Number of Animals**	
			Male	Female
5. Blank microspheres (right knee) Blank microspheres (left knee)	75 (0 TCA) 25 (0 TCA)	1 1	6	6
6. TCA IR suspension (right knee) Diluent (left knee)	0 (18.75 TCA) 0 (0 TCA)	1 1	6	6
7. FX006 microspheres Low Dose (right knee)	25 (6.25 TCA)	1	6	6
8. FX006 microspheres High Dose (right knee)	75 (18.75 TCA)	1	6	6

** Animals were dosed via the intra articular route into the right and/or left knee joint on Days 1, 30 (1 month) and 60/61 (2 months) – 3 doses 1 month apart.
A subset of 3 animals/sex/group was sacrificed 1 month after 3rd and final dose (Day 92 males/Day 93 females and after 6 months recovery from 3rd and final dose (Day 243 males/Day 244 females).

Observation and Results

Parameters Measured

Clinical Findings

Animals were observed for mortality/morbidity twice daily (a.m. and p.m.) and once prior to scheduled sacrifices.

Each animal observed for evidence of death or impending death as per Calvert SOP VET-14.

Clinical observations were recorded prior to dose administration, at approximately 1-2 hours post-dose, and additionally as needed. On non-dosing days, animals were observed once daily.

Animals were also observed once prior to scheduled sacrifice.

Body weights

Body weights were recorded at the time of randomization/selection, prior to dose administration on Day 1 and weekly thereafter.

A fasted terminal body weight was recorded prior to scheduled sacrifice.

Body weights were also recorded prior to each additional dose administration, but not reported.

Food consumption

Full feeder weights and/or feeder weigh backs were recorded daily for determination of food consumption.

Hematology

Parameters Analyzed:

Hematology Parameters	
Red Blood Cell Count (RBC) and Morphology	Platelet count (PLT)
White Blood Cell Count (WBC)*	Hematocrit (HCT)
Mean Corpuscular Hemoglobin (MCH)	Hemoglobin (HGB)
Mean Corpuscular Hemoglobin Concentration (MCHC)	Reticulocyte Count (Retic)
Mean Corpuscular Volume (MCV)	

*Total and differential white blood cell counts, including neutrophils, basophils, eosinophils, monocytes, lymphocytes and large unstained cells.

Parameters Analyzed:

Coagulation Parameters	
Activated Partial Thromboplastin Time (APTT)	Prothrombin Time (PT)

Clinical chemistry

Parameters Analyzed:

Clinical Chemistry Parameters	
Alanine Aminotransferase (ALT)	Globulin (calculated)(GLOB)
Albumin (ALB)	Glucose (GLU)
Albumin/Globulin ratio (calculated)(A/G)	Phosphorus (PHOS)
Alkaline Phosphatase (ALP)	Potassium (K)
Aspartate Aminotransferase (AST)	Sodium (NA)
Calcium (CA)	Total Bilirubin (T-BIL)
Chloride (CL)	Total Protein (TP)
Cholesterol (CHOL)	Triglycerides (TRIG)
Creatinine (CREAT)	Urea Nitrogen (BUN)

Urinalysis

Parameters Analyzed:

Urinalysis Parameters	
Specific gravity	Appearance/Color
pH	Bilirubin
Protein	Blood
Glucose	Leukocytes
Ketone	Microscopic examination of spun deposit
Urobilinogen	Nitrite

Gross pathology

A complete gross necropsy was performed on all animals that were sacrificed during the study. The necropsy included examination of:

- the external body surface
- all orifices
- the cranial, thoracic and abdominal cavities and their contents.

All abnormalities were described completely and recorded.

Organ weights

Organs Weighed	
Adrenals	Testes
Brain	Ovaries
Heart	Spleen
Kidneys	Thyroids/Parathyroids
Liver	Popliteal Lymph Node
Thymus	

Histopathology

Adequate Battery: yes (X), no ( )
Note that an adequate battery of organs was collected but selected tissues were evaluated microscopically. Since a clinical BA study demonstrated that the systemic exposure to TCA following administration of Zilretta is within the reference product, the systemic safety of TCA has been justified and therefore the focus of this study is the local effects of the Zilretta. Nevertheless, TCA is a well-characterized corticosteroid and the target tissues are well known. It appears that the selected tissues were selected with this in mind and therefore, the organs selected for microscopic evaluations (adrenal glands, brain, femur, liver, kidney, knee joint synovium, lymph nodes, patella, pituitary, proximal tibia, sternum with bone marrow, spleen, thymus, thyroid, and organs with gross findings) are considered acceptable.
Peer review: yes (X), no ( )

Other

Synovial fluid samples were collected to measure TCA concentration in the synovial fluid of the knees.

Mortality

No mortality/morbidity was observed during the study. All animals survived until scheduled euthanization.

Clinical Signs

No test article-related clinical signs were observed. Occasional soft feces were observed but these signs did not appear to be test article-related.

Clinical Signs		
Dose Group	**Males**	**Females**
Group 1	N/A	N/A
Group 2	• Emesis (Days 314-315)	• Elevated temperature of 100.0 °C – unknown cause but no other symptoms
Group 3	• Favored the right hind leg (Days 314-317 & 321-234) – no signs of local swelling, redness, or tenderness but from gross necropsy at Day 363, abundant clear yellow fluid in the right synovium and a thickened, joint capsule anterior cruciate ligament and meniscus were observed.	N/A
Group 4	• A red sclera (Days 91-92) – resolved with an ophthalmic ointment treatment (Day 93)	• An inflamed conjunctivas at the right eye (Day 29) – completely cleared with an ophthalmic ointment treatment by Day 34 • An incidental lesion on the tongue (Day 132) – treated with antibiotics and vitamin B supplements and recovered by Day 150 • Decreased activity, salvation and/or a rough hair coat (Days 132-141)
Group 5	N/A	N/A
Group 6	• Areas of red skin discoloration at the injection sites (Day 30) and a small amount of hair loss at the sites (Days 47-48) • A patch of hair loss on the back (Day 133), no ulceration noted.	N/A
Group 7	• An interdigital cyst between digit 3 and 4 with mild swelling and redness in the interdigital space (Day 188), no criptus or swelling noted – recovered by Day 191	• A large amount of brown emesis with food particles (Day 202)
Group 8	• A mild seizure (Day 30), possibly due to a reaction to Ketamine during anesthesia • Favored the left forepaw due to a raised white mass of webbing on the ventral surface between the middle two toes of the left front paw (Days 129-132) – an interstitial wart	N/A

Body Weights

No test article-related body weight changes were observed. There were some changes in body weight (~10%) but they do not appear to be test article-related.

Food Consumption

No test article-related food consumption changes were observed. Both males and females in all groups showed dramatic food consumption reduction (more than 60%) at the second dosing (D90). It was not shown at the third dosing (D181/182). For the subset study, similar findings were observed at the first dosing (D30) but higher fluctuations in food consumption were observed in general.

Ophthalmoscopy

No ophthalmoscopy parameters were monitored in this study.

ECG

No ECG parameters were evaluated in this study.

Hematology

Main Study (Group 1 to 4): Reductions in lymphocytes were observed in TCA-treated animals (Group 2 to 4) at Day 210 but recovered by Day 363. Reductions in WBC were observed in both LD or HD of PLGA-TCA treated males (Group 3 and 4) at Day 363, after a 6-month recovery period. However, considering a lower level and a large variation at predose Day 9 for Group 3 and 4, respectively, compared to the control, it is not considered to be toxicologically significant. Increases in reticulocytes were observed in HD TCA treated males (18.75 mg, Group 2 and 4). Considering large variations at predose, Day 210, and Day 363, these changes were not considered toxicologically significant. Reductions in eosinophil were observed in TCA-treated females (Group 2 to 4) at Day 211 but recovered by Day 364. Increases in platelets were observed in TCA-treated females (Group 2 to 4) at Day 364, after a 6-month recovery period. Considering large variations in all groups at all time points, these changes were not considered toxicologically significant.

Male		Group 1	Group 2	Group 3	Group 4
Lymphocytes ($x10^3$/mcL)	**Predose Day 9**	2.77 ± 0.55	2.25 ± 0.52	2.59 ± 0.70	2.42 ± 0.48
	Day 210	2.42 ± 0.60	**1.14# ± 0.23**	**1.68 ± 0.23**	**1.45* ± 0.26**
	Day 363	2.78 ± 0.36	2.14 ± 0.48	1.87 ± 0.12	2.12 ± 0.39
WBC ($x10^3$/mcL)	**Predose Day 9**	12.03 ± 1.62	9.49 ± 1.59	9.77 ± 1.25	11.22 ± 4.02
	Day 210	12.27 ± 6.06	7.923 ± 2.06	7.507 ± 2.16	9.71 ± 4.46
	Day 363	9.29 ± 0.85	8.38 ± 0.78	**7.03* ± 1.19**	**6.98* ± 0.74**

Reticulocytes ($x10^9$/L)	**Predose Day 9**	33.57 ± 18.25	36.38 ± 21.06	47.88 ± 15.59	33.60 ± 9.44
	Day 210	53.60 ± 9.82	49.53 ± 15.61	37.07 ± 15.21	32.53 ± 10.96
	Day 363	27.80 ± 6.80	**55.00 ± 17.46**	33.63 ± 2.26	**59.30* ± 13.56**
Female		**Group 1**	**Group 2**	**Group 3**	**Group 4**
Lymphocytes ($x10^3$/mcL)	**Predose Day 9**	2.52 ± 0.42	2.67 ± 0.59	2.44 ± 0.73	2.67 ± 0.65
	Day 211	2.30 ± 0.50	1.74 ± 0.21	1.98 ± 0.21	**1.30* ± 0.42**
	Day 364	2.31 ± 0.31	2.12 ± 0.39	1.97 ± 0.42	2.61 ± 0.75
Eosinophil ($x10^3$/mcL)	**Predose Day 9**	0.27 ± 0.17	0.19 ± 0.13	0.16 ± 0.047	**0.32** ± 0.28
	Day 211	0.56 ± 0.01	**0.063# ± 0.035**	**0.25# ± 0.089**	**0.047# ± 0.015**
	Day 364	0.31 ± 0.04	0.263 ± 0.065	0.223 ± 0.023	0.237 ± 0.15
Platelet ($x10^3$/mcL)	**Predose Day 9**	298.3 ± 63.82	325.3 ± 21.44	361.8 ± 57.08	321.2 ± 48.61
	Day 211	339.0 ± 92.70	353.0 ± 19.70	416.3 ± 40.80	357.7 ± 70.12
	Day 364	221.3 ± 30.89	**309.3* ± 23.86**	**298.7* ± 34.27**	**291.3 ± 34.70**

*p<0.05, #p<0.01

No red blood cell morphology changes were observed. Statistically significant reductions (~8%) in prothrombin time were observed in females (Group 3 and 4) on Day 364.

Subset Study (Group 5 to 8): Similar to the main study, slight reductions in lymphocytes were observed in animals treated with TCA (Group 2 to 4) at Day 92/93 and these changes appear to be reversible by Day 243/244. WBC levels were reduced in females treated with HD (Group 8) and it appears to be reversible by Day 244. There were increases in reticulocytes in Group 6 to 8 at Day 243. However, they were not considered to be toxicologically significant because of the reversibility during the recovery period (lymphocytes and WBC) or large standard deviations (reticulocytes).

Male		**Group 5**	**Group 6**	**Group 7**	**Group 8**
Lymphocytes ($x10^3$/mcL)	**Predose Day 9**	2.70 ± 0.35	2.14 ± 0.67	2.52 ± 0.79	2.72 ± 1.24
	Day 92	2.19 ± 0.51	**1.35* ± 0.14**	**1.83 ± 0.37**	**1.41 ± 0.16**
	Day 243	2.96 ± 0.085	2.11 ± 0.82	2.31 ± 0.91	2.92 ± 1.35
WBC ($x10^3$/mcL)	**Predose Day 9**	9.73 ± 1.30	9.89 ± 2.22	11.76 ± 2.78	9.53 ± 1.22
	Day 92	11.88 ± 2.32	**7.10# ± 1.33**	8.84 ± 0.70	9.47 ± 0.50
	Day 243	10.46 ± 1.56	11.38 ± 3.41	9.26 ± 2.94	10.15 ± 2.54

Reticulocytes ($x10^9$/L)	**Predose Day 9**	36.68 ± 14.16	46.58 ± 26.05	52.62 ± 21.61	41.67 ± 7.26
	Day 92	33.27 ± 21.51	46.10 ± 23.78	25.53 ± 6.95	28.5 ± 11.391
	Day 243	49.0 ± 16.97	**94.97 ± 41.94**	**111.83 ± 49.30**	**80.77 ± 22.44**
Female		**Group 5**	**Group 6**	**Group 7**	**Group 8**
Lymphocytes ($x10^3$/mcL)	**Predose Day 9**	2.48 ± 0.60	2.33 ± 0.59	2.31 ± 0.54	2.43 ± 0.64
	Day 93	2.25 ± 0.74	**1.58 ± 0.15**	2.20 ± 0.33	**1.64 ± 0.16**
	Day 244	2.95 ± 0.52	1.71 ± 0.62	1.97 ± 0.0058	2.44 ± 0.19
Eosinophil ($x10^3$/mcL)	**Predose Day 9**	0.22 ± 0.15	0.24 ± 0.087	0.18 ± 0.10	0.477 ± 0.86
	Day 93	0.23 ± 0.076	**0.133 ± 0.15**	0.17 ± 0.092	**0.037 ± 0.058**
	Day 244	0.45 ± 0.12	0.21 ± 0.076	0.33 ± 0.14	0.33 ± 0.12

*p<0.05, #p<0.01

No red blood cell morphology changes were observed. Statistically significant reductions (~10%) in prothrombin time were observed in TCA-treated males at Day 244. All values were within normal historical control range (PT: 6.8-8.4 (male) and 6.8-8.4 (female); APTT 9.6-11.8 (male) and 9.8-12.0 (female)). Therefore, these changes are not considered to be toxicologically significant.

Clinical Chemistry

Main Study (Group 1 to 4): Reduced creatinine levels were observed in Group 2 and 4 at Day 210/211 but these changes were reversible by Day 363/364. Increased albumin levels were observed in Group 2 and 4 at Day 210/211 and these changes were reversible by Day 363/364. Changes in potassium and total protein levels were observed in males and females, respectively, at Day 363/364. Therefore, these changes are not considered to be toxicologically significant.

Male		**Group 1**	**Group 2**	**Group 3**	**Group 4**
Creatinine (mg/dL)	**Predose Day 9**	0.788 ± 0.1232	0.795 ± 0.0774	0.845 ± 0.0853	0.810 ± 0.1723
	Day 210	0.933 ± 0.0416	**0.697# ± 0.0643**	0.820 ± 0.0500	**0.730* ± 0.0985**
	Day 363	0.870 ± 0.0500	0.857 ± 0.1185	0.857 ± 0.0929	0.827 ± 0.0416
K (mEq/L)	**Predose Day 9**	4.28 ± 0.172	4.33 ± 0.225	4.37 ± 0.273	4.23 ± 0.393
	Day 210	4.33 ± 0.153	4.30 ± 0.529	4.27 ± 0.058	4.47 ± 0.058
	Day 363	4.33 ± 0.058	4.33 ± 0.115	**4.63* ± 0.208**	**4.63* ± 0.058**

Female		Group 1	Group 2	Group 3	Group 4
Creatinine (mg/dL)	**Predose Day 9**	0.730 ± 0.0341	0.760 ± 0.1122	0.847 ± 0.0671	0.773 ± 0.0739
	Day 211	0.727 ± 0.0153	0.670 ± 0.0889	0.753 ± 0.0208	**0.553* ± 0.0907**
	Day 364	0.697 ± 0.0306	0.690 ± 0.1253	0.740 ± 0.0755	0.737 ± 0.0321
Albumin (g/dL)	**Predose Day 9**	3.13 ± 0.186	3.18 ± 0.117	3.20 ± 0.126	3.00 ± 0.200
	Day 211	3.37 ± 0.115	**3.63* ± 0.153**	3.17 ± 0.058	**3.53 ± 0.058**
	Day 364	3.07 ± 0.252	3.00 ± 0.000	3.13 ± 0.058	3.23 ± 0.058
Total Protein (g/dL)	**Predose Day 9**	5.68 ± 0.240	5.93 ± 0.273	5.85 ± 0.362	5.52 ± 0.417
	Day 211	5.70 ± 0.346	5.87 ± 0.058	5.60 ± 0.173	6.03 ± 0.208
	Day 364	5.23 ± 0.208	5.23 ± 0.058	5.50 ± 0.100	**5.63* ± 0.208**

*p<0.05, #p<0.01

Subset Study (Group 5 to 8): Similar to the main study, statistically significant reductions in creatinine levels were observed in animals treated with TCA (Group 2 to 4) at Day 92/93 and these changes appear to be reversible. High ALP levels (about 2-fold) were observed in animals of Group 8 but these changes appear to be reversible by Day 243. There were no associated microscopic findings such as necrosis; therefore, it was not considered to be toxicologically significant.

Male		Group 5	Group 6	Group 7	Group 8
Creatinine (mg/dL)	**Predose Day 9**	0.737 ± 0.0674	0.745 ± 0.0612	0.727 ± 0.0776	0.680 ± 0.0645
	Day 92	0.867 ± 0.0404	**0.643# ± 0.0153**	0.747 ± 0.1159	**0.550# ± 0.050**
	Day 243	0.883 ± 0.0635	0.783 ± 0.085	0.897 ± 0.0808	0.853 ± 0.1106
ALP (U/L)	**Predose Day 9**	49.0 ± 19.68	41.2 ± 13.99	50.2 ± 18.15	54.5 ± 19.04
	Day 92	71.0 ± 8.89	72.3 ± 11.24	53.0 ± 29.61	172.7 ± 93.52
	Day 243	37.7 ± 13.43	70.3 ± 44.52	55.3 ± 17.93	42.7 ± 11.93
K (mEq/L)	**Predose Day 9**	4.37 ± 0.137	4.38 ± 0.147	4.27 ± 0.121	4.45 ± 0.197
	Day 92	3.97 ± 0.231	**4.57* ± 0.153**	4.07 ± 0.115	4.50 ± 0.361
	Day 243	4.33 ± 0.379	4.63 ± 0.252	4.40 ± 0.520	4.53 ± 0.153

*p<0.05, #p<0.01

Female		Group 5	Group 6	Group 7	Group 8
Creatinine (mg/dL)	Predose Day 9	0.773 ± 0.077	0.758 ± 0.0902	0.792 ± 0.0999	0.757 ± 0.0350
	Day 93	0.770 ± 0.0985	**0.557* ± 0.0737**	0.673 ± 0.1002	**0.497# ± 0.0058**
	Day 244	0.813 ± 0.0862	0.703 ± 0.0153	0.850 ± 0.0173	0.687 ± 0.0709
TBIL (mg/dL)	Predose Day 9	0.142 ± 0.0183	0.140 ± 0.0141	0.142 ± 0.0382	0.135 ± 0.0122
	Day 93	0.160 ± 0.020	**0.110* ± 0.173**	**0.113* ± 0.0231**	0.147 ± 0.0153
	Day 244	0.103 ± 0.208	0.140 ± 0.0173	0.163 ± 0.0451	0.110 ± 0.0173
ALP (U/L)	Predose Day 9	60.8 ± 16.81	67.7 ± 29.7	72.5 ± 25.82	59.3 ± 22.25
	Day 93	84.3 ± 49.14	61.7 ± 11.06	44.7 ± 12.58	**113.0 ± 4.58**
	Day 244	42.7 ± 13.58	57.7 ± 10.12	49.3 ± 9.61	43.3 ± 12.10

*p<0.05, #p<0.01

Urinalysis

No test article-related changes in urinalysis parameters were observed.

Synovial Fluid

Main Study (Group 1 to 4): Blood urea nitrogen (BUN) and WBC levels in synovial fluid of the treated right knee were lower in animals treated with the test article (Group 2 to 4) at Day 210/211 but these changes appear to be reversible at Day 363/364. As note, for certain samples, insufficient numbers of samples (N=1 or 2) were analyzed (see the table below). There were no noticeable test article-related changes in synovial fluid of the left knee.

Male			Group 1	Group 2	Group 3	Group 4
Right Knee	BUN (mg/dl)	Day 210	11.7 ± 2.9	4.0 (n=1)	5.3 ± 1.5	5.0 ± 1.4 (n=2)
		Day 363	10.0 ± 3.6	6.3 ± 3.1	10.0 ± 3.5	9.3 ± 2.5
	WBC ($x10^3$/mcL)	Day 210	0.10 ± 0.05	0.13 ± 0.025	0.058 ± 0.038	0.075 ± 0.066
		Day 363	0.050 ± 0.00	0.033 ± 0.014	0.15 ± 0.087	0.13 ± 0.025
Left Knee	BUN (mg/dl)	Day 210	8.7 ± 1.53	9.7 ± 4.73	6.7 ± 1.15	5.3 ± 0.58
		Day 363	9.7 ± 1.53	5.7 ± 0.58	11.0 ± 3.61	8.7 ± 2.08
	WBC ($x10^3$/mcL)	Day 210	0.092 ± 0.014	na	na	na
		Day 363	0.033 ± 0.014	na	na	na

N=3 if not noted. na (not available)

Female			Group 1	Group 2	Group 3	Group 4
Right Knee	BUN (mg/dl)	Day 211	12.7 ± 1.2	7.0 (n=1)	2.0 (n=1)	1.5 ± 0.7 (n=2)
		Day 364	9.3 ± 3.8	11.3 ± 4.0	12.3 ± 2.5	9.7 ± 1.2
	WBC ($x10^3$/mcL)	Day 211	0.125 ± 0.075	0.058 ± 0.0144	0.075 ± 0.0000	0.083 ± 0.1010
		Day 364	0.033 ± 0.014	0.067 ± 0.0382	0.100 ± 0.0433	0.075 ± 0.0433
Left Knee	BUN (mg/dl)	Day 211	7.0 ± 2.65	7.7 ± 3.06	6.0 ± 1.73	7.7 ± 3.51
		Day 364	10.0 ± 2.65	10.3 ± 3.06	9.0 ± 1.00	12.3 ± 1.53
	WBC ($x10^3$/mcL)	Day 211	0.067 ± 0.038	na	na	na
		Day 364	0.03 ± 0.0000	na	na	na

N=3 if not noted. na (not available)

Subset Study (Group 5 to 8): Similar to the main study, BUN and WBC levels in synovial fluid of the treated right knee were lower in animals treated with the test article (Group 2 to 4) at Day 92/93 but these changes appear to be reversible at Day 243/244. As note, for certain samples, insufficient numbers of samples (N=1 or 2) were analyzed (see the table below).

Male			Group 5	Group 6	Group 7	Group 8
Right Knee	BUN (mg/dl)	Day 92	12.7 ± 5.5	10.0 ± 0.0 (n=2)	7.3 ± 0.58	5.0 ± 0.00 (n=1)
		Day 243	9.7 ± 2.31	8.0 ± 2.83 (n=2)	13.3 ± 2.52	7.0 ± 1.41 (n=2)
	WBC ($x10^3$/mcL)	Day 92	0.37 ± 0.28	0.17 ± 0.116	1.18 ± 1.59	0.16 ± 0.014
		Day 243	0.108 ± 0.058	0.058 ± 0.014	0.092 ± 0.014	0.142 ± 0.14
Left Knee	BUN (mg/dl)	Day 92	12.7 ± 4.93	7.3 ± 0.58	9.7 ± 3.06	8.0 ± 2.65
		Day 243	11.0 ± 0.0	9.0 ± 2.0	7.3 ± 0.58	8.7 ± 3.51
	WBC ($x10^3$/mcL)	Day 92	na	na	na	na
		Day 243	0.042 ± 0.029	0.108 ± 0.038	na	na
Female			**Group 5**	**Group 6**	**Group 7**	**Group 8**
Right Knee	BUN (mg/dl)	Day 93	15.7 ± 3.5	11.5 ± 3.5 (n=2)	7.0 ± 0.0 (n=2)	5.0 ± 1.73
		Day 244	11.0 ± 1.73	12.0 ± 0.0 (n=1)	13.7 ± 2.08	5.5 ± 4.95 (n=5)
	WBC ($x10^3$/mcL)	Day 93	0.27 ± 0.076	0.33 ± 0.26	0.39 ± 0.35	0.32 ± 0.23
		Day 244	0.108 ± 0.08	0.138 ± 0.12	0.133 ± 0.014	0.10 ± 0.05

Left Knee	**BUN (mg/dl)**	**Day 93**	10.7 ± 0.58	8.7 ± 2.89	8.0 ± 3.61	6.7 ± 1.15
		Day 244	8.7 ± 1.15	11.7 ± 4.16	9.7 ± 1.53	9.7 ± 3.21
	WBC ($x10^3$/mcL)	**Day 93**	0.32 ± 0.19	0.067 ± 0.14	na	na
		Day 244	0.13 ± 0.11	0.125 ± 0.09	na	na

N=3 if not noted. na (not available)

Gross Pathology

No test article-related gross findings were observed.

Main Study (Group 1 to 4): A few animals (two females in Group 1 and one female in Group 3) had either the right or left ovary as being larger than the contralateral ovary. The enlargement of the one side of ovaries was due to the presence of more corpora lutea as a normal estrous cycle variation or due to the presence of a cyst of the left ovary. Therefore, they do not appear to be related to the test article treatment.

Subset Study (Group 5 to 8): One male (Animal Number 0005) had enlarged right kidney and one adrenal gland in Group 6 at Day 92. No other gross findings were observed.

Organ Weights

Main Study (Group 1 to 4): Liver weights were increased in animals treated with HD of TCA (Group 2 and 4) but these changes appear to be reversible by Day 363 in males. Slight increases in spleen and thymus weights were observed in animals of Group 3 and 4. Considering large variations in the control and treatment groups and reversibility by the end of recovery period, it was not considered to be toxicologically significant.

Male (% body weight)		**Group 1**	**Group 2**	**Group 2**	**Group 4**
Liver	**Day 210**	2.36 ± 0.391	**4.23* ± 0.656**	2.51 ± 0.112	3.07 ± 0.443
	Day 363	2.37 ± 0.158	2.32 ± 0.301	2.44 ± 0.279	2.50 ± 0.138
Spleen	**Day 210**	0.85 ± 0.320	0.66 ± 0.158	**1.20 ± 0.184**	**1.16 ± 0.316**
	Day 363	0.88 ± 0.156	0.92 ± 0.347	1.04 ± 0.570	1.13 ± 0.324
Female (% body weight)		**Group 1**	**Group 2**	**Group 2**	**Group 4**
Liver	**Day 211**	2.67 ± 0.169	3.00 ± 0.501	2.37 ± 0.684	3.42 ± 0.422
	Day 364	2.23 ± 0.097	**2.96# ± 0.308**	2.62 ± 0.194	**2.82* ± 0.153**
Spleen	**Day 211**	0.62 ± 0.190	0.95 ± 0.223	0.73 ± 0.100	0.96 ± 0.368
	Day 364	1.04 ± 0.620	1.01 ± 0.275	0.82 ± 0.494	0.69 ± 0.187
Thymus	**Day 211**	0.13 ± 0.037	0.15 ± 0.016	0.15 ± 0.047	**0.22 ± 0.055**
	Day 364	0.16 ± 0.040	0.15 ± 0.064	0.16 ± 0.028	0.16 ± 0.037

N=3, *$p<0.05$, #$p<0.01$

Subset Study (Group 5 to 8): Liver weights were increased in males treated with HD of TCA (Group 6 and 8) but these changes appear to be reversible by Day 243/244.

Male (% body weight)		Group 5	Group 6	Group 7	Group 8
Liver	Day 92	2.87 ± 0.39	**3.76* ± 0.47**	3.01 ± 0.19	**3.58 ± 0.26**
	Day 243	2.51 ± 0.12	2.79 ± 0.43	2.76 ± 0.18	2.36 ± 0.18
Spleen	Day 92	0.51 ± 0.24	0.74 ± 0.25	**1.41# ± 0.086**	0.78 ± 0.089
	Day 243	0.99 ± 0.35	0.90 ± 0.29	0.82 ± 0.22	0.91 ± 0.049
Female (% body weight)		**Group 5**	**Group 6**	**Group 7**	**Group 8**
Liver	Day 93	2.82 ± 0.52	2.63 ± 0.29	2.79 ± 0.28	**3.95# ± 0.15**
	Day 244	2.58 ± 0.279	3.42 ± 0.71	2.76 ± 0.097	3.05 ± 0.17
Spleen	Day 93	0.80 ± 0.41	0.90 ± 0.19	**1.20 ± 0.19**	0.74 ± 0.26
	Day 244	0.79 ± 0.082	0.74 ± 0.45	0.95 ± 0.39	1.00 ± 0.61

N=3, *p<0.05, #p<0.01

Histopathology

Adequate Battery

Tissues Collected	
Cardiovascular	**Urogenital**
Aorta	Kidneys *
Heart	Urinary Bladder
Digestive	Ovaries
Salivary gland(s)	Uterus
Tongue	Cervix
Esophagus	Vagina
Stomach	Testes
Small Intestine	Epididymides
Duodenum	Prostate
Jejunum	**Endocrine**
Ileum	Adrenals *
Large Intestine	Pituitary *
Cecum	Thyroid/Parathyroid *
Colon	**Skin/Musculoskeletal**
Rectum	Skin
Pancreas	Mammary Gland
Liver *	Skeletal Muscle (thigh)
Gallbladder	Femur (distal) with Articular Surface * a)

Respiratory	Proximal Tibia *
Trachea	Knee joint synovium from each synovial sac (suprapatellar synovium from the femoropatellar sac, the left femorotibial sac, and the right femorotibial sac) *
Larynx	Meniscus b)
Lung with mainstem bronchus	Patella *
Lymphoid/Hematopoietic	**Nervous/Special Sense**
Sternum with bone marrow *	Eye with optic nerve
Thymus *	Sciatic Nerve
Spleen *	Brain *
Lymph Nodes *	Spinal Cord – cervical
Mandibular *	Spinal Cord – midthoracic
Mesenteric *	Spinal Cord – lumbar
Popliteal *	Lacrimal Glands
Inguinal *	**Other**
	Unique Animal Identifier b)
	Gross Findings *
	Bone Marrow Smears b)

* Tissues evaluated microscopically

a) Following collection of the synovial fluid, both the right and left knee joint(s) of each animal were opened by initially removing the skin overlying the joint then incising through the joint capsule, patellar tendon, and medial and lateral collateral ligaments. Once the knee joint space was entered, the cruciate ligaments were transected and the femur was separated from the tibia (disarticulation). Subsequently, the menisci were removed from the tibial articular surface. Throughout the dissection, care was taken to prevent damage to articular surfaces. The synovium and articular surfaces were carefully examined and any abnormalities were documented. Additionally, each knee joint was examined for the presence or absence of residual test material, as appropriate. The distal femur, proximal tibia, patella, and a representative sample of the knee joint synovium from each synovial sac (suprapatellar synovium from the femoropatellar sac, the left femorotibial sac, and the right femorotibial sac) were saved in 10% neutral buffered formalin.

b) Collected but not evaluated.

Peer Review

The pathology report was peer-reviewed and signed by a board certified pathologist.

Histological Findings

Macroscopic Findings

Main Study (Group 1 to 4):

Day	Sex	Animal Number (Group Number)	Necropsy Findings	Corresponding Microscopic Findings
210/211	M	85 (4)	Round/fluid-filled cyst (the medulla of the brain)	Cyst, single, medulla oblongata
	F	95 (1), 92 (3)	A larger ovary than the other side (either right or left)	Presence of more corpora lutea
		100 (1)		Presence of a cyst, unilateral

363/364	M	24 (3)	Right knee (femur erosion of cartilage on media condyle, synovium abundant clear yellow fluid, meniscus and joint capsule thickened, anterior cruciate ligament thickened and frayed)	Moderate articular cartilage degeneration, moderate granulation tissue and synovial hyperplasia, fibrosis of the synovium
	F	71 (4)	Right knee (femur erosion of cartilage on medial trochlear ridge)	Marked degeneration of articular cartilage
		43 (3)	Abundant, clear, yellow fluid in both right and left knee joints	Right knee (minimal fibrosis and slight synovial hyperplasia, loss of cartilage to subchondral bone); Left knee (normal)
		45 (2), 70 (2)	Enlarged one side of ovaries	Corpora lutea, bilateral
		40 (4)	Pituitary cyst	Cyst, single, pars intermedia

Subset Study (Group 5 to 8):

Day	Sex	Animal Number (Group Number)	Findings	Associated Microscopic Findings (animal number)
92/93	M	18 (7)	White discoloration of the right/lateral joint capsule	Moderate fat necrosis, joint capsule
	F	1 (8)	Thinner with multiple red foci in the right meniscus	Minimal degeneration and congestion of the meniscus
		50 (8)	Brown fat discoloration in the right popliteal region	Moderate steatitis
		5 (6)	Left kidney missing, right kidney enlarged, left adrenal missing	right kidney: normal morphology
		53 (5), 44 (7)	Agenesis of one uterine horn	No microscopic findings (53), placentation site (44)

Microscopic Findings

Systemic Organ/Tissue Findings

Main Study (Group 1 to 4): Microscopic findings in tissues listed below were attributable to hypercortisolism from TCA. There were no microscopic findings present in systemic organs/tissues related to IA administration of blank microspheres (Group 1).

- Adrenal gland: Dose-dependent and TCA-related atrophy of the zona fasciculate (a decrease in the width of the zona fasciculate of the adrenal cortex) was observed at Day 210/211. Incidence and intensity of adrenocortical atrophy were decreased at Day 363/364, suggesting the reversibility of this TCA-related adverse effect in the adrenal gland.
- Liver: Dose-dependent and TCA-related cellular swelling of hepatocytes with cytoplasmic rarefaction mostly in midzonal regions of the hepatic lobules and some in periportal and centrilobular zones were observed at Day 210/211. These findings were consistent with the entity of steroid hepatopathy in dogs with

hypercortisolism (Haschek et al., 2010). By Day 363/364, these findings appear to be reversible.

- Lymphoid tissues (thymus, lymph nodes, and spleen): TCA-related lymphoid depletion was observed in the thymus, which is a known effect of corticosteroid (Haschek et al., 2010), at Day 210/211. Lower degree/incidence but TCA-related lymphoid depletion was observed in lymph nodes (mandibular, mesenteric, inguinal, left and right popliteal) and spleen. No lymphoid depletion in the thymus, lymph nodes, and spleen was observed by Day 363/364, a complete reversibility.
- Bone marrow (sternum): Minimal hypocellularity of hematopoietic cell elements in the sternal bone marrow was observed in animals at high dose TCA (Group 2 and 4) at Day 210/211, but these findings were not observed at Day 363/364.

Microscopic Findings of Systemic Organs/Tissues in Main Study (Group 1 to 4)

Tissue (N=3)	Day 210/211								Day 363/364							
	G-1		G-2		G-3		G-4		G-1		G-2		G-3		G-4	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Adrenal Gland																
Atrophy: cortex	-	-	3	3	3	3	3	3	-	-	3	2	2	2	3	3
Grade 2	-	-	-	1	-	-	-	-	-	-	2	1	2	2	3	3
Grade 3	-	-	-	1	2	3	1	1	-	-	-	1	-	-	-	-
Grade 4	-	-	3	1	1	-	2	2	-	-	1	-	-	-	-	-
Vacuolation	-	-	3	2	3	3	3	3	-	1	2	2	2	2	3	3
Grade 1	-	-	1	-	2	2	-	-	-	-	-	1	2	1	-	2
Grade 2	-	-	2	1	1	1	3	2	-	1	2	1	-	1	3	1
Grade 3	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-
Bone Marrow, sternum																
Hypocellularity	-	-	3	-	-	-	-	1	-	-	-	-	-	-	-	-
Grade 1	-	-	3	-	-	-	-	1	-	-	-	-	-	-	-	-
Brain																
Mononuclear infiltr.	-	1	-	-	1	-	-	-	-	-	1	-	-	-	-	-
Grade 1	-	1	-	-	1	-	-	-	-	-	1	-	-	-	-	-
Cyst	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Grade 1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Kidney																
Mononuclear infiltr.	1	2	-	-	-	1	1	-	-	1	-	-	1	-	1	-
Grade 1	1	2	-	-	-	1	1	-	-	1	-	-	1	-	1	-
Tubular basophilia	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Grade 1	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Tubular pigment	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glomerular lipidosis	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-
Grade 1	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-
Fibrosis:cortex	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Ligament (Anterior)																
Degeneration	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Granulation tissue	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Hemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Liver																
Cell swelling: hepat.	-	-	3	2	2	-	3	3	-	-	1	1	-	-	1	-
Grade 1	-	-	1	1	-	-	-	-	-	-	1	1	-	-	1	-
Grade 2	-	-	-	1	2	-	2	3	-	-	-	-	-	-	-	-
Grade 3	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-
Mixed cell infiltr.	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-
Grade 1	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-
Pigment:Kupffer cell	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Grade 1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Lymph Node: Inguinal																
Neutrophil infiltr.	1	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lymphoid depletion*	-	-	3	2	1	1	2	3	-	-	-	-	-	-	-	-
Grade 1	-	-	1	2	1	1	2	1	-	-	-	-	-	-	-	-
Grade 2	-	-	1	-	-	-	-	2	-	-	-	-	-	-	-	-
Grade 3	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Erythrophagocytosis	2	-	-	1	-	-	-	-	1	-	-	-	2	-	1	-
Grade 1	2	-	-	1	-	-	-	-	1	-	-	-	2	-	1	-
Eosinophil infiltr.	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lymph Node: Left Popliteal																
Lymphoid depletion	-	-	3	2	1	1	3	3	-	-	-	-	-	-	-	-
Grade 1	-	-	1	1	1	1	3	-	-	-	-	-	-	-	-	-
Grade 2	-	-	2	-	-	-	-	3	-	-	-	-	-	-	-	-
Grade 3	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Lymphoid hyperplasia	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Grade 2	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Erythrophagocytosis	-	-	-	1	-	-	-	-	-	-	1	-	1	-	1	1
Grade 1	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	1
Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-
Lymph Node: Mandibular																
Lymphoid depletion	-	-	3	2	1	-	2	3	-	-	-	-	-	-	-	-
Grade 1	-	-	1	2	1	-	2	3	-	-	-	-	-	-	-	-
Grade 2	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Erythrophagocytosis	2	-	2	1	1	-	-	1	2	-	-	-	-	1	-	-
Grade 1	1	-	1	-	1	-	-	1	2	-	-	-	-	1	-	-
Grade 2	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 3	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Neutrophil infiltr.	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 2	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Vacuolation	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Gade 2	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-

Lymph Node: Mesenteric																
Lymphoid depletion	-	-	3	3	1	1	3	3	-	-	-	-	-	-	-	-
Grade 1	-	-	1	1	1	1	3	-	-	-	-	-	-	-	-	-
Grade 2	-	-	2	2	-	-	-	3	-	-	-	-	-	-	-	-
Erythrophagocytosis	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3
Grade 1	2	3	1	3	3	3	3	2	2	3	2	3	3	2	3	3
Grade 2	1	-	2	-	-	-	-	1	1	-	-	-	-	1	-	-
Lymphoid hyperplasia	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Grade 1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Lymph Node: Right Popliteal																
Lymphoid depletion	-	-	3	2	1	2	3	3	-	-	-	-	-	-	-	-
Grade 1	-	-	1	1	1	2	3	1	-	-	-	-	-	-	-	-
Grade 2	-	-	1	1	-	-	-	2	-	-	-	-	-	-	-	-
Grade 3	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Erythrophagocytosis	-	1	1	-	-	-	-	-	1	-	-	-	1	-	1	1
Grade 1	-	1	1	-	-	-	-	-	1	-	-	-	-	-	1	1
Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Lymphoid hyperplasia	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Grade 2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Meniscus (Right)																
Degeneration	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Hyperplasia: synovium	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Granulation tissue	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Lymphocyte infiltr.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Plasma cell infiltr.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Parathyroid Glands																
Cyst	-	-	1	1	-	-	-	-	-	1	1	1	-	-	-	-
Grade 1	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-
Grade 2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Grade 3	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-
Pituitary Gland																
Cyst	-	-	-	2	1	2	-	2	1	1	1	-	1	-	-	1
Grade 1	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-
Grade 2	-	-	-	1	1	-	-	2	1	1	1	-	-	-	-	1
Grade 3	-	-	-	1	1	1	-	-	-	-	-	-	1	-	-	-
Mononuclear infiltr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Spleen																
Lymphoid depletion	-	-	3	2	1	-	-	1	-	-	-	-	-	-	-	-
Grade 1	-	-	1	2	1	-	-	1	-	-	-	-	-	-	-	-
Grade 2	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-

Thymus																
Lymphoid depletion	-	-	-	2	2	3	3	3	-	-	-	-	-	-	-	-
Grade 2	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Grade 3	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-
Grade 4	-	-	-	2	1	1	2	3	-	-	-	-	-	-	-	-
Involution	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thyroid Gland																
Mononulear infiltr.	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Grade 2	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-

**N=2; **N=1; infiltr (infiltratration); multinuc. (multinucleated giant cell infiltration); hepat. (hepatocytes with cytoplasmic rarefaction); synov. (synoviocyte cytoplasm)*

Local Findings in Knees (Injection Site)

Local microscopic findings such as multinuclear macrophages, lymphocyte/plasma cell/neutrophil infiltration, fibrosis, neovascularization, and debris/microspheres appear to be related to both microspheres and TCA. Although most of findings were not observed in the active comparator, Kenalog-40,-treated animals, it appears that TCA attenuates these local adverse effects because animals at HD (Group 4) showed a slightly higher number of incidence and severity than the vehicle control (Group 1). Microspheres and debris were observed at Day 210/211, a month after the last dosing, but disappeared by the end of the recovery period (Day 363/364). At the end of recovery period, the local microscopic findings described above were only partially recovered and other findings such as synovial hyperplasia, degeneration, and mineralization were present in animals treated with TCA (Group 2, 3, and 4). These findings were consistent with the cellular/structural joint score results.

Microscopic Findings of Systemic Organs/Tissues in Main Study (Group 1 to 4)

Tissue (N=3)	Day 210/211								Day 363/364							
	G-1		G-2		G-3		G-4		G-1		G-2		G-3		G-4	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Femur (Right Distal)**																
Degeneration	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Knee Joint Synovial Left																
Vacuolation: synov.	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 1	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hyperplasia: synov.	2	2	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Grade 1	2	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Grade 2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lymphocyte infiltr.	-	2	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Grade 1	-	2	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Plasma cell infiltr.	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Grade 1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Grade 2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Neovascularization	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Knee Joint Synovial Right																	
Macrophage/multinuc.	3	3	-	-	2	3	3	3	-	2	-	1	-	1	1	2	
Grade 1	2	-	-	-	1	3	1	1	-	2	-	1	-	1	1	2	
Grade 2	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	
Grade 3	1	2	-	-	1	-	1	2	-	-	-	-	-	-	-	-	
Lymphocyte infiltr.	2	3	-	-	2	1	3	2	-	2	1	1	3	1	1	2	
Grade 1	2	3	-	-	-	1	1	-	-	2	1	1	1	-	-	1	
Grade 2	-	-	-	-	2	-	2	2	-	-	-	-	2	1	1	1	
Plasma cell infliltr.	1	1	-	-	2	-	2	2	-	2	2	1	3	2	1	3	
Grade 1	1	1	-	-	-	-	-	-	-	2	2	1	1	1	-	1	
Grade 2	-	-	-	-	2	-	2	2	-	-	-	-	2	1	1	2	
Neutrophil infiltr.	-	-	-	-	1	-	2	2	-	-	-	-	-	-	-	-	
Grade 1	-	-	-	-	1	-	2	2	-	-	-	-	-	-	-	-	
Fibrosis	1	2	-	-	-	2	2	2	-	-	-	1	2	1	2	1	
Grade 1	1	2	-	-	-	2	2	2	-	-	-	1	-	1	-	1	
Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	
Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	
Neovascularization	2	3	-	-	2	1	2	2	-	-	-	-	-	-	-	-	
Grade 1	2	1	-	-	-	1	-	1	-	-	-	-	-	-	-	-	
Grade 2	-	2	-	-	2	-	2	1	-	-	-	-	-	-	-	-	
Granulation tissue	1	1	-	-	1	-	1	1	-	-	-	-	1	-	1	-	
Grade 1	1	1	-	-	1	-	1	1	-	-	-	-	1	-	1	-	
Debris	-	1	1	-	2	2	3	3	-	-	-	-	-	-	-	-	
Grade 1	-	1	-	-	-	2	1	1	-	-	-	-	-	-	-	-	
Grade 2	-	-	1	-	2	-	2	1	-	-	-	-	-	-	-	-	
Grade 3	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	
Microspheres	3	3	-	-	2	1	2	3	-	-	-	-	-	-	-	-	
Rarefaction: synov.	-	-	3	3	-	-	-	-	-	-	-	-	-	-	-	-	
Grade 2	-	-	3	3	-	-	-	-	-	-	-	-	-	-	-	-	
Hemorrhage: synovium	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	
Grade 1	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	
Hyperplasia: synov.	2	-	1	-	-	-	-	-	-	-	1	1	3	2	2	-	
Grade 1	2	-	1	-	-	-	-	-	-	-	-	-	1	-	2	-	
Grade 2	-	-	-	-	-	-	-	-	-	-	1	1	1	2	-	-	
Grade 3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	
Pigment: macrophage	1	-	-	-	-	-	-	-	-	1	-	1	1	1	1	2	
Grade 1	1	-	-	-	-	-	-	-	-	1	-	1	1	1	-	1	
Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
Eosinophil infiltr.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	
Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	
Mineralization	-	-	-	-	-	-	-	-	-	-	1	1	2	1	3	1	
Grade 1	-	-	-	-	-	-	-	-	-	-	1	1	2	1	3	1	
Degeneration	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	1	
Grade 1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	
Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	
Edema	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	
Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	

**N=2; **N=1; infiltr (infiltratration); multinuc. (multinucleated giant cell infiltration); hepat. (hepatocytes with cytoplasmic rarefaction); synov. (synoviocyte cytoplasm)*

At Day 210/211, minimal to marked macrophage and multinucleated giant cell infiltration in the right knee joint synovium was observed in animals treated with microspheres (Group 1, 3, and 4). These macrophages and multinucleated cells at the synovial surface were in direct apposition with multiple, variable-sized round space consistent with the PLGA microspheres. The morphological character of cellular responses to the microspheres was similar among microsphere-treated groups but the intensity of microphage/multinucleated giant cell responses was dose dependent. A few additional cellular responses in the microsphere-treated right knee of Group 1 compared to the diluent-treated left knee of Group 1 include lymphocyte and plasma cell infiltrates. Also, minimal fibrosis, neovascularization, neutrophil infiltrates, and granulation tissue were observed in the right synovium of animals treated with microspheres (Group 1, 3, and 4). Debris in the synovial surface was observed in all groups. Cytoplasmic rarefaction and hyperplasia of synoviocytes were observed in Group 2.

At Day 363/364, microspheres were no longer apparent microscopically and macrophage/multinucleated giant cell infiltrates were reduced in both incidence and intensity, indicating that the PLGA microspheres were degraded with reversibility of accumulated macrophage and multinucleated giant cells. Fibrosis, lymphocyte infiltration, synovial hyperplasia, and plasma cell infiltration persisted in animals, which had structural alterations in articular cartilage, with reduced intensity (minimal to slight). Minimal mineralization of the synovium was also observed in Group 2 to 4. The presence of debris and cytoplasmic rarefaction of synoviocytes were not apparent.

Summary of Combined Group Incidence (M and F) of Foreign Body Response (Macrophage/ Multinucleated Giant Cell Infiltrate) to Microspheres in the Right Knee Joint Synovium

mg/ml		Group Number	Study Day		Grade of Foreign Body Response*				
Microspheres	TCA		Day	Number of Dogs	0	1	2	3	4
75	0	1	210/211	6	0	2	1	3	0
			363/364	6	4	2	0	0	0
25	6.25	3	210/211	6	1	4	0	1	0
			363/364	6	5	1	0	0	0
75	18.75	4	210/211	6	0	2	1	3	0
			363/364	6	3	3	0	0	0
Total Number of Animals (Percentage of Total)			Day 210/211	18	1 (5.6%)	8 (44.4%)	2 (11.1%)	7 (38.9%)	0 (0%)
			Day 363/364	18	12 (66.7%)	6 (33.3%)	0 (0%)	0 (0%)	0 (0%)

* H&E sections of right synovium were evaluated using a subjective grading scale as follows: grade 0 = no findings; grade 1 = minimal with barely detectable change usually of focal distribution; grade 2 = slight with easily detectable change in tissues but still limited in extent, usually of focal to multifocal distribution; grade 3 = moderate with readily detectable change in tissues of notable extent, usually of multifocal distribution; and grade 4 = marked with very evident to profound change in tissues, usually diffuse in distribution.

Combined Group Incidence and Severity of Selected Changes Noted in the Right and Left Knee Joint Synovium at Day 210/211

Dose Group	1 (Left Knee)	1 (Right Knee)	2	3	4
Concentration Microspheres/TCA (mg/ml)	0/0	75/0	0/18.75	25/6.25	75/18.75
Number/Group	6	6	6	6	6
Lymphocyte Infiltration					
Grade 1*	2	5	0	1	1
Grade 2*	0	0	0	2	4
Neutrophil Infiltration					
Grade 1*	0	0	0	1	4
Plasma Cell Infiltration					
Grade 1*	1	2	0	0	0
Grade 2*	1	0	0	2	4
Fibrosis					
Grade 1*	0	3	0	2	4

* H&E sections of right and left synovium were evaluated using a subjective grading scale as follows: grade 0 = no findings; grade 1 = minimal with barely detectable change usually of focal distribution; grade 2 = slight with easily detectable change in tissues but still limited in extent, usually of focal to multifocal distribution; grade 3 = moderate with readily detectable change in tissues of notable extent, usually of multifocal distribution; and grade 4 = marked with very evident to profound change in tissues, usually diffuse in distribution.

Combined Group Incidence and Severity of Selected Changes Noted in the Right and Left Knee joint Synovium at Day 363/364

Dose Group	1 (Left Knee)	1 (Right Knee)	2	3	4
Concentration Microspheres/FX005 (mg/ml)	0/0	75/0	0/18.75	25/6.25	75/18.75
Number/Group	6	6	6	6	6
Lymphocyte Infiltration					
Grade 1*	1	2	2	1	1
Grade 2*	0	0	0	3	2
Eosinophil Infiltration					
Grade 1*	0	0	0	1	0
Plasma Cell Infiltration					
Grade 1*	0	2	3	2	1
Grade 2*	0	0	0	3	3
Fibrosis					
Grade 1*	0	0	1	1	2
Grade 2*	0	0	0	1	1
Grade 3*	0	0	0	1	0

* H&E sections of right and left synovium were evaluated using a subjective grading scale as follows: grade 0 = no findings; grade 1 = minimal with barely detectable change usually of focal distribution; grade 2 = slight with easily detectable change in tissues but still limited in extent, usually of focal to multifocal distribution; grade 3 = moderate with readily detectable change in tissues of notable extent, usually of multifocal distribution; and grade 4 = marked with very evident to profound change in tissues, usually diffuse in distribution.

Subset Study (Group 5 to 8): Similar microscopic findings to the main study were observed. There were no microscopic findings present in systemic organs/tissues related to IA administration of blank microspheres (Group 1).

- Adrenal gland: Dose-dependent and TCA-related atrophy of the zona fasciculate was observed at Day 92/93. Incidence and severity of adrenocortical atrophy were decreased at Day 234/244, suggesting the reversibility of this TCA-related adverse effect in the adrenal gland.
- Liver: Dose-dependent and TCA-related cellular swelling of hepatocytes with cytoplasmic rarefaction mostly in midzonal regions of the hepatic lobules and some in periportal and centrilobular zones were observed at Day 92/93. By Day 243/244, these findings appear to be reversible.
- Lymphoid tissues (thymus, lymph nodes, and spleen): TCA-related lymphoid depletion was observed in the thymus at Day 92/93. Lower degree/incidence but TCA-related lymphoid depletion was also observed in lymph nodes (mandibular, mesenteric, inguinal, left and right popliteal) and spleen. Lymphoid depletion appears to be reversible by Day 243/244. As note, the thymus was not identified microscopically in a number of animals in Group 6 to 9.
- Bone marrow (sternum): Minimal hypocellularity of hematopoietic cell elements in the sternal bone marrow was observed in TCA-treated animals at Day 92/93, but these findings were not observed at Day 243/244.

Systemic Findings of Subset Study (Group 5 to 8)

Tissue (N=3)	Day 92/93								Day 243/244							
	G-5		G-6		G-7		G-8		G-1		G-2		G-3		G-4	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Adrenal Gland																
Atrophy: cortex	-	-	3	3	3	3	3	3	-	-	3	1	3	1	2	1
Grade 2	-	-	-	-	-	-	-	-	-	-	2	1	3	1	2	1
Grade 3	-	-	-	1	2	2	2	-	-	-	1	-	-	-	-	-
Grade 4	-	-	3	2	1	1	1	3	-	-	-	-	-	-	-	-
Vacuolation	-	-	-	3	1	3	2	2	-	-	2	2	-	1	1	1
Grade 1	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-
Grade 2	-	-	-	2	-	3	2	1	-	-	2	-	-	-	1	1
Grade 3	-	-	-	1	1	-	-	1	-	-	-	1	-	-	-	-
Bone Marrow, sternum																
Hypocellularity	-	-	3	3	2	2	3	2	-	-	-	-	-	-	-	-
Grade 1	-	-	3	3	2	2	3	2	-	-	-	-	-	-	-	-
Fat**																
Steatitis	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Grade 3	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Pigment: macrophage	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Grade 2	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-

Kidney																
Mononuclear infiltr.	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tubular basophilia	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Grade 1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Atrophy: parenchyma	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glomerular lipidosis	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Grade 1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Fibrosis:cortex	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Liver																
Cell swelling: hepat.	-	-	3	1	-	-	3	3	-	-	-	2	-	-	-	-
Grade 1	-	-	1	1	-	-	1	-	-	-	-	2	-	-	-	-
Grade 2	-	-	-	-	-	-	1	3	-	-	-	-	-	-	-	-
Grade 3	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-
Fibrosis: capsule	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Grade 1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Mixed cell infiltr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Lymph Node: Inguinal																
Lymphoid depletion	-	-	1**	2*	3	3	2*	2*	-	-	-	-	-	-	-	-
Grade 1	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
Grade 2	-	-	1	1	2	3	2	2	-	-	-	-	-	-	-	-
Erythrophagocytosis	1	2	-	-	-	-	-	-	1	-	3	-	1	-	-	-
Grade 1	-	1	-	-	-	-	-	-	-	-	2	-	1	-	-	-
Grade 2	1	1	-	-	-	-	-	-	1	-	1	-	-	-	-	-
Lymphoid hyperplasia	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Neutrophil infiltr.	-	-	-	-	-	-	-	-	1	-	1	-	-	-	1	-
Grade 3	-	-	-	-	-	-	-	-	1	-	1	-	-	-	1	-
Lymph Node: Left Popliteal																
Erythrophagocytosis	-	1	-	-	2	3	-	-	-	-	2	-	2	-	-	-
Grade 1	-	1	-	-	2	3	-	-	-	-	1	-	2	-	-	-
Grade 2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Plasmacytosis	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Grade 3	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Lymphoid depletion	-	-	3	3	3	3	3	3	-	-	-	-	-	-	-	-
Grade 1	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-
Grade 2	-	-	2	2	2	2	3	3	-	-	-	-	-	-	-	-
Grade 3	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Lymph Node: Mandibular																
Lymphoid depletion	-	-	3	3	3	3	3	3	-	-	-	-	-	-	-	-
Grade 1	-	-	-	2	1	2	-	-	-	-	-	-	-	-	-	-
Grade 2	-	-	3	1	2	1	3	3	-	-	-	-	-	-	-	-
Erythrophagocytosis	-	1	-	1	1	-	1	1	-	-	-	-	-	-	-	-
Grade 1	-	-	-	1	1	-	1	1	-	-	-	-	-	-	-	-
Grade 2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neutrophil infiltr.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Grade 3	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-

Lymph Node: Mesenteric																
Lymphoid depletion	-	-	3	3	3	3	3	3	-	-	-	-	-	-	-	-
Grade 1	-	-	-	-	2	1	1	-	-	-	-	-	-	-	-	-
Grade 2	-	-	1	2	1	2	2	3	-	-	-	-	-	-	-	-
Grade 3	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-
Erythrophagocytosis	3	3	3	3	2	3	3	3	3	2	3	3	3	2	3	3
Grade 1	3	3	3	2	2	3	3	2	2	2	3	3	2	1	3	2
Grade 2	-	-	-	1	-	-	-	1	1	-	-	-	1	1	-	1
Lymph Node: Right Popliteal																
Lymphoid depletion	-	-	3	3	3	3	3	3	-	-	-	-	-	-	-	-
Grade 1	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-
Grade 2	-	-	3	3	2	2	3	3	-	-	-	-	-	-	-	-
Erythrophagocytosis	1	-	-	-	2	2	-	-	-	-	-	-	-	1	-	-
Grade 1	1	-	-	-	2	2	-	-	-	-	-	-	-	1	-	-
Plasmacytosis	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Grade 3	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Meniscus (Right)																
Degeneration	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Grade 1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Congestion	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Grade 1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Parathyroid Glands																
Cyst	2	-	-	-	-	1	-	1	-	-	1	1	-	1	1	-
Grade 2	2	-	-	-	-	1	-	1	-	-	1	1	-	1	1	-
Pituitary Gland																
Cyst	-	2	-	3	3	1	1	-	1	3	-	2	-	2	2	1
Grade 1	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-
Grade 2	-	2	-	2	3	-	-	-	-	2	-	1	-	1	-	1
Grade 3	-	-	-	-	-	-	1	-	1	1	-	1	-	1	2	-
Mononuclear infiltr.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Spleen																
Lymphoid depletion	-	-	3	2	2	3	3	3	-	-	-	-	-	-	1	-
Grade 1	-	-	3	2	2	3	3	3	-	-	-	-	-	-	1	-
Thymus																
Lymphoid depletion	-	-	1	2*	3	2*	2*	3	-	-	-	-	1	-	-	-
Grade 2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Grade 3	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Grade 4	-	-	1	2	2	2	2	3	-	-	-	-	-	-	-	-
Thyroid Gland																
Squamous cyst	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Grade 2	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-

**N=2; **N=1; infiltr (infiltratration); multinuc. (multinucleated giant cell infiltration); hepat. (hepatocytes with cytoplasmic rarefaction); synov. (synoviocyte cytoplasm)*

Local Findings in Knees (Injection Site)

Similar microscopic findings to the main study were observed. Local microscopic findings appear to be related to both microspheres and TCA. Although most of findings were not observed in the active comparator, Kenalog-40,-treated animals, it appears

that TCA attenuates these local adverse effects because animals at HD (Group 8) showed a slightly higher number of incidence and severity than the vehicle control (Group 5). Microspheres and debris were observed at Day 92/93 but disappeared by the end of the recovery period (Day 243/244). At the end of recovery period, microsphere-related microscopic findings were only partially recovered and other findings such as synovial hyperplasia, degeneration, and mineralization were present in animals treated with TCA (Group 2, 3, and 4). As noted, the incidence and severity of microscopic findings at the end of the recovery period were lower in the subset study than in the main study.

Local Findings of Subset Study (Group 5 to 8)

Tissue (N=3)	Day 92/93								Day 243/244							
	G-5		G-6		G-7		G-8		G-1		G-2		G-3		G-4	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Knee Joint Synovial Left																
Macrophage/multinuc.	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 2	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 3	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lymphocyte infiltr.	3	2	-	-	-	-	-	-	2	-	1	-	-	-	-	-
Grade 1	2	2	-	-	-	-	-	-	2	-	1	-	-	-	-	-
Grade 2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plasma cell infiltr.	1	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Grade 1	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Grade 2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neutrophil infiltr.	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fibrosis	3	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Grade 1	3	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Neovascularization	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 1	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Microspheres	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vacuolation: synov.	-	-	3	3	-	-	-	-	-	-	1	1	-	-	-	-
Grade 1	-	-	3	3	-	-	-	-	-	-	1	1	-	-	-	-
Pigment: macrophage	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-
Grade 1	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-
Knee Joint Synovial Right																
Macrophage/multinuc.	3	3	-	-	3	3	3	3	-	1	-	-	-	-	1	3
Grade 1	1	3	-	-	-	2	-	1	-	1	-	-	-	-	1	2
Grade 2	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	1
Grade 3	2	-	-	-	3	-	2	1	-	-	-	-	-	-	-	-
Lymphocyte infiltr.	3	3	-	-	3	-	3	1	3	1	2	-	2	2	2	3
Grade 1	3	3	-	-	1	-	-	1	3	1	2	-	2	2	2	2
Grade 2	-	-	-	-	1	-	3	-	-	-	-	-	-	-	-	-
Grade 3	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
Eosinophil infiltr.	-	-	-	-	-	-	-	-	-	1	-	-	-	1	1	-
Grade 1	-	-	-	-	-	-	-	-	-	1	-	-	-	1	1	-
Plasma cell infliltr.	2	-	-	-	2	-	2	1	2	-	1	-	2	-	2	3
Grade 1	1	-	-	-	1	-	1	1	2	-	1	-	2	-	2	2
Grade 2	1	-	-	-	1	-	1	-	-	-	-	-	-	-	-	1
Neutrophil infiltr.	-	-	-	-	1	-	2	-	-	-	-	-	-	-	-	-
Grade 1	-	-	-	-	1	-	2	-	-	-	-	-	-	-	-	-

Fibrosis	2	3	-	-	2	-	2	1	-	-	1	-	2	-	1	2
Grade 1	1	2	-	-	2	-	2	1	-	-	1	-	2	-	-	2
Grade 2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Neovascularization	3	3	-	-	3	1	3	1	-	-	-	-	-	-	-	-
Grade 1	2	2	-	-	2	1	2	1	-	-	-	-	-	-	-	-
Grade 2	1	1	-	-	1	-	1	-	-	-	-	-	-	-	-	-
Debris	1	1	-	-	3	3	3	3	-	-	-	-	-	-	-	-
Grade 1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grade 2	1	-	-	-	-	3	-	2	-	-	-	-	-	-	-	-
Grade 3	-	-	-	-	3	-	3	1	-	-	-	-	-	-	-	-
Microspheres	3	3	-	-	3	3	3	3	-	-	-	-	-	-	-	-
Rarefaction: synov.	-	-	3	3	-	-	-	2	-	-	-	-	-	-	-	-
Grade 2	-	-	3	3	-	-	-	2	-	-	-	-	-	-	-	-
Fat necrosis	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Grade 3	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Hyperplasia: synov.	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	1
Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Grade 2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Pigment: macrophage	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
Grade 1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
Mineralization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Grade 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Degeneration	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Grade 3	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-

**N=2; **N=1; infiltr (infiltratration); multinuc. (multinucleated giant cell infiltration); hepat. (hepatocytes with cytoplasmic rarefaction); synov. (synoviocyte cytoplasm)*

At Day 92/93, microspheres were present microscopically in all synovial specimens of microsphere-treated groups (Group 5, 7, and 8) in association with macrophages and multinucleated cells at the synovial surface. There were increased incidence and/or intensity of lymphocyte and plasma cell infiltrates, neovascularization, and neutrophil infiltrates for microsphere-treated groups as compared to diluent-treated and TCA IR-treated animal group (Group 6). The incidence of fibrosis was slightly increased for blank microsphere-treated joints versus the test article-treated joints. Similar to the main study, these findings appears to be expected foreign body responses to PLGA microspheres. The presence of debris on the synovial surface/vacuolation of synoviocyte cytoplasm, or cytoplasmic rarefaction of synoviocytes was observed in diluent-treated specimen or TCA IR-treated specimens, respectively.

At Day 243/244, microspheres were no longer apparent microscopically. Foreign responses were reduced in both incidence and intensity, suggesting the reversibility of macrophage and multinucleated giant cell infiltration related to microsphere treatment. Similar to the main study, lymphocyte infiltration and plasma cell infiltration persisted with minimal intensity and sporadic/minimal fibrosis was observed. Other sporadic synovial changes at Day 243/244 included synovial hyperplasia, mineralization, degeneration, neutrophil infiltration, eosinophil infiltration, and pigmented macrophages. Debris was not apparent microscopically and cytoplasmic vacuolation of synoviocytes was noted in only two diluent-treated samples, indicating its reversibility.

Summary of Combined Group Incidence (M and F) of Foreign Body Response (Macrophage/ Multinucleated Giant Cell Infiltrate) to Microspheres in the Right and Left Knee Joint Synovium

mg/ml		Group Number	Study Day		Grade of Foreign Body Response*				
Microspheres	TCA		Day	Number of Dogs	0	1	2	3	4
25	0	5 (Left Knee)	92/93	6	0	0	4	2	0
			243/244	6	6	0	0	0	0
75	0	5 (Right knee)	92/93	6	0	1	0	5	0
			243/244	6	5	1	0	0	0
25	6.25	7	92/93	6	0	2	1	3	0
			243/244	6	6	0	0	0	0
75	18.75	8	92/93	6	0	1	2	3	0
			243/244	6	2	3	1	0	0
Total Number of Joints for Each Grade of Foreign Body Response- All Groups Combined (Percentage of Total)			Day 92/93	24	0 (0%)	4 (16.67%)	7 (29.17%)	13 (54.17%)	0 (0%)
			Day 243/244	24	19 (79.17%)	4 (16.67%)	1 (4.17%)	0 (0%)	0 (0%)

* H&E sections of right and left synovium were evaluated using a subjective grading scale as follows: grade 0 = no findings; grade 1 = minimal with barely detectable change usually of focal distribution; grade 2 = slight with easily detectable change in tissues but still limited in extent, usually of focal to multifocal distribution; grade 3 = moderate with readily detectable change in tissues of notable extent, usually of multifocal distribution; and grade 4 = marked with very evident to profound change in tissues, usually diffuse in distribution.

Combined Group Incidence and Severity of Selected Changes Noted in the Right and Left Knee Joint Synovium at Day 92/93

Dose Group	5 (Left Knee)	5 (Right Knee)	6 (Left Knee)	6 (Right Knee)	7	8
Concentration Microspheres/TCA (mg/ml)	25/0	75/0	0/0	0/18.75	25/6.25	75/18.75
Number/Group	6	6	6	6	6	6
Lymphocyte Infiltration						
Grade 1*	4	6	0	0	1	1
Grade 2*	1	0	0	0	1	3
Grade 3*	0	0	0	0	1	0
Neutrophil Infiltration						
Grade 1*	1	0	0	0	1	2
Plasma Cell Infiltration						
Grade 1*	0	1	0	0	1	2
Grade 2*	1	1	0	0	1	1
Fibrosis						
Grade 1*	6	3	0	0	2	3
Grade 2*	0	2	0	0	0	0

* H&E sections of right and left synovium were evaluated using a subjective grading scale as follows: grade 0 = no findings; grade 1 = minimal with barely detectable change usually of focal distribution; grade 2 = slight with easily detectable change in tissues but still limited in extent, usually of focal to multifocal distribution; grade 3 = moderate with readily detectable change in tissues of notable extent, usually of multifocal distribution; and grade 4 = marked with very evident to profound change in tissues, usually diffuse in distribution.

Combined Group Incidence and Severity of Selected Changes Noted in the Right and Left Knee Joint Synovium at Day 243/244

Dose Group	5 (Left Knee)	5 (Right Knee)	6 (Left Knee)	6 (Right Knee)	7	8
Concentration Microspheres/TCA (mg/ml)	25/0	75/0	0/0	0/18.75	25/6.25	75/18.75
Number/Group	6	6	6	6	6	6
Lymphocyte Infiltration						
Grade 1*	2	4	1	2	4	4
Grade 3*	0	0	0	0	0	1
Neutrophil Infiltration						
Grade 1*	0	0	0	0	0	1
Eosinophil Infiltration						
Grade 1*	0	1	0	0	1	1
Plasma Cell Infiltration						
Grade 1*	2	2	0	1	5	4
Grade 2*	0	0	0	0	0	1
Fibrosis						
Grade 1*	0	0	1	1	2	2

* H&E sections of right and left synovium were evaluated using a subjective grading scale as follows: grade 0 = no findings; grade 1 = minimal with barely detectable change usually of focal distribution; grade 2 = slight with easily detectable change in tissues but still limited in extent, usually of focal to multifocal distribution; grade 3 = moderate with readily detectable change in tissues of notable extent, usually of multifocal distribution; and grade 4 = marked with very evident to profound change in tissues, usually diffuse in distribution.

Semi-Quantitative Mankin Scoring Evaluation

The following modified Mankin scoring system was used by the pathologist to aid in the evaluation.

Category	Score
Structure	
Normal	0
Surface irregularities	1
Pannus and surface irregularities	2
Clefts to transitional zone	3
Clefts to tidemark	4
Clefts to subchondral bone	5
Complete disorganization	6
Cells	
Normal	0
Diffuse hypercellularity	1
Cloning	2
Hypocellularity (necrosis)	3
Safranin O staining	
Normal	0
Slight reduction, tangential zone	1
Moderate reduction (to tidemark)	2
Severe reduction (to subchondral bone in at least 1 area)	3
No dye observed	4
Tidemark Integrity	
Intact	0
Crossed by blood vessels	1
Total	0-14

A separate microscopic scoring system for the host tissue response to blank microspheres and FX006 microspheres was based on the semi-quantitative scoring system set forth in ISO 10993 Part 6. Parameters that were evaluated included, but were not limited to, inflammation as characterized by inflammatory cell infiltrates (heterophils/neutrophils, eosinophils, mast cells, lymphocytes, plasma cells, macrophages, and multinucleated giant cells) and the associated tissue response parameters to include necrosis, vascularization, granulation tissue, fibrosis, mineralization, granulomas, tissue ingrowth into the device, foreign debris (other than implant), and hemorrhage.

The severity scale employed for the implant site evaluation in accordance with ISO 10993-6 was on a scale of 0-4 as follows:

0 = Not present
1 = Minimal/Slight - 1 to 25 % of the implant site is involved or 1 - 5 cells per high power field (400x)
2 = Mild - 26 to 50% of the implant site is involved or 5 - 10 cells per high power field (400x)
3 = Moderate - 51 to 75% of the implant site is involved or a heavy infiltrate of cells per high power field (400x)
4 = Marked /Severe - 76 to 100% of the implant site is involved or the cells are packed per high power field (400x)

Main Study (Group 1 to 4): At Day 210/211, there were no clear dose-dependent trends in the test article treated groups (Group 3 and 4). Additionally, the average values for the test article-treated groups were comparable to the average values for the TCA IR-treated Group 2. Increased Mankin score values were related to a consistent decrease in Safranin O staining due to loss of extracellular matrix (glycosaminoglycans), which is a known effect of corticosteroids when administered directly into synovial joints (Jubb et al., 1993). There were no articular cartilage changes relative to structure, cellularity, or tidemark integrity in these groups (Group 2 to 4). A slight reduction in articular cartilage Safranin O staining in the left knee joints for Group 2 to 4 was observed, which likely represented a systemic corticosteroid effect in these animals. At 363/364, similar to Day 210/211, there were no clear dose-dependent trends in the test article treated groups (Group 3 and 4) and the average values for the test article-treated groups were comparable to the average values for the TCA IR-treated Group 2. There was reversibility of decreased Safranin O Staining in animals of Group 2 to 4, but there were changes in the structure, cellularity, and/or tidemark integrity present in the articular cartilage from bones of the right knee joint from at least one animal/sex/group.

At Day 210/211, there were no articular cartilage changes in the diluent-treated control (left knee joint of Group 1). The average total join scores the blank microsphere-treated control (right knee of Group 1) were minor and there were alterations limited to surface irregularities, chondrocyte cloning with a slight decrease in Safranin O staining in two distal femur specimens. At Day 363/364, there were no changes in articular cartilage in joints treated with the diluent or blank microspheres.

Average Joint Score for Males and Females

Group	Study Day		Males	Females
1	Day 210/211	Right Knee Joint Total Average	0.00	0.89
		Left Knee Joint Total Average	0.00	0.00
	Day 363/364	Right Knee Joint Total Average	0.00	0.00
		Left Knee Joint Total Average	0.00	0.00
2	Day 210/211	Right Knee Joint Total Average	2.67	2.67
		Left Knee Joint Total Average	0.89	0.67
	Day 363/364	Right Knee Joint Total Average	3.22	0.78
		Left Knee Joint Total Average	0.00	0.00
3	Day 210/211	Right Knee Joint Total Average	2.33	2.67
		Left Knee Joint Total Average	0.00	0.44
	Day 363/364	Right Knee Joint Total Average	3.44	3.11
		Left Knee Joint Total Average	0.00	0.00
4	Day 210/211	Right Knee Joint Total Average	2.89	2.78
		Left Knee Joint Total Average	0.78	0.78
	Day 363/364	Right Knee Joint Total Average	3.67	2.67
		Left Knee Joint Total Average	0.00	0.11

Safranin O Staining Score

Day	Tissues (N=2)		Group 1 Control	Group 2 Kenalog IR	Group 3 LD	Group 4 HD
Male						
Day 210	Tibial Plateau	R	0.00	1.33	1.67	2.33
		L	0.00	0.00	0.00	0.33
	Distal Femur	R	0.00	3.33	2.33	3.00
		L	0.00	1.67	0.00	1.00
	Patella	R	0.00	3.33	3.00	3.33
		L	0.00	1.00	0.00	1.00
Day 363	Tibial Plateau	R	0.00	1.33	1.33	1.00
		L	0.00	0.00	0.00	0.00
	Distal Femur	R	0.00	1.33	1.00	2.00
		L	0.00	0.00	0.00	0.00
	Patella	R	0.00	1.67	1.67	1.67
		L	0.00	0.00	0.00	0.00
Female						
Day 211	Tibial Plateau	R	0.00	1.00	1.67	2.00
		L	0.00	0.00	0.00	0.33
	Distal Femur	R	0.67	3.33	3.00	3.33
		L	0.00	1.00	0.33	1.00
	Patella	R	0.00	3.67	3.33	3.00
		L	0.00	1.00	1.00	1.00

Day 364	Tibial Plateau	R	0.00	**0.67**	**1.00**	**1.00**
		L	0.00	0.00	0.00	0.00
	Distal Femur	R	0.00	**1.00**	**1.00**	**1.00**
		L	0.00	0.00	0.00	0.00
	Patella	R	0.00	**0.33**	**1.67**	**0.67**
		L	0.00	0.00	0.00	**0.33**

Subset Study (Group 5 to 8): At Day 92/93, the average total join score values for Group 8 were comparable to the average values for the TCA IR-treated Group 6. Increased Mankin score values were related to a consistent decrease in Safranin O staining due to loss of extracellular matrix. There were dose-dependent Mankin score increase in Group 7 and Group 8. At Day 243/244, reduction in Mankin scores were observed compared to Day 92/93 and it appears to be dose-dependent. There were structural and cellular articular cartilage surface changes but the incidence and severity of these changes did not worsen during the recovery period.

At Day 92/93, there were no articular cartilage changes in the low concentration of blank microsphere-treated control (25 mg/ml, left knee joint of Group 5) while there were tibial alterations in articular cartilage structure in the high concentration of blank microsphere-treated control (75 mg/mL, right knee).

Average Joint Score for Males and Females

Group	Study Day		Males	Females
5	Day 92/93	Right Knee Joint Total Average	0.22	0.33
		Left Knee Joint Total Average	0.00	0.00
	Day 243/244	Right Knee Joint Total Average	0.00	0.44
		Left Knee Joint Total Average	0.00	0.00
6	Day 92/93	Right Knee Joint Total Average	2.11	2.44
		Left Knee Joint Total Average	0.78	0.89
	Day 243/244	Right Knee Joint Total Average	1.44	2.22
		Left Knee Joint Total Average	0.00	0.00
7	Day 92/93	Right Knee Joint Total Average	2.33	2.67
		Left Knee Joint Total Average	0.56	0.67
	Day 243/244	Right Knee Joint Total Average	0.22	2.33
		Left Knee Joint Total Average	0.11	0.00
8	Day 92/93	Right Knee Joint Total Average	3.11	3.11
		Left Knee Joint Total Average	1.44	1.00
	Day 243/244	Right Knee Joint Total Average	1.22	1.22
		Left Knee Joint Total Average	0.00	0.00

Safranin O Staining Score

Day	Tissues (N=2)		Group 5 Control	Group 6 Kenalog IR	Group 7 LD	Group 8 HD
Male						
Day 92	Tibial Plateau	R	0.33	1.00	1.00	1.67
		L	0.00	0.33	0.00	1.00
	Distal Femur	R	0.00	2.33	3.00	3.00
		L	0.00	1.00	1.00	1.67
	Patella	R	0.00	3.00	2.33	3.00
		L	0.00	1.00	0.67	1.33
Day 243	Tibial Plateau	R	0.00	1.00	0.67	1.00
		L	0.00	0.00	0.33	0.00
	Distal Femur	R	0.00	1.33	0.00	1.33
		L	0.00	0.00	0.00	0.00
	Patella	R	0.00	1.67	0.00	1.33
		L	0.00	0.00	0.00	0.00
Female						
Day 93	Tibial Plateau	R	0.33	1.67	1.67	1.00
		L	0.00	0.67	0.33	0.67
	Distal Femur	R	0.00	2.67	3.00	3.33
		L	0.00	1.00	1.00	1.33
	Patella	R	0.00	3.00	3.00	3.00
		L	0.00	1.00	0.67	1.00
Day 244	Tibial Plateau	R	0.00	1.00	1.00	1.33
		L	0.00	0.00	0.00	0.00
	Distal Femur	R	0.33	1.67	1.67	0.67
		L	0.00	0.00	0.00	0.00
	Patella	R	0.00	2.00	0.33	1.00
		L	0.00	0.00	0.00	0.00

Synovial Tissue

Synovial fluid samples were collected from the stifle joint and synovial fluid slides for the injected knees were stained with Wright-Giemsa stain. The adjusted white blood cell (WBC) count of the synovial fluid was obtained by multiplying the measured WBC count by a diluted factor calculated with the plasma and synovial fluid levels of urea (Krause et al., 2002). Large mononuclear cells are large macrophages with or without cytoplasmic vacuoles and/or phagocytized elements. Small mononuclear cells are small monocytes or small macrophages not yet containing vacuoles or phagocytized elements. The

Applicant noted that a term of small mononuclear cells were used in this report because lymphocytes may be hard to differentiate from very small early macrophages based on light microscopy alone if the macrophage nuclei are still round.

Main Study (Group 1 to 4):
With limited samples, for example, only a single value available for some samples, it is difficult to make a definitive conclusion but, in general, there was no dose-dependent response of WBC, neutrophils, small mononuclear cells, or large mononuclear cells at both Day 210/211 and 363/364. No eosinophils, plasma cells, or mast cells were observed in synovial fluids.

Mean Values of White Blood Cells, Neutrophils, Small Mononuclear Cells, and Large Mononuclear Cells at Day 210/211

	Day 210/211 Animals							
	White Blood Cells (x 10^3/µL)		Neutrophils (x 10^3/µL)		Small Mononuclear Cells (x 10^3/µL)		Large Mononuclear Cells (x 10^3/µL)	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Group	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)
1 (Right Knee)	0.159 (0.114)	0.159 (0.109)	0.00 (0.00)	0.00 (0.00)	0.10 (0.09)	0.14 (0.10)	0.06 (0.03)	0.02 (0.02)
1 (Left Knee)	0.195 (0.085)	0.182 (0.136)	0.00 (0.00)	0.00 (0.00)	0.07 (0.03)	0.08 (0.06)	0.13 (0.06)	0.10 (0.08)
2	0.350 (NA)	0.100 (NA)	0.00 (NA)	0.00 (NA)	0.17 (NA)	0.08 (NA)	0.18 (NA)	0.02 (NA)
3	0.154 (0.073)	0.640 (NA)	0.02 (0.01)	0.00 (NA)	0.11 (0.06)	0.22 (NA)	0.08 (0.01)	0.42 (NA)
4	0.119 (0.062)	0.240 (0.170)	0.01 (0.01)	0.01 (0.01)	0.07 (0.04)	0.10 (0.04)	0.05 (0.02)	0.15 (0.13)

NA= Not Applicable; Group 1 right knee= blank microspheres; Group 1 left knee= diluent; Group 2= TCA IR; Group 3= FX006 low dose; Group 4= FX006 high dose

Mean Values of White Blood Cells, Neutrophils, Small Mononuclear Cells, and Large Mononuclear Cells at Day 363/364

	Day 363/364 Animals							
	White Blood Cells (x 10^3/µL)		Neutrophils (x 10^3/µL)		Small Mononuclear Cells (x 10^3/µL)		Large Mononuclear Cells (x 10^3/µL)	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Group	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)
1 (Right Knee)	0.085 (0.035)	0.074 (0.058)	0.00 (0.00)	0.00 (0.00)	0.08 (0.06)	0.06 (0.02)	0.02 (0.01)	0.02 (0.03)
1 (Left Knee)	0.058 (0.015)	0.047 (0.013)	0.00 (0.00)	0.00 (0.00)	0.05 (0.01)	0.03 (0.01)	0.01 (0.01)	0.02 (0.01)
2	0.096 (0.041)	0.108 (0.039)	0.00 (NA)	0.00 (0.00)	0.07 (NA)	0.08 (0.03)	0.02 (NA)	0.03 (0.01)
3	0.260 (0.130)	0.155 (0.080)	0.00 (0.00)	0.00 (0.00)	0.22 (0.12)	0.10 (0.06)	0.05 (0.03)	0.04 (0.04)
4	0.210 (0.009)	0.116 (0.061)	0.00 (0.00)	0.00 (0.00)	0.17 (0.01)	0.10 (0.05)	0.04 (0.01)	0.02 (0.01)

NA= Not Applicable; Group 1 right knee= blank microspheres; Group 1 left knee= diluent; Group 2= TCA IR; Group 3= FX006 low dose; Group 4= FX006 high dose

Subset Study (Group 5 to 8):
With limited samples, for example, only a single value available for some samples, it is difficult to make a definitive conclusion but, in general, there was no dose-dependent response of WBC, neutrophils, small mononuclear cells, or large mononuclear cells at both Day 210/211 and 363/364. Only males in Group 7 had 0.03 ± 0.05 x 10^3/mcL eosinophils. No plasma cells or mast cells were observed in synovial fluids.

Mean Values of White Blood Cells, Neutrophils, Small Mononuclear Cells, and Large Mononuclear Cells at Day 92/93

	Day 92/93 Animals							
	White Blood Cells (x 10^3/µL)		Neutrophils (x 10^3/µL)		Small Mononuclear Cells (x 10^3/µL)		Large Mononuclear Cells (x 10^3/µL)	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Group	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)
5 (Right Knee)	0.459 (0.276)	0.330 (0.087)	0.00 (0.00)	0.00 (0.01)	0.47 (0.07)	0.19 (0.11)	0.15 (0.13)	0.14 (0.06)
5 (Left Knee)	NA (NA)	0.525 (0.256)	NA (NA)	0.02 (0.02)	NA (NA)	0.34 (0.17)	NA (NA)	0.16 (0.09)
6 (Right Knee)	0.295 (0.177)	0.652 (0.415)	0.00 (0.00)	0.00 (0.00)	0.11 (0.04)	0.31 (0.40)	0.19 (0.13)	0.34 (0.01)
6 (Left Knee)	NA (NA)	0.125 (0.036)	NA (NA)	0.00 (0.00)	NA (NA)	0.04 (0.01)	NA (NA)	0.08 (0.03)
7	2.158 (2.595)	1.105 (0.257)	0.20 (0.27)	0.03 (0.04)	1.18 (1.41)	0.77 (0.26)	0.76 (0.87)	0.32 (0.04)
8	0.468 (NA)	0.688 (0.405)	0.04 (NA)	0.03 (0.03)	0.20 (NA)	0.51 (0.32)	0.23 (NA)	0.15 (0.08)

NA= Not Applicable; Group 5 right knee= blank microspheres high dose; Group 5 left knee= blank microspheres low dose; Group 6 right knee= TCA IR; Group 6 left knee= diluent; Group 7= FX006 low dose; Group 8= FX006 high dose

Mean Values of White Blood Cells, Neutrophils, Small Mononuclear Cells, and Large Mononuclear Cells at Day 243/244

	Day 243/244 Animals							
	White Blood Cells (x 10^3/µL)		Neutrophils (x 10^3/µL)		Small Mononuclear Cells (x 10^3/µL)		Large Mononuclear Cells (x 10^3/µL)	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Group	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)	Mean (Standard Deviation)
5 (Right Knee)	0.196 (0.066)	0.150 (0.093)	0.00 (0.00)	0.00 (NA)	0.12 (0.05)	0.11 (NA)	0.08 (0.03)	0.02 (NA)
5 (Left Knee)	0.070 (0.040)	0.241 (0.208)	0.00 (NA)	0.00 (0.01)	0.03 (NA)	0.16 (0.18)	0.02 (NA)	0.07 (0.03)
6 (Right Knee)	0.115 (0.038)	0.307 (NA)	0.00 (0.00)	0.01 (NA)	0.08 (0.01)	0.20 (NA)	0.04 (0.03)	0.10 (NA)
6 (Left Knee)	0.194 (0.063)	0.179 (0.098)	0.00 (0.00)	0.00 (0.00)	0.10 (0.06)	0.11 (0.08)	0.09 (0.01)	0.07 (0.03)
7	0.130 (0.022)	0.193 (0.022)	0.00 (0.00)	0.00 (0.00)	0.11 (0.03)	0.14 (0.04)	0.04 (0.01)	0.05 (0.02)
8	0.370 (0.431)	0.635 (0.694)	0.00 (0.00)	NA (NA)	0.10 (0.10)	NA (NA)	0.27 (0.34)	NA (NA)

NA= Not Applicable; Group 5 right knee= blank microspheres high dose; Group 5 left knee= blank microspheres low dose; Group 6 right knee= TCA IR; Group 6 left knee= diluent; Group 7= FX006 low dose; Group 8= FX006 high dose

ISO 10993 Part 6 Evaluation

Semi-quantitative evaluation of the host tissue foreign tissue response to blank microspheres and the test article (FX001 microspheres) was performed in accordance with the ISO 10993 Part 6 guidelines.

Total irritancy scores = [(sum of inflammation scores)X2] + (sum of tissue response scores)
The ranked irritancy score = $[\text{average irritancy score}]_{\text{test article}}$ – $[\text{average irritancy score}]_{\text{blank microsphere}}$

Non-irritant = 0.0 up to 2.9
Slight irritant = 3.0 up to 8.9
Moderate irritant = 9.0 up to 15.0
Severe Irritant = > 15

Main Study (Group 1 to 4):
At Day 210/211, the total irritancy score of Group 1 ranged from 5.0 to 19.0 with an average irritancy score of 13.0 and the total irritancy score of Group 4 ranged from 4.0 to 26.0 with an average irritancy score of 20.2. The test article at 75 mg/ml microsphere was determined to be a slight irritant. At Day 363/364, there were no microspheres present so this evaluation could not be performed.

Summary of ISO 10993 Part 6 Evaluation Scores at Day 210/211

Group	Concentration Microspheres/TCA (mg/ml)	Study Day	Average Irritancy Score	Ranked Irritancy Score Compared to Control	Irritancy Conclusion
1 (Right Knee)	75/0	210/211	13.0	NA	NA
3 (Right Knee)	25/6.25	210/211	15.7	NA	NA
4 (Right Knee)	75/18.75	210/211	20.2	7.2	Slight Irritant

NA= Not Applicable

Subset Study (Group 5 to 8):
At Day 92/93, the ranked irritancy scores for the FX006 microspheres at 25 mg/ml or 75 mg/ml were 0.0. Therefore, the test article at these concentrations was determined to be a non-irritant at Day 92/93. At Day 243/244, there were no microspheres present so this evaluation could not be performed.

Summary of ISO 10993 Part 6 Evaluation Scores at Day 92/93

Group	Concentration Microspheres/TCA (mg/ml)	Study Day	Average Irritancy Score	Ranked Irritancy Score Compared to Control	Irritancy Conclusion
5 (Left Knee)	25/0	92/93	14.3	NA	NA
5 (Right Knee)	75/0	92/93	16.2	NA	NA
7 (Right Knee)	25/6.25	92/93	13.2	0.0	Non-Irritant
8 (Right Knee)	75/18.75	92/93	15.0	0.0	Non-Irritant

NA= Not Applicable

Toxicokinetics

The systemic TK values (both C_{max} and AUC) of TCA were higher in animals treated with TCA IR (Group 2) compared to the test article (an ER formulation, Group 3 and 4). The systemic levels of TCA were dose-dependent but do not appear to accumulate over time in animals treated with the test article (Group 3 and 4). There was no significant difference of systemic TCA levels in males and females.

Groups 1-4:

On Days 1, 90 and 181/182, whole blood samples (approximately 1 mL/sample) were collected from 3 animals/sex in Groups 1-4 at the following timepoints:

0 hour (pre-dose)	8 hours post-dose
1 hour post-dose	12 hours post-dose
2 hours post-dose	24 hours post-dose
4 hours post-dose	48 hours post-dose

In addition, 7, 14, 30 and 60 days after each dose, whole blood samples (approximately 1 mL/sample) were collected from three animals in Groups 1-4 (3/sex/group).

Whole blood samples (approximately 1 mL/sample) were collected from three animals in Groups 1-4 (3/sex/group) on Days 210 (1 month recovery) 270 (3 month recovery), 300 (4 month recovery) and 360 (6 month recovery).

Groups 5-8:

On Days 1, 30 and 60/61, whole blood samples (approximately 1 mL/sample) were collected from 3 animals/sex in Groups 5-8 at the following timepoints:

0 hour (pre-dose)	8 hours post-dose
1 hour post-dose	12 hours post-dose
2 hours post-dose	24 hours post-dose
4 hours post-dose	48 hours post-dose

In addition, 7 and 14 days, after each dose, whole blood samples (approximately 1 mL/sample) were collected from three animals in Groups 5-8 (3/sex/group).

Whole blood samples (approximately 1 mL/sample) were collected from three animals in Groups 5-8 (3/sex/group) on Days 90 (1 month recovery), 120 (2 month recovery), 150 (3 month recovery), 180 (4 month recovery) and 240 (6 month recovery).

Toxicokinetic Parameters for TCA in Plasma Following Repeated Intra-Articular Injections

N=3/sex/group		Main Study (3-month dosing interval)				Subset Study (1-month dosing interval)			
		1 CT	2 Kenalog-40	3 6.25 mg	4 18.75 mg	1 CT	2 Kenalog-40	3 6.25 mg	4 18.75 mg
Dose 1	C_{max} (ng/mL)	-	47.2 ± 9.87	1.38 ± 0.532	3.68 ± 2.380	-	49.6 ± 11.21	1.10 ± 0.491	3.64 ± 2.208
	$AUC_{0\text{-}1\ day}$ (ng*day/mL)	-	26.0 ± 5.089	1.0 ± 0.45	2.5 ± 1.57	-	28.3 ± 8.565	0.84 ± 0.393	2.8 ± 1.83
	$AUC_{0\text{-}\infty}$ (ng*day/mL)	-	83.7 ± 14.69	18.0 ± 9.77	38.4 ± 7.75	-	85.4 ± 25.26	11.4 ± 3.51	34.0 ± 15.57
	$T_{1/2,e}$ (day)	-	15.4 ± 22.70	21.9 ± 17.8	29.3 ± 20.15	-	16.5 ± 18.00	15.9 ± 13.86	14.6 ± 9.54
Dose 2	C_{max} (ng/mL)	-	42.2 ± 12.85	1.54 ± 0.686	6.23 ± 3.599	-	48.3 ± 22.71	2.35 ± 0.640	3.61 ± 1.616
	$AUC_{0\text{-}1\ day}$ (ng*day/mL)	-	22.9 ± 7.70	1.2 ± 0.53	4.3 ± 1.52	-	24.3 ± 12.55	2.0 ± 0.57	2.7 ± 1.24
	$AUC_{0\text{-}\infty}$ (ng*day/mL)	-	74.3 ± 9.98	24.4 ± 15.26	53.6 ± 13.47	-	85.5 ± 19.71	19.2 ± 6.35	50.8 ± 12.50
	$T_{1/2,e}$ (day)	-	22.6 ± 19.55	54.1 ± 78.49	25.0 ± 23.70	-	13.7 ± 11.01	6.7 ± 2.71	22.4 ± 15.00
Dose 3	C_{max} (ng/mL)	-	35.3 ± 20.59	1.26 ± 0.895	4.94 ± 1.071	-	35.7 ± 22.81	1.95 ± 1.142	3.81 ± 0.883
	$AUC_{0\text{-}1\ day}$ (ng*day/mL)	-	18.6 ± 9.33	1.0 ± 0.68	3.5 ± 0.81	-	20.6 ± 13.97	1.6 ± 0.95	3.0 ± 0.52
	$AUC_{0\text{-}\infty}$ (ng*day/mL)	-	85.7 ± 29.2	13.6 ± 4.11	46.2 ± 8.70	-	71.5 ± 26.67	16.7 ± 5.56	48.1 ± 6.26
	$T_{1/2,e}$ (day)	-	60.1 ± 84.92	18.5 ± 13.29	20.9 ± 14.88	-	8.6 ± 3.57	12.7 ± 11.67	14.1 ± 3.26

CT (blank microspheres/diluent)

The Applicant also submitted a synovial TCA evaluation report, but it does not appear to provide a meaningful data because there appear to be large variations with a low number of samples measured. Most of samples were categorized with aliquot not received, sample volume insufficient for analysis, or not reportable value. Therefore, it is not reviewed fully.

Dosing Solution Analysis

Acceptable

Vehicle control and blank microsphere preparations were devoid of TCA. All TCA dosing formulations were considered acceptable with regard to concentration and homogeneity.

FX006 low dose preparations had an overall label claim of (b) (4)%, FX006 high dose preparations had an overall label claim of (b) (4)%, and the TCA IR dose preparations had an overall label claim of (b) (4)% and (b) (4)% during the first and last week of dosing, respectively and were all considered acceptable.

4 Page(s) has been Withheld in Full as b4 (CCI/TS) immediately following this page

(b) (4)

7 Genetic Toxicology

No genetic toxicology studies were conducted but the Applicant nor required to support this 505(b)(2) application. There are no data in the referenced product labeling or identified via a literature search. The Applicant performed computational toxicity assessment on impurities using DEREK and Leadscope evaluations. No potential risks of genotoxicity were predicted for impurities including (b) (4) using DEREK and Leadscope analyses. These studies were briefly summarized below.

Title: DEREK Evaluation of FX006 Structures

Study Number: FX006TOX-2013-001

Method: Six FX006 structure (b) (4) were evaluated for potential toxicity and genetic toxicity using DEREK 3.0.1 Nexus 1.5.

(b) (4)

Summary: None of these chemical structures generated genotoxicity or carcinogenicity alert except (b) (4) (Structure 4). Structure 4 alerted for mutagenicity in vitro ((b) (4) (b) (4)

Reviewer's Note: [redacted] was re-evaluated by CDER/OTS/OCP/DARS for bacterial mutagenicity using (Q)SAR models (DEREK Nexus 5.0.2 (DX), Leadscope Model Applier 2.2.1-1 (LMA), and CASE Ultra 1.6.2.1 (CU). Consistent with the Applicant's result, [redacted] was predicted to be positive in Salmonella and *E. coli* TA100 and TA102 mutagenicity in DEREK analysis due to the presence of the [redacted] adequate genetic toxicology studies for the reference product, Kenalog-40 IR, have not been completed. [redacted] Data were not supplied in the NDA to support that conclusion. However, daily exposure to [redacted] is below acceptable daily intake [redacted] per ICH M7 guidance on genotoxic impurities in therapeutic products given the current specification and in vitro dissolution data discussed previously.

Title: Computational Toxicity Assessment Using the Leadscope Model Applier

Study Number: FX006TOX-2015-001

Method: [redacted] was examined for bacterial mutagenicity using the Leadscope model applier, which is a statistical-based (Q)SAR method that meets the ICH M7 recommendation for a statistical-based method.

Summary: [redacted] was within the Leadscope domain of applicability of bacterial mutagenicity models and it is predicted to be negative for Ames.

[redacted]

Title: DEREK and Leadscope Evaluation of FX006 Unspecified Impurities

Study Number: FX006TOX-2016-001

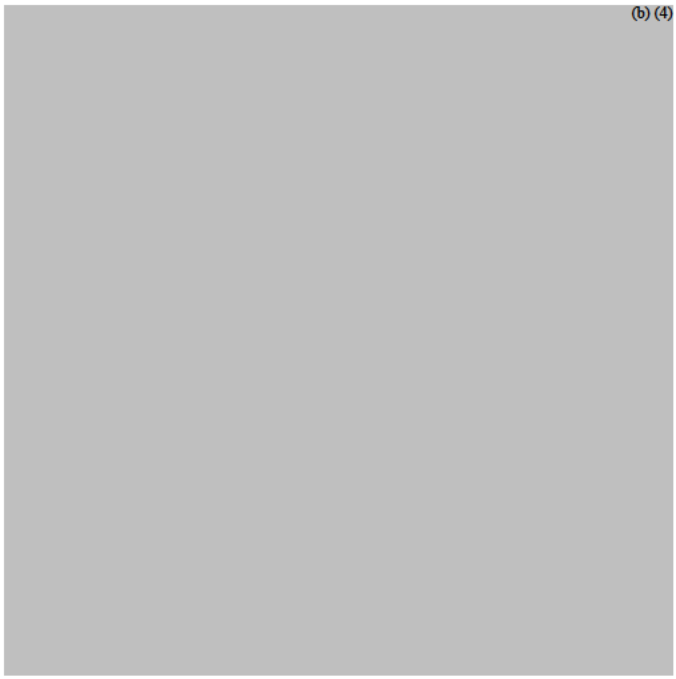
Method: TCA and three impurities of TCA (impurities at RT [redacted] see the table below) were evaluated for potential toxicity associated with their chemical structures using DEREK and Leadscope systems.

Relative Retention Time	Substance (original name)	Specified Substance (chemical name)
RRT (b) (4)	Impurity A	Unspecified
RRT	Impurity B	(b) (4)
RRT	Impurity C	Unspecified

* Impurity B (b) (4)
+ Impurity C (b) (4)

Note that Impurity B is now known as (b) (4)

(b) (4)



Summary: DEREK and Leadscope analyses indicate that all four chemical structures have no significant alerts relating to genotoxicity.

8 Carcinogenicity

No carcinogenicity studies were conducted by the Applicant or were required to support this NDA. There are no carcinogenicity data in the referenced product labeling.

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicology studies were submitted with the NDA or required for this 505(b)(2) application. As part of the requirement under the Pregnancy Lactation and Labeling Rule (PLLR), the Applicant conducted a review of the literatures. Thirty one studies were identified via an adequate search of relevant databases and the articles were submitted with the NDA. Among those literature studies, 12 studies were selected for review and summarized in this section.

9.1 Fertility and Early Embryonic Development

Craniofacial and Central Nervous System Malformations Induced by Triamcinolone Acetonide in Nonhuman Primates (NHPs): I. General Teratogenicity. Hendrickx AG, Pellegrini M, Tarara R, Parker R, Silverman S, and Steffek AJ. Teratology 22:103-114 (1980)

- Methods: Pregnant NHPs (18 rhesus monkeys (*Macaca mulatta*), 15 bonnet monkeys (*Macaca radiate*), and 6 baboons (*Papio cynocephalus*)) were treated with 5-20 mg/kg triamcinolone acetonide (TAC) between 21 and 43 days of gestation on single- or multiple-day treatment schedule.

Species and animal #	Days of treatment	Dose mg/kg	Pregnancy outcome				
			Delivery	Birth wt.	Sex	Gestational age	Age sacrificed
Rhesus monkeys							
1	23	20	CS-live	350	M	169	1 y. 9 mo.
2	25	20	V-live	250	M	144	1 y. 8 mo.
3	27	20	CS-live	425	M	168	1 y. 8 mo.
4	29	20	V-live	375	F	171	1 y. 7 mo.
5	21, 23, 25	10	CS-dead	222	M	165	–
6	25, 27, 29	10	CS-live	320	M	162	1 y. 9 mo.
7	25, 27, 29	10	V-live	407	M	169	1 y. 9 mo.
8	25, 27, 29	10	CS-live	525	M	175	DOB
9	23, 25, 27 29, 31	10	CS-live	300	F	170	DOB
10	23, 25, 27 29, 31	10	V-stillborn	410	M	153	–
11	23, 25, 27 29, 31	10	CS-live	306	F	158	DOB
12	23, 25, 27 29, 31	10	CS-dead	24	M	147	–
13	23, 25, 27 29, 31	10	V-abortion	221	F	137	–
14	23, 25, 27 29, 31	10	CS-live	112	F	118	DOB
15	27, 29, 31 39, 41, 43	10	CS-live	18	F	71	DOB
16	33, 35, 37 39, 41	10	CS-live	129	F	102	DOB
17	33, 35, 37 39, 41	10	CS-live	198	F	141	DOB
18	33, 35, 37 39, 41	10	CS-live	340	M	160	3 d.

Bonnet monkeys							
19	25	10	V-live	400	M	157	2 y. 6 mo.
20	29	10	V-live	360	F	169	2 y. 4 mo.
21	33	10	V-live	520	M	170	2 y. 4 mo.
22	25	15	V-stillborn	377	M	163	–
23	27	15	V-live	425	F	165	2 y.
24	25, 27, 29	10	V-neonatal death	412	M	176	1 d.
25	25, 27, 29	10	V-live	370	M	162	15 d.
26	23, 25, 27 29	10	CS-live	176	M	145	DOB
27	23, 25, 27 29, 31	10	CS-live	65	F	102	DOB
28	23, 25, 27 29, 31	10	CS-dead	14	M	99	–
29	23, 25, 27 29, 31	10	CS-dead	182	F	146	–
30	23, 25, 27 29, 31	10	CS-dead	56	M	151	–
31	23, 25, 27 29, 31	10	CS-dead	33	F	147	–
32	23, 25, 27 29, 31	10	CS-dead	60	F	146	–
33	23, 25, 27 29, 31	10	CS-live	261	M	148	DOB
Baboons							
34	23, 25, 27 29, 31	5	CS-live	104.6	F	100	DOB
35	23, 25, 27 29, 31	5	CS-live	112.2	F	102	DOB
36	23, 25, 27 29, 31	5	CS-live	118.3	M	99	DOB
37	23, 25, 27 29, 31	10	CS-live	81.9	F	102	DOB
38	23, 25, 27 29, 31	10	CS-live	75.6	F	100	DOB
39	23, 25, 27	10	resorption		–	49	–

CS, cesarean section; V, vaginal delivery; DOB, day of birth.

- o Key Findings related to teratogenic effects of TAC: Treatment with TAC induced severe craniofacial and CNS malformations, accompanied by an increased number of nonviable offspring and prenatal deaths, in all groups tested.

  - Prenatal deaths (from 80 days gestation to term) were tripled in the bonnet monkey and doubled in the rhesus monkey. No significant increase in pre- or perinatal loss was observed in the baboon.
  - The incidence of prenatal mortality and stillbirth was 40%, 22%, and 16% in the TAC-treated bonnet monkey, rhesus monkey, and baboon, respectively, compared with 14.2%, 11.8%, and 0% incidence in the control colonies.
  - All prenatal deaths and stillbirths occurred in the multiple-day treatment groups.
  - One resorption occurred at 49 days gestation in a baboon treated on a multiple-day schedule with 10 mg/kg TAC.

	Rhesus monkeys (*Macaca mulatta*)		Bonnet monkeys (*Macaca radiate*)		Baboons (*Papio cynocephalus*)	
Treatment Day(s)	**Single**	**Multiple**	**Single**	**Multiple**	**Single**	**Multiple**
Cranial Malformation						
Craniofacial dysmorphia	4/4	14/14	3/5	10/10		5/5
Aplasia cutis congenita	1/4	1/14	3/5	2/10		1/5
Cranum bifidum occultum	2/4	2/14	4/5	2/10		1/5
Cranium bifidum	2/4	12/14	1/5	7/10		2/5
Interparietal sutures	0/4	3/14	0/5	0/10		0/5
Arched palate	3/4	10/14	0/5	5/10		0/5
Cleft palate	0/4	2/14	0/5	3/10		0/5
Exophthalmia	1/4	10/14	0/5	3/10		3/5
Low-set ears	0/4	7/14	0/5	3/10		1/5
CNS malformation						
Meningocele	2/4	5/14	1/5	7/10		1/5
Encephalocele	0/4	7/14	0/5	0/10		1/5
Mild occipital lobe hypoplasia	2/4	0/14	1/5	0/10		4/5
Occipital lobe hypoplasia	2/4	5/14	3/5	3/10		
Temporal lobe hyperplasia						1/5
Mild cerebellar hypoplasia	2/4	3/14	2/5	2/10		4/5
Moderate cerebellar hypoplasia	0/4	4/14	0/5	3/10		0/5
Severe cerebella distortion	0/4	4/14	0/5	0/10		1/5
Mild hydrocephalus	3/4	3/14	0/5	0/10		1/5
Hydrocephalus	0/4	3/14	0/5	3/10		
Mild midbrain beaking	1/4	0/14	2/5	0/10		
Moderate midbrain beaking	0/4	3/14	0/5	2/10		
Severe midbrain beaking/discortion	0/4	4/14	0/5	0/10		
Agyria/polygyria	0/4	5/14	0/5	0/10		
Thinned/absent corpus callosum	0/4	3/14	0/5	0/10		2/5
Miscellaneous skeletal/visceral malformations						
Growth retardation	1/4	5/14	0/5	4/10		5/5
Scoliosis	0/4	1/14	0/5	2/10		2/5
Leg hyperextension	0/4	0/14	0/5	4/10		
Talipes	0/4	0/14	0/5	4/10		
Syndactyly/webbed digits	3/4	7/14	4/5	5/10		
Visceral abnormalities	1/4	9/14	3/5	4/10		2/5

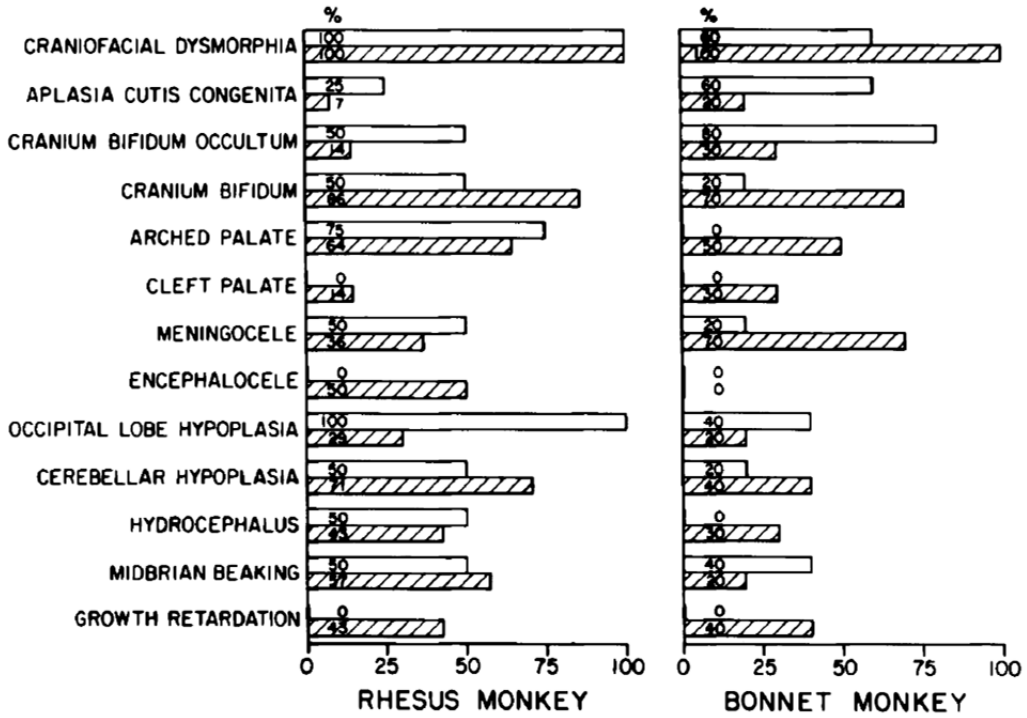


Fig. 1. Percent major malformations associated with single and multiple days of TAC treatment in rhesus and bonnet monkeys. Defects were of similar distribution with single (▭) and multiple (▨) day treatments, but increased in severity with multiple doses.

- Reviewer's note: Following review of the literature, TAC treatment during the organogenesis appears to induce severe craniofacial and CNS malformations, accompanied by an increased number of nonviable offspring and prenatal deaths in three different NHPs. The TAC dosages tested in NHPs (5 to 20 mg/day) were higher (167 – 387 mg daily based on the HED conversion) than the range of therapeutic dosage (32 mg) used in human. Nonetheless, there are risks of teratogenic effects to human and it is considered appropriate for the Zilretta label.

Comparative Teratogenicity of Triamcinolone Acetonide and Dexamethasone in the Rhesus Monkey (*Macaca mulatta*), Jerome CP and Hendrickx AG. J Med Primatol. 17:195-203 (1988)

- Method: Ten adult rhesus females (*Macaca multta*) were treated with TAC (Kenalog-40, ER. Squibb and Sons, Inc., Princeton, New Jersey) at 0.5 or 2.5 mg/kg IM on 22-50 ± 3 gestational day (GD). DEX (decadron phosphate, Merck, Sharp and Dohme, West Point, PA) was administered at 1.0 or 10 mg/kg IM to 12 animals at various times between 23 and 49 GD. A control group consisted of 2 concurrent untreated animals and 9 animals which had served as untreated controls in previous studies (Hendrickx AG et al, 1980). Pregnancies were terminated by hysterotomy on 100 ± 3 GD with the exception of 2 fetuses exposed to 2.5 mg/kg TAC and 1 historical control which were removed on 60 GD.

Drug	Dose Group (mg/kg)	(N)	Days of treatment	Total dose (mg)
TAC	0.5	(3)	22–50 ± 3	13.5
	2.5	(7)	22–50 ± 3	67.5
DEX	1.0	(3)	23,25,27,29,31	5.0
		(3)	23–49 (alternate days)	14.0
	10.0	(2)	33	10.0
		(2)	33,35,37	30.0
		(2)	23,25,27,29,31	50.0

- Key Findings related to teratogenic effects of TAC:
  - Two fetuses treated with 2.5 mg/kg TAC aborted spontaneously prior to 100 GD: a nongravid uterus by rectal palpation on GD 50 and fragments of placental tissue recovered on 97 GD from the other.
  - Three fetuses treated with 2.5 mg/kg TAC were dead at 100 GD.
  - The incidence and severity of gross malformations were greater for those treated with TAC than those treated with DEX.
  - TAC-treated fetus showed similar findings from the previous study (Hendrickx AG et al, 1980); occipital encephalocele and hydrocephalus, occipital meningoceles, poor development of the underlying calvarium and large vascular sinuses within the tentorium cerebelli, a cleft in the hard plate, aplasia curtis congenita.
  - Significant body weight losses were observed in fetuses treated with 0.5 mg/kg TAC and brain weights were observed in animals treated with 0.5 mg/kg TAC and 10.0 mg/kg DEX due to occipital lobe hypoplasia.
  - Markedly decreased ossification of the frontal and parietal bones (cranium bifidum) was observed in TAC-treated animals (see table below).
    - The MTD (transverse diameter of mild cranial fossa), PTD (transverse diameter of posterior cranial fossa), and lesser wings of the sphenoid were significantly smaller in animals treated with 2.5 mg/kg TAC and 10 mg/kg DEX.

Pregnancy outcome and malformations in fetuses treated with TAC or DEX

Drug	Dose group (mg/kg)	Deaths	Malformations[1]					
			E	M	CB	ACC	CP	Total[2]
DEX	1.0	0/6	0/6	0/6	0/6	2/6	0/6	2/6
	10.0	0/6	0/6	0/6	1/6	3/6	0/6	4/6
TAC	0.5	0/3	0/3	0/3	2/3	2/3	0/3	3/3
	2.5	⩾5/7[3]	1/5	2/5	2/5	0/5	1/5	4/5[2]

[1]E=encephalocele; M=meningocele; CB=cranium bifidum; ACC= aplasia cutis congenita; CP=cleft palate.
[2]Some animals had multiple malformations
[3]Two viable fetuses were removed on GD 60.

Mean weights and cephalometric measurements in 100-day rhesus monkey fetuses treated with TAC and DEX compared with controls[1]

Drug	Dose group (mg/kg)	(N)	Body weight (g)	Brain weight (g)	MTD (mm)	PTD (mm)
Control	—	(7–10)	135.3 ± 16.1	17.2 ± 1.5	32.2 ± 1.2	24.1 ± 1.4
TAC	0.5	(3)	109.7 ± 4.5*	15.3 ± 0.5**	29.8 ± 1.0**	22.5 ± 0.5
DEX	1.0	(6)	136.1 ± 7.8	17.7 ± 0.8	32.6 ± 1.6	23.7 ± 1.2
	10.0	(6)	125.3 ± 15.3	15.5 ± 1.8**	30.4 ± 1.5**	22.0 ± 1.4**

[1]MTD = transverse diameter of middle cranial fossa; PTD = transverse diameter of posterior cranial fossa.
*$p < 0.025$.
**$p < 0.05$.

- Reviewer's note: Following review of the literature, TAC treatment during the organogenesis appears to induce craniofacial and CNS malformations, accompanied by an increased number of nonviable offspring and prenatal deaths in NHPs. The TAC dose ranges tested in NHPs (0.5 to 2.5 mg/day, which correspond to 9.7 and 48 mg based on the HED conversion) falls within the range of therapeutic dosage (32 mg) in human. Therefore, there are some risks of teratogenic effects to human and it is considered appropriate for the Zilretta label.

Induction of Cleft Palate in Rabbits by Several Glucocorticoids. Walker BE. Proc Soc Exp Biol Med. 125(4):1281-1284 (1967)

- Method: New Zealand White or American Dutch female rabbits were treated with various doses of glucocorticoids (see the tables below) intramuscularly for 4 days, starting 13.3 days post conception. Animals were euthanized on the 21st day post conception.

- Key Findings related to teratogenic effects of TAC:
  - TAC induced cleft palate in daily doses ranging from 0.10 to 1 mg/day and litter resorptions were observed at higher doses (1 to 5 mg/day) in New Zealand White rabbits.
  - TAC also induced cleft palates in American Dutch rabbits treated from 0.2 to 2 mg/day and litter resorptions were observed.
  - Although American Dutch rabbits weigh less than half compared to the New Zealand White rabbits, Cleft palates were observed at higher (2X) dose ranges.

Litter Resorption, Fetal Resorption and Cleft Palate Development in New Zealand White Rabbits after Various Doses of Three Glucocorticoids

Drug	Dose, mg/day*	No. of litters		No. of fetuses		Palate morphology	
		Treated	Resorbed	Living	Resorbed†	Normal	Cleft
Triamcinolone	5.00	1	1				
	2.00	3	3				
	1.00	7	2	32	6	15	17
	.75	2	0	14	2	10	4
	.50	3	0	11	3	9	2
	.30	1	0	4	0	4	0
	.25	4	0	28	0	24	4
	.10	3	0	23	0	19	4
	.01	8	0	55	1	55	0
Dexamethasone	4.00	2	2				
	3.00	2	2				
	2.50	2	0	11	0	3	8
	2.00	2	1	4	0	4	0
	1.00	7	3	26	3	10	16
	.50	3	1	14	3	11	3
	.25	1	0	1	5	0	1
	.10	3	0	25	0	25	0
Prednisolone	8.00	2	2				
	4.00	6	4			11	2
	3.00	4	1	17	10	13	4
	2.00	4	1	16	2	14	2
	1.50	3	1	9	10	8	1
	1.00	5	1	36	0	36	0

* Dose listed was given each of 4 days from 13⅓ to 16⅓ days postconception.
† Resorbed embryos in litters having one or more living embryos.

Fetal Resorption and Cleft Palate Development in American Dutch Rabbits after Various Doses of Triamcinolone

Dose, mg/day	No. of litters	No. of fetuses		Palate morphology	
		Living	Re-sorbed	Normal	Cleft
4.00	5	0	32	0	0
2.00	2	5	5	0	5
1.00	3	4	12	0	4
.60	2	9	5	4	5
.30	4	24	4	13	11
.20	7	27	8	26	1
.10	7	32	5	32	0
.01	3	16	1	16	0

- Reviewer's note: Following review of the literature, TAC treatment for 4 days from GD 13 to 16 appears to induce teratogenic effects such as cleft palate and fetal resorption. The TAC dose ranges showing teratogenic effects in rabbits (0.1 to 5 mg/day which corresponds 2 to 96 mg based on the HED conversion) fall within the range of therapeutic dosages used in human (32 mg). Therefore,

there are some risks of teratogenic effects to human and it is considered appropriate for the Zilretta label.

Comparative Teratogenicity of Triamcinolone Acetonide, Triamcinolone, and Cortisol in the Rat. Rowland JM and Hendrickx AG. Teratogenesis, Carcinogenesis and Mutagenesis. 3:313-319 (1983a)

- Method: Pregnant Sprague-Dawley rats were injected with TAC (Kenalog) via IM. At 20 GD, pregnant females were euthanized by cervical dislocation and the uterus was examined for the number of live and dead fetuses and resorption. Live fetuses were weighed, sexed, and examined for external malformations.

Test	Drug Tested	Dose	Days of Treatment
1	TAC	0.5 mg/kg	12, 13, or 14 GD
2	TAC	0.5, 2.5, or 5 mg/kg	13
	Triamcinolone (TA)	10, 20, or 40 mg/kg	13
	Cortisol	125 or 500 mg/kg	13

- Key findings related to teratogenic effects of TAC:
  - While all TAC-treated group at GD 12, 13, or 14 (Test 1) showed an increased proportion of fetuses with cleft palate compared to an untreated control group ($p<0.05$), TAC-treated group at 13 GD showed the highest incidence of cleft palate, suggesting that GD 13 is the most sensitive day for cleft palate induction by TAC in the rat. Fetal weights were also reduced in TAC-treated group but there were no increases in the rate of resorption or fetal death in Test 1.
  - Among three different drug tested in Test 2 (see the study design table above), TAC was 59 times as potent as TA in inducing cleft palate: ED_{50} of 1.1 mg/kg and 65 mg/kg, respectively. TAC treatment also induced weight reduction. Cortisol treatment induced partial cleft palate but it appears to be much less potent compared to TAC. Other developmental abnormalities from TAC treatment include umbilical hernias, resorption, fetal growth retardation and fetal death.

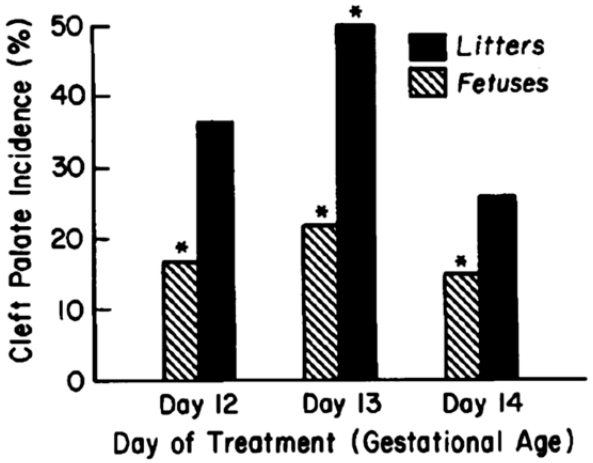


Fig. 1. Cleft palate incidence following a single injection of 0.5 mg/kg TAC. The solid bars represent the percentage of affected litters, and the hatched bars represent the percentage of affected fetuses. The asterisks denote a significant difference ($P < 0.05$) from the control values.

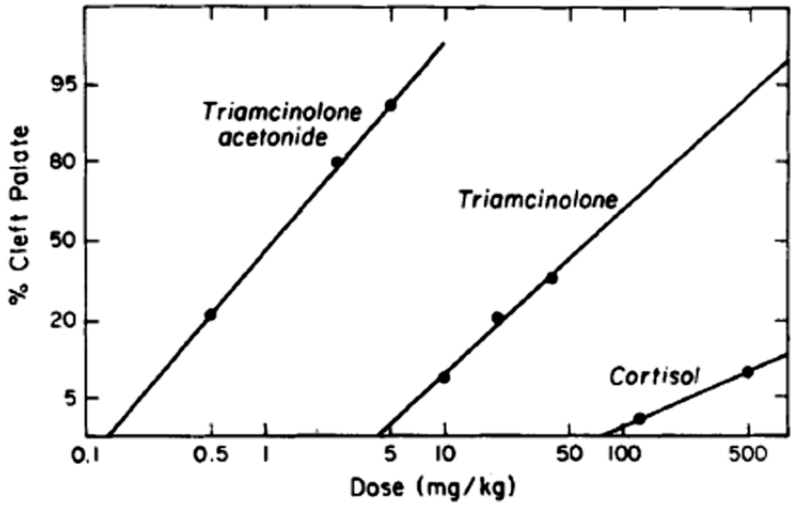


Fig. 2. Dose-response for corticosteroid-induced cleft palate. Triamcinolone acetonide, triamcinolone, or cortisol was injected intramuscularly on day 13 of gestation and fetuses were examined for cleft palate on day 20. The results are presented in a log-probit plot and represent the percentage of affected fetuses at each dose (51–156 fetuses/point). The ED_{50} for cleft palate induction was calculated to be 1.1 mg/kg for triamcinolone acetonide and 65 mg/kg for triamcinolone. The efficacy of cortisol in inducing cleft palate was too low to calculate an ED_{50}.

TABLE I. Teratogenicity of Corticosteroids Administered on Day 13 of Gestation

Agent	Dose	Number of litters	Number of fetuses	Percent cleft palate		Percent umbilical hernias		Fetal weight $\bar{X} \pm SD$	
				Litters	Fetuses	Litters	Fetuses	Males	Females
Physiological saline	1 cc/kg	10	140	0	0	0	0	4.03 ± 0.32	3.76 ± 0.57
Triamcinolone acetonide	0.5	12	140	50[a]	21.4[c]	16.7	3.6	3.55 ± 0.33[b]	3.42 ± 0.36
	2.5	10	91	100[c]	79.1[c]	100.0[b]	94.5[c]	1.88 ± 0.64[c]	1.81 ± 0.59[b]
	5.0	6	51	100[c]	92.2[c]	100.0[a]	82.4[c]	1.98 ± 0.48[c]	1.90 ± 0.44[c]
Triamcinolone	10	11	147	45.5	7.5[c]	9.1	0.7	3.67 ± 0.25	3.52 ± 0.26
	20	10	133	80.0[c]	20.3[c]	30.0	3.0	3.27 ± 0.44[c]	3.24 ± 0.39[a]
	40	11	150	90.9[c]	34.0[c]	9.1	0.7	3.18 ± 0.44[c]	3.03 ± 0.43[c]
Cortisol	125	10	133	30	3.0	0	0	3.82 ± 0.19	3.63 ± 0.24
	500	12	156	50[a]	8.3[c]	8.3	0.6	3.07 ± 0.36[c]	2.99 ± 0.36[c]

[a]Significant difference from controls ($P < 0.05$).
[b]Significant difference from controls ($P < 0.01$).
[c]Significant difference from controls ($P < 0.005$).

- Reviewer's note: Following review of the literature, the most sensitive day of gestation for cleft palate induction by TAC in rats is GD 13. TAC was the most potent inducing cleft palate compared to TA or cortisol. The TAC dose ranges tested in SD rats (0.5 to 5 mg/day which corresponds 4.8 to 48 mg based on the HED conversion) fall within the range of therapeutic dosages used in human (32 mg). Therefore, there are risks of teratogenic effects to human and it is considered appropriate for the Zilretta label.

Teratogenicity of Triamcinolone Acetonide in Rats. Rowland JM and Hendrickx AG. Teratology. 27:13-18 (1983b)

- Method: Pregnant female Sprague-Dawley rats were treated with triamcinolone acetonide (TAC) intramuscularly once daily for three consecutive days (GD 9-11, 12-14, or 15-17) with vehicle alone, 0.125, 0.25, or 0.5 mg/kg TAC and they were euthanized on GD 20.

- Key findings related to teratogenic effects of TAC:
  - GD 12-14 was the most sensitive period of teratogenic effects of TAC and within the periods, dose-dependent fetal malformation and fetal mortality were observed.
  - Umbilical hernias and undescended testes were also observed in the high-dose group treated on GD 12-14.
  - Reduced degree of ossification was found in all TAC-treated groups but no specific skeletal malformation was observed.
  - Fetal weight reduction was also observed in all TAC-treated groups.
  - Teratogenic effects of TAC were elicited at doses causing no maternal lethality.
  - Fetal growth retardation can be produced at doses below those required to produce malformations and during GD where no malformation are produced.

TABLE 1. Effects of triamcinolone acetonide on prenatal development

Days gestation	Dose (mg/kg/day)	No. of litters	No. of fetuses	% Viable fetuses/litters (X ± S.D.)	% of litters with malformed fetuses	% of malformed fetuses/litter (X ± S.D.)
9–11	0	8	92	95.78 ± 4.88	0	0.00 ± 0.00
	0.125	7	76	97.22 ± 4.30	0	0.00 ± 0.00
	0.25	7	71	89.17 ± 51.12	0	0.00 ± 0.00
	0.5	7	101	82.93 ± 33.48	14.29	2.04 ± 5.40
12–14	0	11	130	97.04 ± 1.32	9.09	1.52 ± 1.96
	0.125	11	121	89.06 ± 13.68	45.46	28.79 ± 40.89
	0.25	10	87	77.49 ± 23.12**	80.00**	61.14 ± 37.09**
	0.5	10	97	58.33 ± 33.23*	100.00**	84.17 ± 33.44**
15–17	0	9	127	97.87 ± 3.20	0	0.00 ± 0.00
	0.125	8	111	95.88 ± 4.87	0	0.00 ± 0.00
	0.25	8	113	100.00 ± 0.00	0	0.00 ± 0.00
	0.5	8	108	91.92 ± 6.97**	62.5**	32.01 ± 33.37**

*Significant difference from controls ($p \leq 0.01$).
**Significant differences from controls ($p \leq 0.05$).

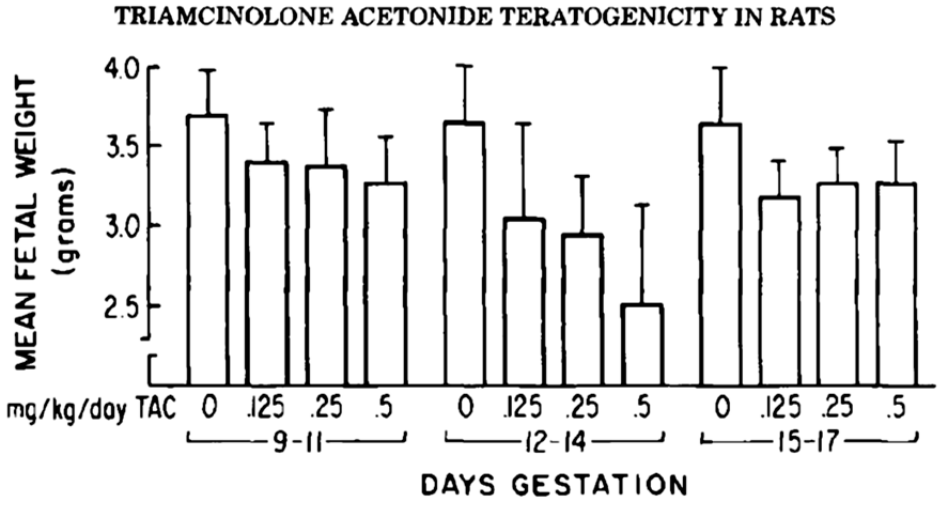


Fig. 1. Effect of TAC treatment on fetal body weight.

TABLE 2. Incidence of specific malformations induced by triamcinolone acetonide

Days gestation	Dose (mg/kg/day)	No. of fetuses/litter	% of fetuses/litter with: Cleft palate	Umbilical hernias	Undescended testes	Hypoplastic thymus
9–11	0–0.5	161/29	0/0	0/0	0/0	0/0
12–14	0	63/11	0/0	0/0	0/0	0/0
	0.125	60/11	23.33*/45.46*	3.33/9.09	0/0	0/0
	0.25	41/10	53.66*/60.00*	20.37*/50.00*	6.67/12.50	0/0
	0.5	54/10	70.37*/80.00*	57.85*/80.00*	27.59*/60.00*	0/0
15–17	0	64/9	0/0	0/0	0/0	0/0
	0.125–0.25	111/16	0/0	0/0	0/0	0/0
	0.5	54/8	18.52*/50.00	0/0	0/0	22.22*/37.50

*Significant difference from control values ($p \leq 0.05$).

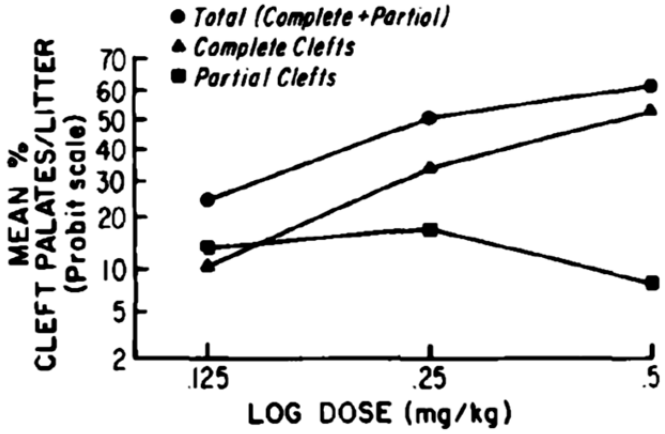


Fig. 2. Cleft palate incidence following treatment with TAC on days 12–14 of gestation.

- Reviewer's note: Following review of the literature, GD 12-14 was the most sensitive period of teratogenic effects of TAC and within the periods, dose-dependent fetal mortality and fetal malformation such as cleft palate, umbilical hernias, undescended testes, and fetal weight reduction were observed. Reduced degree of ossification without specific skeletal malformation was observed. The TAC dose ranges tested in SD rats (up to 0.5 mg/day which corresponds up to 4.8 mg based on the HED conversion) fall within the range of therapeutic dosages used in human (32 mg). Therefore, there are risks of teratogenic effects to human and it is considered appropriate for the Zilretta label.

Palatal slit and cleft palate in rats treated with glucocorticoids II. Comparative Teratogenicity of Prednisolone, Triamcinolone Acetonide and Hydrocortisone. Watanabe C, Ishizuka Y, and Nagao T. Cong. Anom. 35:133-140 (1995)

- Method: Pregnant female Sprague-Dawley rats were treated with either prednisolone (12.5 to 100 mg/kg/day), triamcinolone acetonide (0.25 to 2 mg/kg/day), or hydrocortisone (100 mg/kg/day) subcutaneously on GD 14 and 15.

- Key findings related to teratogenic effects of TAC:
  - The frequencies of cleft palate were significantly higher in the group treated with 100 mg/kg/day prednisolone and all groups treated with TAC than the control.
  - TAC was 70 times as potent as prednisolone in inducing palatal slit, with ED_{50} of 1.0 mg/kg/day and 70 mg/kg/day, respectively.
  - Hydrocortisone showed no potentiality for cleft palate and palatal slit induction.
  - Other developmental abnormalities induced by TAC and prednisolone included omphalocele and general edema, late resorption, and growth retardation.

Table 1 Frequency of cleft palate (CP), palatal slit (PS) and late resorptions in rat fetuses treated with various glucocorticoids on days 14 and 15 of gestation

Dose (mg/kg/day)	No. of dams	CP/live fetuses		PS/(live–CP) fetuses		Late resorptions/implants	
		No.	% ± SE	No.	% ± SE	No.	% ± SE
Control	21	0/326	0	0/326	0	1/345	0.25 ± 0.25
Prednisolone							
12.5	12	0/176	0	9/176	5.77 ± 6.03	0/179	0
25	14	0/220	0	9/220	4.63 ± 2.18	6/241	2.61 ± 0.84
50	11	6/170	3.41 ± 1.85	45/164	27.51 ± 9.47**	5/188	2.23 ± 1.54
100	9	13/123	10.59 ± 7.38**	87/110	77.69 ± 7.52**	19/145	8.13 ± 3.72*
Triamcinolone acetonide							
0.25	10	0/157	0	0/157	0	10/176	5.54 ± 1.93
0.5	10	13/153	8.56 ± 6.60*	30/140	26.27 ± 8.55**	5/169	2.89 ± 1.56
1	11	35/146	26.06 ± 6.61**	35/111	42.65 ± 11.70**	20/158	15.72 ± 4.76*
2	10	77/125	58.34 ± 8.87**	35/ 48	80.46 ± 7.83**	18/156	11.38 ± 3.70*
Hydrocortisone							
100	17	0/252	0	0/252	0	0/277	0

* Significantly different from control by Mann-Whitney's U test ($P < 0.05$)

** Significantly different from control by Mann-Whitney's U test ($P < 0.01$)

Table 2 Teratogenic potential of various glucocorticoids to induce cleft palate (CP) and palatal slit (PS) in rat fetuses

Compound	ED_{50}[a] (mg/kg/day)	
	CP	PS
Prednisolone	not calculated	70
Triamcinolone acetonide	1.4	1.0
Hydrocortisone	> 100	> 100
Dexamethasone[b]	6.0	3.2

[a] Effective dose for producing cleft palate or palatal slit with a frequency of 50% (data from Fig. 1).

[b] Data from Ishizuka et al. (1993)

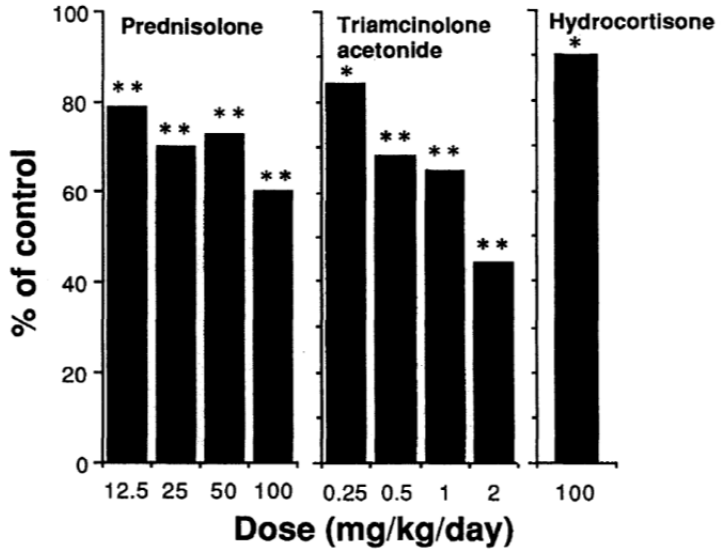


Fig. 2 Body weight (% of control) of fetuses of rats treated with prednisolone, triamcinolone acetonide, and hydrocortisone on days 14 and 15 of gestation.
* Significantly different from control by Mann-Whitney's U test ($P < 0.05$).
** Significantly different from control by Mann-Whitney's U test ($P < 0.01$).

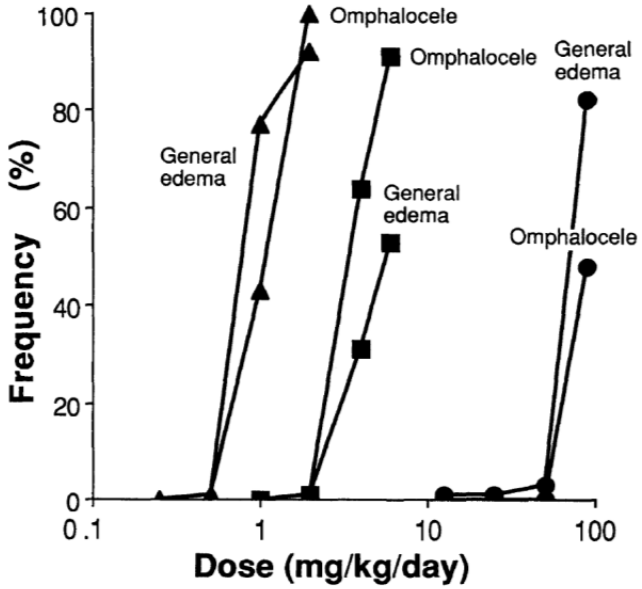


Fig. 3 Dose vs. frequency relations of omphalocele and general edema observed in fetuses of rats treated with prednisolone (●), triamcinolone acetonide (▲), and dexamethasone (■, data from Ishizuka et al., 1993) on days 14 and 15 of gestation.

- Reviewer's note: Following review of the literature, TAC was significantly more potent than prednisolone in inducing cleft palate and palatal slit. The most prevalent malformations other than palatal defects were omphalocele and general edema. The TAC dose ranges tested in SD rats (up to 2 mg/day which corresponds up to 19 mg based on the HED conversion) fall within the range of

therapeutic dosages used in human (32 mg). Therefore, there are risks of teratogenic effects to human and it is considered appropriate for the Zilretta label.

Reversible Effects of Triamcinolone and Lack of Effects With Aspirin or L-656,224 on External Genitalia of Male Sprague-Dawley Rats Exposed in Utero. Wise LD, Vetter CM, Anderson CA, Antonello JM, and Clark RL. Teratology. 44:507-520 (1991)

- Method:
  - Study 1: SD female rats were treated with TAC (Kenalog-10) subcutaneously at dose levels of 0.05 or 0.1 mg/kg/day in a volume of 2 mL/kg once daily from GD 11 to 19. Aspirin (75 or 150 mg/kg/day), L-656224 (a specific, orally active lipoxygenase inhibitor 1000 or 2000 mg/kg/day) were administered by oral gavage in a dose volume of 5 mL/kg. (Note: Data of aspirin or L-656224 were not included in Key findings listed below.)
  - Study 2: SD rats were subcutaneously treated with 0.1 or 0.25 mg/kg/day of TA either with or without co-administration of 100 mg/kg/day of arachidonic acid (AA) once daily from GD14 to 19. Control groups were treated with the vehicle or 100 mg/kg/day AA. All females were euthanized on GD 20 and examined for malformation.

- Key findings related to teratogenic effects of TAC:
  - Study 1:
    - F_0 females:
      - No mortality observed
      - Body weight reduction observed in animals treated with 0.05 or 0.1 mg/kg/day of TAC
    - F_1:
      - Mortality at birth observed and moribund litters during the first postnatal week in the 0.05 or 0.1 mg/kg/day TAC-treated groups
      - Unusual external appearance of the perineal region in all pups of TAC-treated groups at GD 23 (flattened genital tubercle and a strip of darkened skin (thinned and unusually glossy) between the genital tubercle and the anus)
      - Omphaloceles and cleft palate in pups of the 0.1 mg/kg/day of TAC-treated group
      - Statistically significant reduction (35-46%) in pup weights on GD23 observed in 0.05 and 0.1 mg/kg/day TCA-treated groups
      - Significant and dose-related reduction in absolute male anogenital distance (AGD) on GD23 (11 and 19% below the control value) observed

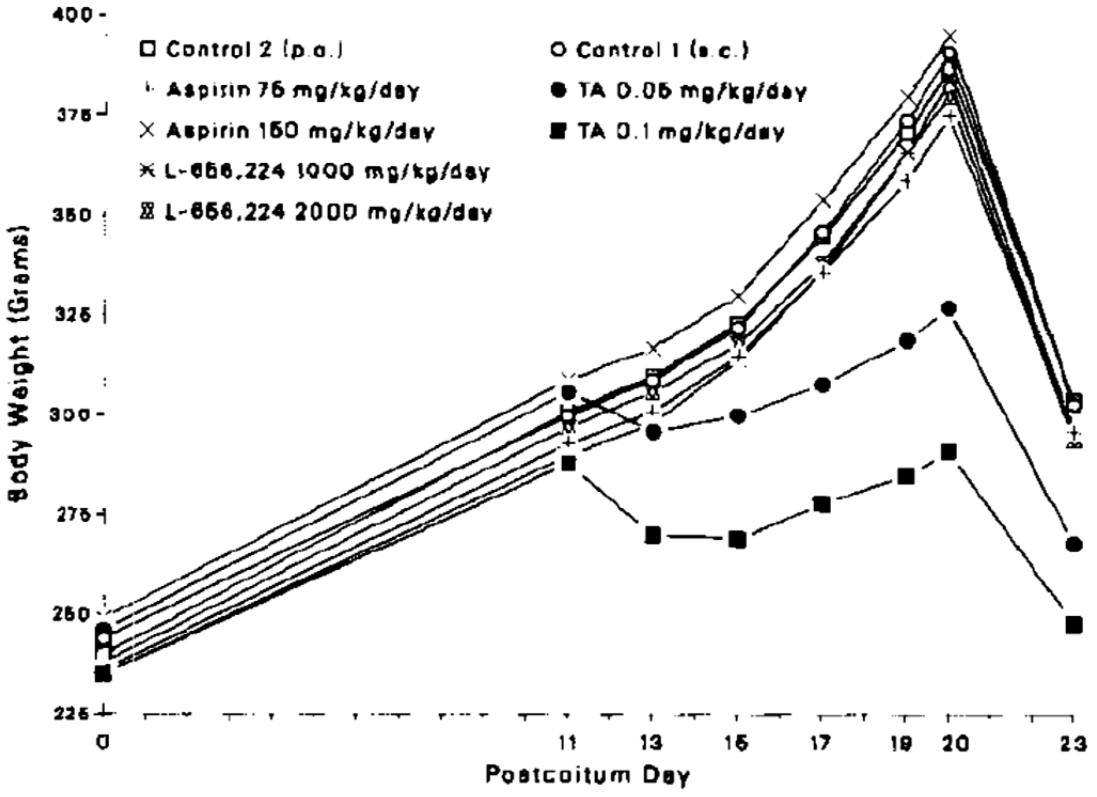


TABLE 1. Study 1—Summary of F1 generation (prior to weaning) exposed in utero to triamcinolone acetonide, aspirin, or L-656,224 from gestational days 11 to 19

Dose Group:	Control 1	Triamcinolone acetonide		Control 2	Aspirin		L-656,224	
mg/kg/day:	0	0.05	0.1	0	75	150	1000	2000
Route:	S.C.	S.C.	S.C.	P.O.	P.O.	P.O.	P.O.	P.O.
Pregnant females	10	10	10	10	10	10	10	10
Length of gestation (days)	22.2	22.6**	22.9***	22.2	22.1	22.4	22.2	22.2
Females with live pups on day 0	10	10	8	10	10	10	10	10
Females with live pups on day 21	9	7	0	10	9	10	0	10
Live pups per litter at birth	14.2	12.9	4.7***	13.3	12.1	14.5	14.8	14.5
% live pups at birth	98.7	96.7	45.3	99.3	93.3	99.2	98.7	100
Pup deaths (%)								
Postpartum days 0–3	1 (0.7)	42 (37)	47 (93)	0	0	1 (0.7)	10 (8)	1 (0.6)
Postpartum days 0–7	10 (6.0)	52 (45)	48 (100)	2 (1.5)	0	2 (1.4)	10 (8)	1 (0.6)
Live female pup weight								
Postcoitum day 23	6.5[a]	4.2***	3.5***	6.6	6.6	6.6	6.2	6.1
Postpartum day 7	15.6	9.4**		16.2	15.3	15.9	14.4	13.9*
Postpartum day 14	31.5	22.7**		31.6	31.1	31.5	b	29.3
Postpartum day 21	51.6	39.9**		51.7	51.1	52.5		48.3
Live male pup weight								
Postcoitum day 23	7.0	4.5***	3.8***	7.0	6.6	7.1	6.4	6.5
Postpartum day 7	16.8	10.0***		17.0	14.9	16.7	15.2	15.2
Postpartum day 14	33.8	24.9***		32.7	31.6	32.7	b	30.9
Postpartum day 21	55.4	42.2***		54.1	52.4	54.5		51.3

[a]Weights in grams.
[b]Group discarded on PND 7–11 due to no observable effects.
*,**,***Trend statistically significant through indicated dose ($P \leq 0.05, 0.01, 0.001$).

TABLE 2. Study 1—Summary of external alterations of pups exposed in utero to triamcinolone acetonide, aspirin, or L-656,224 on gestational days 11 to 19

Dose group:	Control 1	Triamcinolone acetonide		Control 2	Aspirin		L-656,224	
mg/kg/day:	0	0.05	0.1	0	75	150	1000	2000
Route:	S.C.	S.C.	S.C.	P.O.	P.O.	P.O.	P.O.	P.O.
No. examined externally	144	133	90	134	123	146	150	145
No. with malformations	1	0[1]	5(7)[1,2]	1	0	1	0	0
No. of malformations	1	0	12	1	0	1	0	0
No. of variations	0	0	0	0	0	0	0	0
Type + no. of alterations[1] (affected fetuses/litters)								
Anophthalmia	1	0	0	0	0	1	0	0
Microphthalmia	0	0	0	1	0	0	0	0
Omphalocele	0	0	10/2	0	0	0	0	0
Cleft palate	0	0	2/1	0	0	0	0	0

[1]Excludes anomalous external genitalia which occurred in all pups in triamcinolone groups as discussed in text.
[2]Numbers in parentheses represent pups dead at first observation.

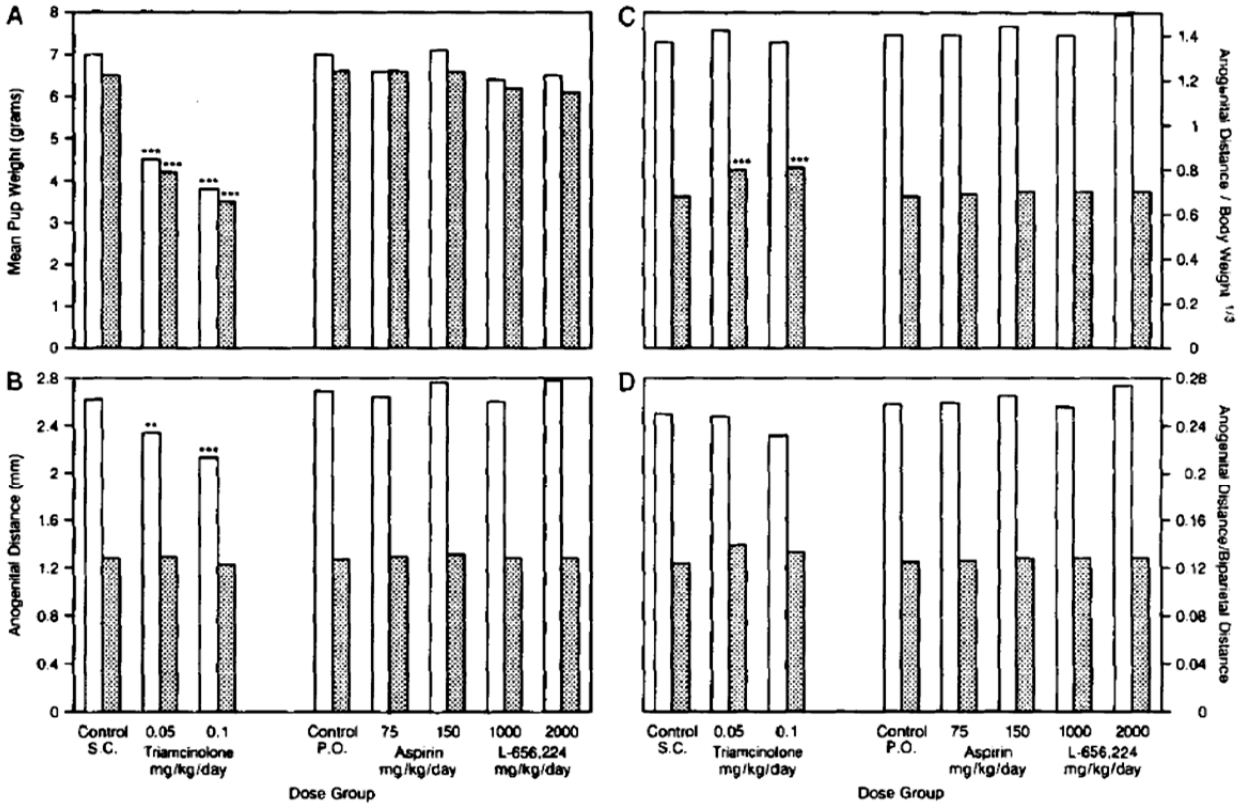


Fig. 2. Postcoitum day 23 results from Study 1 where dams received the various treatments on gestational days 11–19. **A.** Group means of pup weight. **B.** Absolute anogenital distance (AGD). **C.** Relative AGD, group means of AGD/cube root of body weight. **D.** Relative AGD, group means of AGD/biparietal distance (BPD). Open bars, males; stippled bars, females. **, ***: Trend statistically significant through indicated dose ($P \leq 0.05$, 0.01, 0.001, respectively).

TABLE 3. Study 1—Summary of developmental signs of F1 animals exposed in utero to triamcinolone acetonide, aspirin, or L-656,224 on gestational days 11 to 19

Dose group:	Control 1	Triamcinolone acetonide	Control 2	Aspirin	L-656,224
mg/kg/day:	0	0.05	0	150	2000
Route:	S.C.	S.C.	P.O.	P.O.	P.O.
Vaginal canalization					
Postnatal day 37					
Number examined	18	13			
Percent positive	83	64			
Preputial separation					
Postnatal day 39					
Number examined	18	14	20	20	20
Percent positive	0	0	5	15	0
Postnatal day 43					
Number examined	18	14	20	20	20
Percent positive	29	14	50	45	45
Postnatal day 47					
Number examined	18	14	18	20	20
Percent positive	67	64	80	90	75
Postnatal day 51					
Number examined	18	14	20	20	20
Percent positive	83	93	90	95	90
Testes descent					
Postnatal day 43					
Number examined	18	14	20	20	20
Percent positive	100	100	100	100	100

- Study 2:
  - F_0 females:
    - No mortality observed
    - Body weight reduction observed in animals treated with 0.1 or 0.25 mg/kg/day TAC with/without 100 mg/kg/day AA during GD 14 to 20
  - F_1:
    - Mortality observed in TAC-treated groups (0.1 and 0.25 mg/kg/day) but reduced mortality in TAC/AA-treated groups
    - Similar alterations in the appearance of the perineal region above (Study 1) and no improvement by co-administration of AA
    - Micrognathia and omphalocele were observed in 0.25 mg/kg/day TAC-treated groups and AA co-administration reduced the incidence of these malformations.
    - Cleft palate were only observed in the TAC-only treated group (0.25 mg/kg/day)
    - Dose-dependent reduction in fetal weight were observed in TAC groups (slightly lower body weight reduction by co-administration of AA)
    - Significant reduction in absolute male AGD in all TAC-treated groups on GD 20 and little effect by co-administration of AA

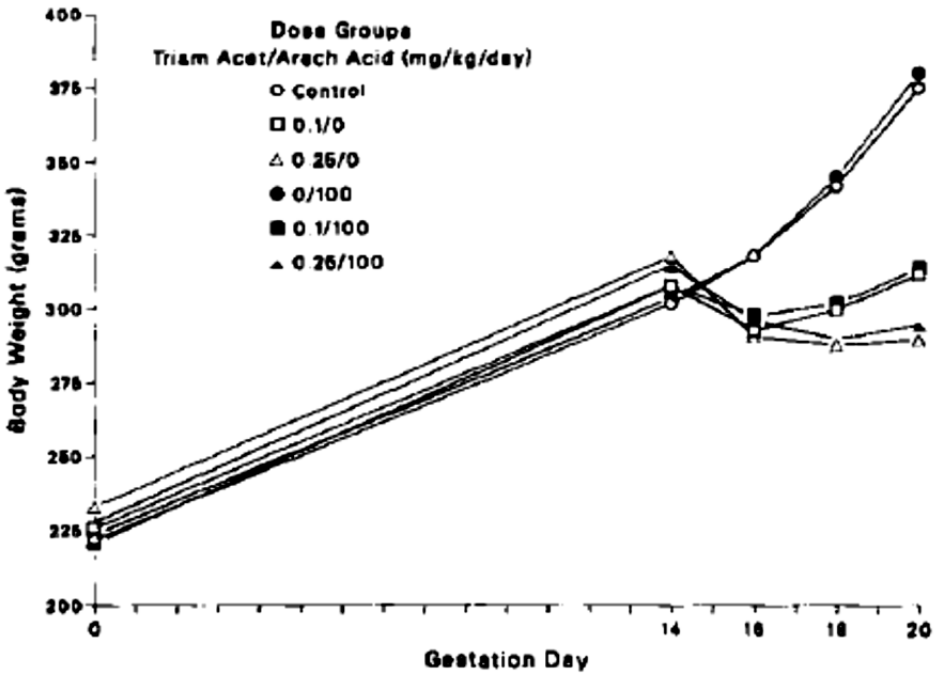


Fig. 3. Average maternal body weights of rats receiving triamcinolone acetonide and/or arachidonic acid on gestational days 14–19.

TABLE 4. Study 2—Summary of laparotomy data from females given triamcinolone acetonide and/or arachidonic acid on gestational days 14 to 19

Dose group:	Control	Triamcinolone acetonide		Arachidonic acid	Triamcinolone/ Arachidonic acid	
mg/kg/day:	0	0.1	0.25	100	0.1/100	0.25/100
Total females	8	6	6	8	6	6
Live pregnant	8	6	6	8	6	6
Implants/pregnant female	12.8	13.3	13.7	14.4*	14.0	13.3
Resorptions	3	1	3	7	6	6
Dead fetuses	0	2	3	0	0	1
% (resorptions + dead fetuses)/implants	2.9	3.6	7.5	6.3	7.7	8.8
Live fetuses/pregnant female	12.4	12.8	12.7	13.5	13.0	12.2

*Significantly different ($P \leq 0.05$) from control group.

TABLE 5. Study 2—Summary of external alterations of fetuses exposed in utero to triamcinolone acetonide and/or arachidonic acid on gestational days 14 to 19

Dose Group:	Control	Triamcinolone acetonide		Arachidonic acid	Triamcinolone Acet/ arachidonic acid	
mg/kg/day:	0	0.1	0.25	100	0.1/100	0.25/100
Fetuses						
Number examined[1]	99	77 (2)	76 (3)	108	78	73 (1)
Number with malformations[1]	0	77 (2)	76 (3)	9	71	73 (1)
Number of malformations[1]	0	78 (2)	168 (5)	9	71	103 (1)
Number with variations	0	0	0	0	0	0
Number of variations	0	0	0	0	0	0
Litters						
Number examined	8	6	6	8	6	6
Number with malformations	0	6	6	3	6	6
Number with variations	0	0	0	0	0	0
Affected fetuses/litters (%)						
Micrognathia	0	0	47/5 (59)	0	0	3/6 (4)
Cleft palate	0	0	1	0	0	0
Omphalocele	0	1 (1)	45/6 (55)	0	0	27/6 (35)
Perineal malformation	0	79/6 (100)	78/6 (99)	9/3 (8)	71/6 (92)	74/6 (100)
Tail malformation	0	0	1	0	0	0
Domed head	0	0	1	0	0	0

[1]Numbers in parentheses represent dead fetuses.

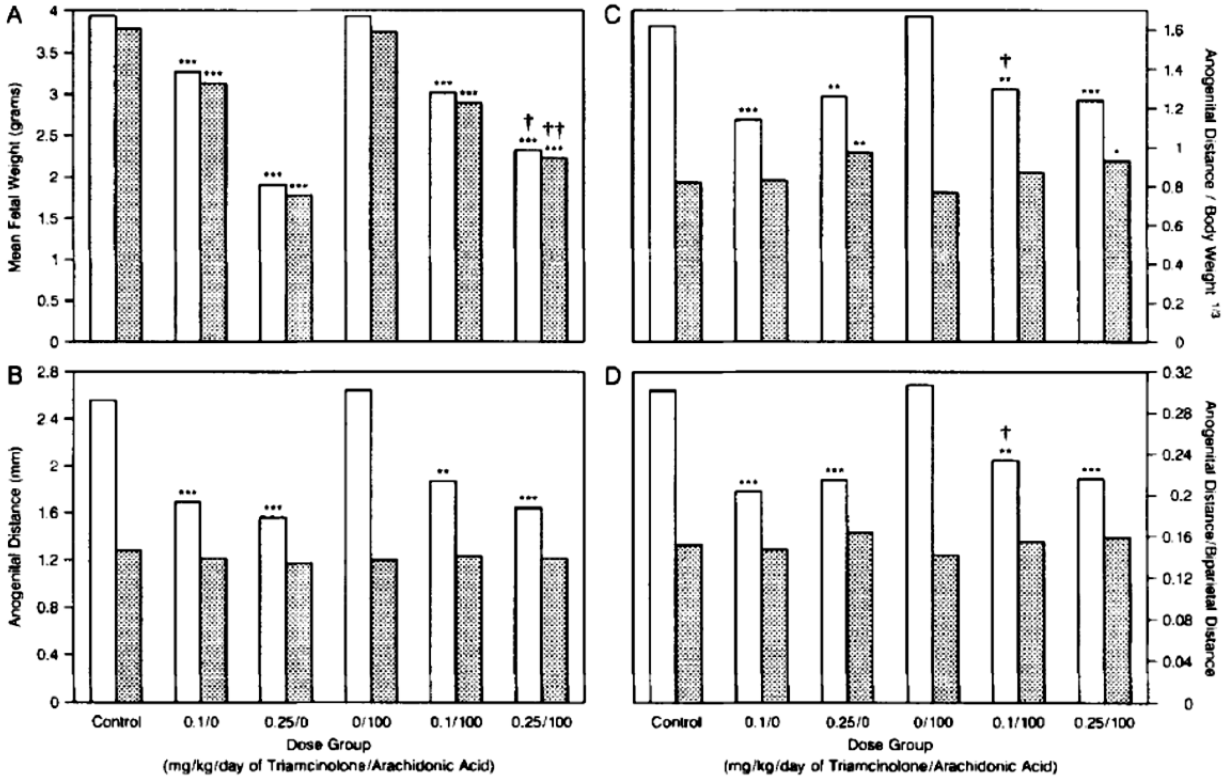


Fig. 5. Fetal results from study 2, in which dams received the various treatments on gestational days 14–19. **A.** Group means of fetal weight. **B.** Group means of absolute anogenital distance (AGD). **C.** Group means of AGD/cube root of body weight. **D.** Group means of AGD/biparietal distance. Open bars, males; stippled bars, females. *, **, ***: trend statistically significant through indicated dose ($P \leq 0.05$, 0.01, 0.001, respectively); †, ‡: statistically different from the group given the same dose of TA-alone ($P \leq 0.05$, 0.01, respectively).

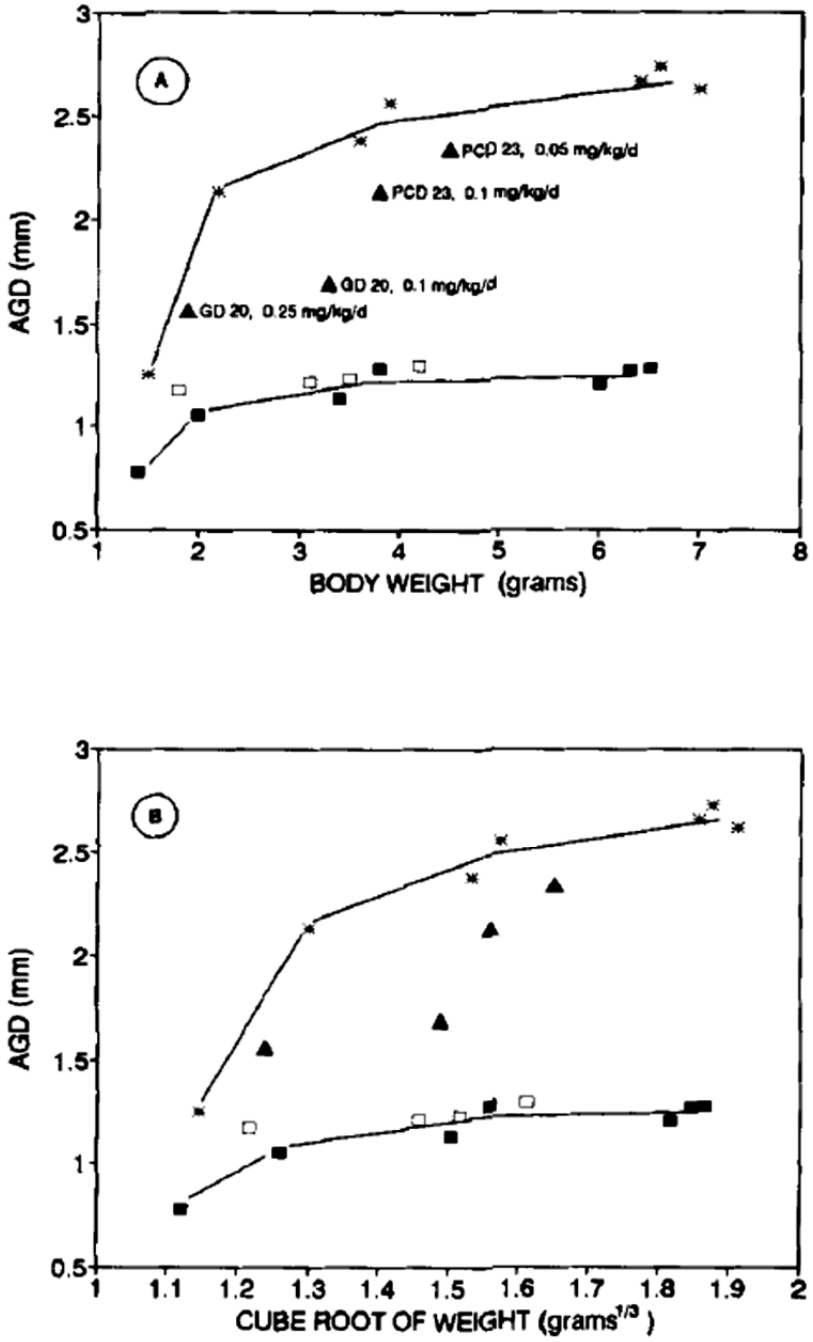


Fig. 6. **A. Relationship between AGD and weight in rat fetuses (GD 17, 18, and 20) and pups (PCD 23). Data (group means) from present studies as well as other unpublished studies conducted in this laboratory. ■, control females; □, TA-exposed females; *, control males; ▲, TA-exposed males. B. Relationship between AGD and cube root of body weight. Source of data and symbols are the same as in A. This transformation results in slightly smoother curves, but does not linearize the data.**

- Reviewer's note: Following review of the literature, TAC between GD 11 to 19 appears to induce mortality, body reduction of fetus and other malformations such as omphalocele and cleft palate in rats. The TAC dose ranges tested in SD rats (from 0.05 to 0.25 mg/day which corresponds to 0.49-2.4 mg based on the HED conversion) fall within the range of therapeutic dosages used in human (32 mg). Therefore, there are risks of teratogenic effects to human and it is considered appropriate for the Zilretta label.

Histopathological Findings of Cleft Palate in Rat Embryos Induced by Triamcinolone Acetonide. Furukawa S, Usuda K, Abe M, and Ogawa I. J. Vet. Med. Sci. 66(4):397-402 (2004)

- Method: Pregnant Wister Hannover rats were treated with 0.5 mg/kg of TAC intramuscularly at GD 12, 13, and 14. Animals were euthanized on Day 14.5, 15, 16, and 20 to examine embryonic/fetal external malformation.

- Key findings related to teratogenic effects of TAC:
  - On GD 20, the numbers and weights of alive embryos/fetuses were statistically significantly reduced and cleft palate was induced in 100% of fetuses as shown in the table below.

Table 1. Effect on embryo/fetus weight and cleft palate with triamcinolone acetonide

Gestation day	Treatment	No. of pregnant animals	Total No. of live embryos/ fetuses	Embryo/ fetus weight (g)	Incidence of open palate (%)
14.5	Control	4	40	0.19 ± 0.02	100.0 ± 0.00
	TAC	4	44	0.14 ± 0.00	100.0 ± 0.00
15	Control	4	30	0.28 ± 0.04	100.0 ± 0.00
	TAC	4	28	0.33 ± 0.04	100.0 ± 0.00
16	Control	4	43	0.71 ± 0.01	21.0 ± 12.20
	TAC	4	38	0.57 ± 0.05	100.0 ± 0.00*
20	Control	4	51	3.27 ± 0.06	0.0 ± 0.00
	TAC	4	9	1.63 ± 0.02$^{\$\$\$}$	100.0 ± 0.00*

Mean ± SE.
*: Significantly different from control at $p<0.05$ (Wilcoxon rank sum test).
$^{\$\$\$}$: Significantly different from control at $p<0.001$ (Student *t*-test).

- Reviewer's note: Authors claims that the major factor of TAC-induced cleft palate is through inhibition of mesenchymal cell proliferation in the palatal shelves at the stage of development. However, the reviewer was not fully convinced with the data; therefore, it is not included as key findings. However, the data showed that 0.5 mg/kg/day of TAC treatment during GD 12 to 14 induced cleft palate by GD 20. The TAC dose ranges tested in WH rats (0.5

mg/kg/day which corresponds to 4.8 mg based on the HED conversion) fall within the range of therapeutic dosages used in human (32 mg). Therefore, there are risks of teratogenic effects to human and it is considered appropriate for the Zilretta label.

Cleft Palate Produced in Mice by Human-Equivalent Dosage with Triamcinolone. Walker BE. Science. 149:862-863 (1965)

- Method: Five strains of mice (A/J, 129/J, DBA/1J, C57BL/6J, and C3H/HeJ) were treated with TAC, desoxycorticosterone acetate, or desoxycorticosterone trimethylacetate intramuscularly or subcutaneously from GD 11 to 14 once or for 4 days as shown in the table below. Animals were euthanized at GD 18 and fetal palate morphology was examined.

- Key findings related to teratogenic effects of TAC:
  - Excessive resorptions were observed in A/J mice treated with 0.025 mg/day or higher for 4 days. Cleft palates were observed in A/J mice treated with 0.001 mg/day or higher for 4 days.
  - In 129/J mice, resorptions were observed in animals treated with 0.003 mg/day or higher and cleft palate was observed at dosage of 0.0125 mg/day or higher for 4 days.
  - C3H/HeJ, C57BL/6J, and DBA/1J mice treated with 0.0125 mg/day for 4 days showed cleft palate.
  - The A/J strain was more susceptible to TAC compared to the 129/J strain.
  - TAC was 200X and 10X more potent as a cleft palate inducing teratogen than cortisone and dexamethasone acetate, respectively.

- Reviewer's note: Following review of the literature, TAC was more potent as a cleft palate teratogen than cortisone or desoxycorticosterone acetate. Cleft palate was observed in all five different strains of mice treated with at least 0.0125 mg/day of TAC for 4 days. The TAC dose ranges tested in several strains of mice (up to 0.5 mg/day which corresponds up to 2.4 mg based on the HED conversion) fall within the range of therapeutic dosages used in human (32 mg). Therefore, there are risks of teratogenic effects to human and it is considered appropriate for the Zilretta label.

Table 1. Palate morphology 18 days after conception in fetuses from mice treated with triamcinolone or desoxycorticosterone. Dosage was intramuscular except where otherwise indicated. CLCP, cleft lip and cleft palate.

Drug administration		Litters		Palate morphology			Palate stage (avg.)
Dosage (mg)	Days after conception*	No.	Resorbed	Normal	Cleft	CLCP	
		Triamcinolone acetonide: strain A/J					
0.5 × 4	11 to 14	5	5				
0.2 × 4	11 to 14	3	3				
0.05 × 4	11 to 14	4	4				
0.025 × 4	11 to 14	4	3		2		0.63
0.0125 × 4	11 to 14	7			43	2	0.55
0.006 × 4	11 to 14	5		13	20		0.83
0.003 × 4	11 to 14	3		15	8	1	0.90
0.001 × 4	11 to 14	2		14	3		0.96
0.0005 × 4	11 to 14	5		38		1	1.00
0.05	11	1			7		0.71
0.0125	12	2		4	2	1	0.92
0.0125	13	2		9		1	1.00
0.0125	14	4		14	3		0.96
0.006 × 4†	11 to 14	5		9	15	2	0.76
		Triamcinolone diacetate: strain A/J					
0.02 × 4‡	11 to 14	3		12	7		0.91
0.01 × 4‡	11 to 14	3		22			1.00
0.02‡	14	3		26			1.00
		Triamcinolone acetonide: strain 129/J					
0.025 × 4	11 to 14	6	3	6	5		0.95
0.0125 × 4	11 to 14	9	5	13	5		0.92
0.006 × 4	11 to 14	3	1	8			1.00
0.003 × 4	11 to 14	4	1	27			1.00
0.001 × 4	11 to 14	1		5			1.00
		Triamcinolone acetonide: strain C3H/HeJ					
0.0125 × 4	11 to 14	5		8§	13		0.76
		Triamcinolone acetonide: strain C57BL/6J					
0.0125 × 4	11 to 14	3	2		3		0.75
		Triamcinolone acetonide: strain DBA/1J					
0.0125 × 4	11 to 14	14	1	29	42		0.80
		Desoxycorticosterone acetate: strain A/J					
0.10 × 4	11 to 14	2		7		1	
0.15 × 4	11 to 14	2		11			
0.50 × 4	11 to 14	4		28		1	
1.00 × 4	11 to 14	2		11			
1.25 × 4‖	11 to 14	2		12#		1	

* Vaginal plug seen on day 0. † Administered subcutaneously. ‡ Administered orally. § Two embryos had cleft uvula. ‖ Desoxycorticosterone trimethylacetate. #One embryo had spina bifida.

Induction of Cleft Palates: Effects of Triamcinolone Acetonide on Transcription in Isolated Nuclei. Anne L and Bekhor I. Teratology. 18:343-352 (1978)

- Method: TAC (13 mg/kg, a dose resulting in cleft palate up to 90% in incidence as measured on GD 18.5) was administered to A/J mice on GD 12.5 and animals were euthanized at 0, 2, 4, 6, 8, and up to 24 h at 4-h increments.

- Reviewer's note: Though this article reports intriguing findings of TAC effects on the transcriptional activity in nuclei isolated from maternal A/J mouse livers and

embryonic maxillary processes, it is not clear whether it is one of molecular mechanisms inducing teratogenic effects of TAC such as cleft palate shown in mice. Additionally, the dose used in this study (13 mg/kg) corresponds to 63.4 mg of human equivalent dose based on HED conversion, which exceeds the proposed clinical dose (32 mg). (b) (4) .

Occurrence of Cleft Palate, Palatal Slit, and Fetal Death in Mice Treated with a Glucocorticoid: An Embryo Transfer Experiment. Kusanagi T. Teratology. 27:395-400 (1983)

- Method: SWV and C57BL/6 mice were treated with TAC subcutaneously in a single dose of 2.5 mg/kg on GD 12 and animals were euthanized on GD 18 for examining fetal malformation such as cleft palate. Embryo transfer procedure was performed by a surgical transfer of blastocysts recovered from the uterus on GD 3 to the right uterine horn of Day 2 pseudopregnant females anesthetized with 0.5 mL/kg sodium pentobarbital. Treatment with TAC was same as described above (a single dose of 2.5 mg/kg on GD 12).

- Key findings related to teratogenic effects of TAC:
  - Cleft palate was significantly increased in both strains of mice treated with TAC. The incidence (%) of cleft palate was much higher in TAC-treated SWV mice (49.7%) compared to the one in TAC-treated C57BL/6 mice (13.4%).
  - Palatal slit (PS) were only observed in C57BL/6 mice and TAC treatment increased the incidence of PS significantly ($p<0.01$).
  - Fetal mortality in SWV mice treated with TAC was significantly increased ($p<0.01$) compared to the control while C57BL/6 mice did not show fetal mortality. A higher incidence of resorption was observed in SWV mice treated with TAC while TAC treatment did not change the number of resorption rate in C57BL/6 mice.
  - There were strain-dependent TAC-related teratogenic effects.
  - Embryo transfer data suggested that uterus environment appeared to have greater effects on TAC-induced cleft palate and fetal genotypes appeared to have greater effects on TAC-induced PS.

TABLE 1. Frequency of cleft palate (CP), palatal slit (PS), and resorptions in mouse embryos treated with triamcinolone on day 12 of pregnancy

Group no.	Strain	Dose (mg/kg)	No. of dams	CP/live fetuses		PS/(live−CP) fetuses		Resorptions/ implantations	
				No.	%	No.	%	No.	%
1	C57BL/6	—	14	0/101	0	4/101	4.0	11/112	9.8
2	C57BL/6	2.5	16	16/119	13.4	36/103	35.0	12/131	9.2
3	SWV	—	15	0/198	0	0/198	0	15/213	7.0
4	SWV	2.5	16	79/159	49.7	0/80	0	61/220	27.7

TABLE 2. Frequency of cleft palate (CP), palatal slit (PS), and resorptions in mouse embryos transferred to pseudopregnant females and treated with triamcinolone on day 12 of pregnancy

Group[a] no.	Transfer class: Embryos		Transfer class: Females	Dose (mg/kg)	No. of dams	CP/live fetuses: No.	CP/live fetuses: %	PS/(live−CP) fetuses: No.	PS/(live−CP) fetuses: %	Resorptions/implantations: No.	Resorptions/implantations: %
5	C57BL/6	→	C57BL/6	—	12	0/28	0	6/28	21.4	3/31	9.7
6	C57BL/6	→	C57BL/6	2.5	15	3/52	5.8	21/49	42.9	8/60	13.3
7	C57BL/6	→	SWV	—	17	0/32	0	0/32	0	16/48	33.3
8	C57BL/6	→	SWV	2.5	24	7/45	15.6	6/38	15.8	25/70	35.7
9	SWV	→	SWV	—	17	0/34	0	0/34	0	22/56	39.3
10	SWV	→	SWV	2.5	25	17/44	38.6	0/27	0	30/74	40.5
11	SWV	→	C57BL/6	—	19	0/49	0	0/49	0	4/53	7.5
12	SWV	→	C57BL/6	2.5	15	2/48	4.2	0/46	0	6/54	11.1

[a]Group numbers continue from Table 1.

TABLE 3. Examination of the significance of treatment, uterine environment, fetal genotype, and embryo transfer procedure effects on cleft palate, palatal slit, and fetal mortality frequencies as determined by various group pair comparisons

Factors examined	Common factors	Comparison (Group No.[a])	Cleft palate (%)	Palatal slit (%)	Resorption (%)
A. Normal mating groups					
(i) Treatment	C57BL/6	2−1	13.4[b]	31.0[b]	−0.6
	SWV	4−3	49.7[b]	0	20.7[b]
(ii) Strain	Untreated groups	1−3	0	4.0	2.8
	Treated groups	2−4	−36.3[b]	35.0[b]	−18.5[b]
B. Embryo transfer groups					
(i) Treatment	C57BL/6→C57BL/6	6−5	5.8	21.5	3.6
	C57BL/6→SWV	8−7	15.6[c]	15.8	2.4
	SWV→SWV	10−9	38.6[b]	0	1.2
	SWV→C57BL/6	12−11	4.2	0	3.6
(ii) Uterine environment	Untreated C57BL/6 fetus	5−7	0	21.4	−23.6
	Untreated SWV fetus	11−9	0	0	−31.8[b]
	Treated C57BL/6 fetus	6−8	−9.8	27.1[c]	−22.4[c]
	Treated SWV fetus	12−10	−34.4[b]	0	−29.4[b]
(iii) Fetal genotype	Untreated C57BL/6 dam	5−11	0	21.4	2.2
	Untreated SWV dam	7−9	0	0	−6.0
	Treated C57BL/6 dam	6−12	1.6	42.9[b]	2.2
	Treated SWV dam	8−10	−23.0	15.8	−4.8
C. Between normal mating and embryo transfer groups					
(i) Embryo transfer procedure	Untreated C57BL/6	5−1	0	17.4[d]	−0.1
	Untreated SWV	9−3	0	0	32.3[d]
	Treated C57BL/6	6−2	−7.6	7.9	4.1
	Treated SWV	10−4	−11.1	0	12.8

[a]See group numbers in Tables 1 and 2.
[b]Significantly different by Goodman's test ($p < 0.01$); note, in comparisons involving strain differences, the negative sign indicates that the frequency in SWV is higher than that in C57BL/6.
[c]Significantly different by Goodman's test ($p < 0.05$).
[d]Significantly different by the chi-square test ($p < 0.01$).

- Reviewer's note: Following review of the literature, it appears that the effects of uterus environment are more important in TAC-induced cleft palate formation and fetal genotypes are more important in palatal slit occurrence. The TAC dose tested in two strains of mice (2.5 mg/day which corresponds 12 mg based on the HED conversion) fall within the range of therapeutic dosages used in human (32 mg). This study reports consistent teratogenic effects of TAC in two different strains of mice. Therefore, there are risks of teratogenic effects to human and it is considered appropriate for the Zilretta label.

Congenital Anomalies Induced by Triamcinolone Acetonide in Murine Embryos. Miyagi H, Kubota Y, Tsuda T, Sasaki Y, Ono S, Kimura O, and Iwai N. Eur. J. Pediatr. Surg. 18:164-167 (2008)

- Method: TAC was administered intramuscularly in a single dose (15 or 30 mg/kg) to ICR-SLC mice on Embryonic Day 7 (E7), 8, 9, and 10. Then, mice were euthanized on E18 to examine internal and external malformation.

- Key Findings related to teratogenic effects of TAC:
  - All group treated with TAC showed a high survival rate and no macroscopic abnormalities except cleft palate.
  - Cleft palate was observed in all TAC-treated mice and the highest incidence (100%) of cleft palate was mice treated with 15 mg/kg of TAC on E9.
  - Glucocorticoid receptor expression was induced in the epithelial and subepithelial layers of the palatal shelf of mice treated with TAC, but not in the ones of the control group on E18.

Table 1 Results of survival rates. TAC = triamcinolone acetonide

Group	TAC (mg/kg)	Survival no.	Survival rate (%)
E7	15	20/20	100
	30	29/30	96.6
E8	15	30/32	93.8
	30	22/22	100
E9	15	30/31	96.8
	30	38/39	97.4
E10	15	28/28	100
	30	17/17	100
Control	0	21/21	100

Table 2 Results of the incidence of cleft palate and other anomalies. TAC = triamcinolone acetonide, ARM = anorectal malformation

Group	TAC (mg/kg)	n	ARM	Urinary malformation	Spina bifida	Omphalocele	Cleft palate	Limb malformation
E7	15	20	0%	0%	0%	0%	5.0%	0%
	30	29	0%	0%	0%	0%	62.1%	0%
E8	15	30	0%	0%	0%	0%	40.0%	0%
	30	22	0%	0%	0%	0%	45.5%	0%
E9	15	30	0%	0%	0%	0%	100%	0%
	30	38	0%	0%	0%	0%	28.9%	0%
E10	15	28	0%	0%	0%	0%	57.1%	0%
	30	17	0%	0%	0%	0%	64.7%	0%
Control	0	21	0%	0%	0%	0%	0%	0%

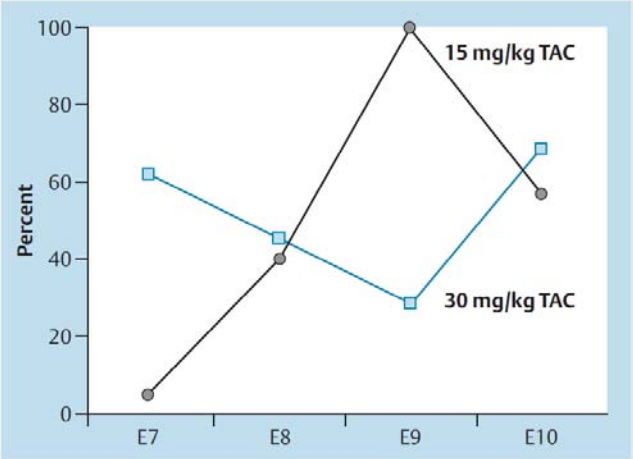


Fig. 3 Incidence of cleft palate. The groups given 15 mg/kg TAC had one peak on E9, and the groups given 30 mg/kg TAC had two peaks on E7 and E10.

- Reviewer's note: Following review of the literature, it is consistent that TAC treatment in early embryonic stages (E7 to E10) induces cleft palate in ICR-SLC mice. The TAC dose ranges tested this study (15 and 30 mg/day which corresponds up to 4.9 and 9.8 mg based on the HED conversion) fall within the range of therapeutic dosages used in human (32 mg). Therefore, this study also supports teratogenic risks to human and it is considered appropriate for the Zilretta label.

9.2 Embryonic Fetal Development

No embryonic fetal development studies were conducted and no relevant literature articles were submitted for review. Several of the above studies test TAC during the typical EFD dosing range and therefore the studies reviewed above contribute to our understanding of the teratogenic risk of TAC.

9.3 Prenatal and Postnatal Development

No prenatal and postnatal studies were conducted and no relevant literature was submitted for review.

10 Special Toxicology Studies

10.1 Extractable Study

Title: Extractable Study Report for Components of FX006 – A Dry Powder for Injection Drug Product

Study Number: RPT49852.02 AB4890

The object of the study: To perform an extraction study on the rubber stopper and glass vials used as the primary container closure system for FX006 dry powder for injection drug product as well as for the FX006 companion diluent used to reconstitute the dry powder.

Drug Product and Packaging Summary

Product	Contents and process	Vial	Stopper
FX006 Drug Product, (b) (4) Glass			(b) (4)
FX006 Drug Product, (b) (4)			
FX006 Diluent			

The extraction study was performed for the following materials:

- For dry powder:
  - two types of glass vials
    - Vial 1: (b) (4) 5-mL 20 mm (b) (4) vial of Type (b) (4) glass (b) (4) vial
    - Vial 2: (b) (4) 5-cc, 20 mm (b) (4) glass vial made of Type (b) (4) (b) (4) glass (b) (4)
  - Stopper 1: (b) (4) rubber stopper (20 mm)
- For diluent
  - Mock diluent vial: a glass vial (Type (b) (4) glass) with stopper
  - Stopper 2: (b) (4) rubber stopper (13 mm)

Extraction Solvents used in the extraction study

- Lab prepared FX006 diluent (a)
- Isopropanol (b)
- Hexane (c)
- Dry - no solvent (d)
- 5% nitric acid (e)

Extraction Methods

Extraction Material	Extraction Amount	Extraction Method	Extraction Solvent	Extraction Duration	Extraction Temp
Rubber stoppers	10 stoppers (b) (4)	Incubation technique	50 mL of FX600 diluent	2 h	121 °C
	2 stoppers (stopper 1: (b) (4) stopper 2:	(b) (4)	70 mL of isopropanol and hexane	2 h (rinse time: 30 m conc. Time: 5 min)	
	1 stopper	Incubation technique	No solvent	2 h	100 °C
	1 stopper	Incubation technique	30 mL of water, FX600 diluent or 5% nitric acid	1 h	121 °C
Glass vials		Incubation technique	5 or 10 mL of extraction solvents	1 h	121 °C

Extractable	Analytical Technique	Extraction Solvents
Non-volatile extractables	HPLC/MS	a,b,c
Semi-volatile extractables	Direct injection GC/MS	a,b,c
Volatile extractables	Headspace GC/MS	d
Metal and elemental extractables	ICP/MS	e

- Headspace GC/MS analysis: (b) (4)
- Liquid Injection GS/MS analysis: (b) (4) .
- LC/MS analysis: (b) (4) .

Results

Identifications were classified into 4 levels using the following criteria.

Identification Level	Criteria
Confirmed (CI)	Retention time and mass spectral match to reference standard material
Confident (Conf)	High quality matches to library spectrum. Expected that the identification could be confirmed with injection of reference standard at a later date.
Tentative (TI)	Library search produced a medium quality match or spectral characteristics suggest that the identification is possible.
Unknown (U)	Library search did not produce a match or spectral characteristics could not be interpreted for identification.

The reporting threshold of the extraction study: (b) (4)

- No non-volatile extractables were observed in the FX006 diluent extraction solvent. More aggressive extraction solvents (b) (4) generated non-volatile extractables (b) (4) which are common in pharmaceutical grade rubber components. A few unknown extractables were detected in isopropanol or hexane extraction conditions. Further analyses were conducted in leachable studies (see the review in **10.2 Leachable Studies**).
- Semi-volatile extractable compounds observed in the FX006 diluent extraction solvent are listed in the table below. The Applicant noted that these extractables are common in pharmaceutical grade rubber components.

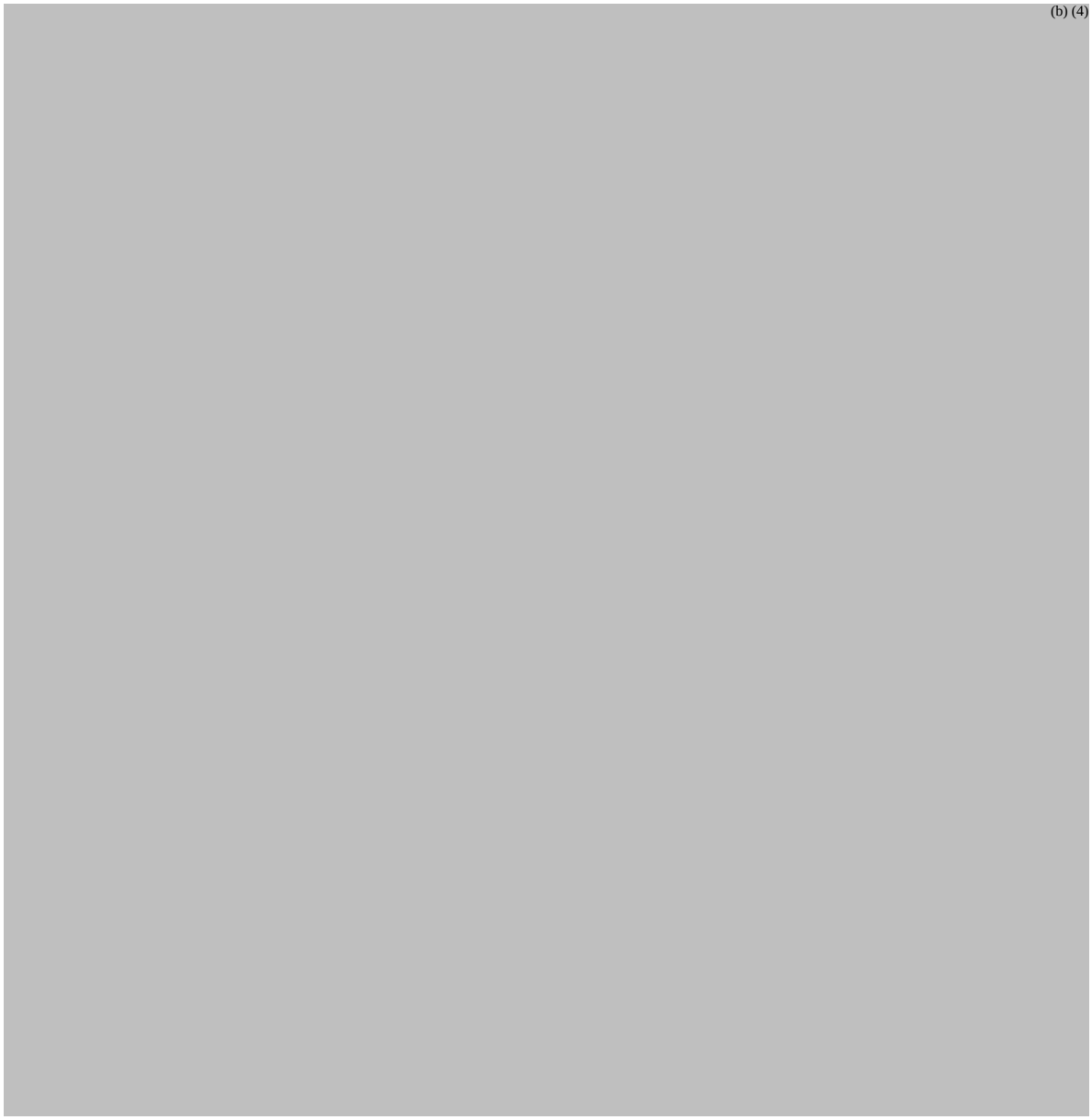
Identification	ID Level	CAS#	Estimated Concentration (µg/stopper)
Rubber Stopper 1 (Product)			
(b) (4)	Confirmed	(b) (4)	(b) (4)
	Confident		
	Confirmed		
	Confirmed		
Rubber Stopper 2 (Diluent)			
	Confirmed	(b) (4)	
	Confident		
	Confirmed		
	Confirmed		

More aggressive extraction solvents (b) (4) generated more semi-volatile extractables (b) (4) which are common in pharmaceutical grade rubber components. Additionally (b) (4) were detected but these extraction conditions are considered to be extreme conditions, which may not be relevant to the clinical setting.

- Volatile extractables observed (b) (4) extracts of the rubber stopper are listed in the tables below. These extractables include (b) (4) which are also common in pharmaceutical grade rubber stoppers.

Identification	ID Level	CAS#	Estimated Concentration (µg/stopper)
Rubber Stopper 1 (Product)			
(b) (4)	Confident	(b) (4)	(b) (4)
	Confirmed		
	Confirmed		
	Confident		
	Confident		
	Confirmed		
	Confirmed		
	Confident		
	Confident		
	Confirmed		
	Confirmed		
	Tentative		
	Unknown		
	Confident		
	Confident		
	Rubber Stopper 2 (Diluent)		
	Confirmed	(b) (4)	
	Confirmed		
	Confirmed		
	Confident		
	Confident		

- The metal and elemental extractables (b) (4) were not detected above the limits listed in ICH Q3D.



10.2 Leachable Studies

Title: Leachables Simulation Study Report for Vial Adapter Used with the FX006 Product

Study Number: 05120-001

The object of the study: To assess potential leachable compounds from the vial adaptor for the FX006 product

Screening Methods
The vial adaptor was exposed to a lab-prepared diluent at ambient conditions for 4 h to simulate actual use conditions or exposed for 24 h as a worst-case exposure condition.

Target Compounds	Analytical Technique
Non-volatile Extractables	HPLC/MS
Semi-volatile Extractables	Direct injection GC/MS
Volatile Extractables	Headspace GC/MS
Inorganic and Metal Extractables	ICP-MS

Configuration 1: Needle and Luer
Preparation:
Amount of material One needle and one luer with excess removed
Volume of Diluent 5 mL
Extraction Time 4 hours & 24 Hours

Configuration 2: Whole Vial Adapter
Preparation:
Amount of material 1 whole vial adapter
Volume of Diluent 50 mL
Extraction Time 4 Hours & 24 Hours

The reporting threshold was (b) (4) mcg/day, which is (b) (4)% (an uncertainty factor for analytical evaluation threshold selected by the Applicant) of the most conservative safety concern threshold (SCT) for carcinogens and irritants (1.5 mcg/day, ICH M7 TTC).

Acceptable Intakes for an Individual Impurity (ICH M7)

Duration of treatment	≤ 1 month	>1 - 12 months	>1 - 10 years	>10 years to lifetime
Daily intake [µg/day]	120	20	10	1.5

Results

- No non-volatile extractables above the reporting threshold were observed in any of the extracted samples.
- No semi-volatile extractables above the reporting threshold were observed in any of the extracted samples.
- No volatile extractables above the reporting threshold were observed in any of the extracted samples.
- From the needle/luer extractions, the only metal/elemental leachable from needle/luer extractions above the sensitivity threshold (b) (4) mcg/needle-luer assembly) was (b) (4) mcg/needle-luer assembly at 4 h and 24 h (extraction time), respectively. As note, the parenteral PDE for (b) (4)

Titles: 1) Assessment of Leachables for FX006 – A Dry Powder for Injection Drug Product and 2) Leachable Study Report for FX006 Dry Powder for Injection Drug Product

Study Numbers: **1)** PT53289.01 S-AB7882 and **2)** RPT61564.01 05321-001

Note: Two leachable studies are reviewed together below in order to look at the timeline batch analyses better.

The object of two leachable studies: To provide an assessment of leachable compounds from FX006 dry powder for injection and FX006 diluent

Sample Batches Tested for Leachables

Sample	Assigned Number	Lot Number	Interval (months)	Storage Conditions	Position	Sample Number
FX006 Drug Product	A	FL1022 (A)	25	5 °C	Inverted	TB227398
			34	5 °C	Upright	TB227397
	B	FL1101 (B)	12	25 °C/60% RH	Not specified	TB0048597
			26	5 °C	Upright	TB227399
	C	862-125 (C)	24	25 °C/60% RH	Not specified	TB0048598
Diluent	#1	023C15	7	40 °C/75% RH	Inverted	TB0048650
			20	5 °C	Inverted	TB227388
			20	25 °C	Inverted	TB227389
	#2	024C15	20	5 °C	Inverted	TB227390
			20	25 °C	Inverted	TB227391
	#3	008C16	5	5 °C	Inverted	TB227392
			5	25 °C	Inverted	TB227393
			5	40 °C	Inverted	TB227394
	#4	009C16	5	40 °C	Inverted	TB227395
	#5	014H16-1	3	40 °C	Inverted	TB229376
	#6	ENG 14041	12	40 °C/75% RH	Upright	TB0048649

Timeline Batch Analyses

Drug Product	12 months	24 months	25 months	26 months	34 months
5 °C			A (I)	B (U)	A (U)
25 °C	B	C			

Diluent	3 months	5 months	7 months	12 months	20 months
5 °C		#3 (I)			#1, #2 (I)
25 °C		#3 (I)			#1, #2 (I)
40 °C	#5 (I)	#3, #4 (I)	#1 (I)	#6 (U)	

Methods

Leachable Class	Analytical Technique
Non-volatile Leachables	HPLC/MS
Semi-volatile Leachables	Direct injection GC/MS
Volatile Leachables	Headspace GC/MS
Metal and elemental Leachables	ICP/MS

Identification Level	Criteria
Confirmed	Retention time and mass spectral match to reference standard material
Confident	High quality matches to library spectrum. Expected that the identification could be confirmed with injection of reference standard at a later date.
Tentative	Library search produced a medium quality match or spectral characteristics suggest that the identification is possible.
Unknown	Library search did not produce a match or spectral characteristics could not be interpreted for identification.

The reporting threshold for leachable compounds is (b) (4) mcg/day.

Acceptable Daily Intakes for an Individual Impurity (ICH M7)

Duration of treatment	≤ 1 month	>1 - 12 months	>1 - 10 years	>10 years to lifetime
Daily intake [µg/day]	120	20	10	1.5

For FX006, the proposed daily dose (a single injection use) is 40 mg (32 mg dispensed). Therefore, the acceptable daily intake (ADI) for a mutagenic impurity (leachables) would be (b) (4) mcg/day assuming all of the compound were released from the materials within (b) (4). For leachables in the diluent, which are reconstituted at the time of use, this is a logical assumption. If a leachable enters the polymeric microsphere, the release could be extended over the duration of microsphere integrity. Therefore, the ADI for carcinogenesis concerns would be (b) (4) mcg/day. In terms of general toxicity perspectives, requested qualification threshold was designated to be (b) (4) mcg/day by the Division. Therefore, the safety concern threshold (SCT) of impurity (leachables) should be (b) (4) mcg/day for this drug product.

Results

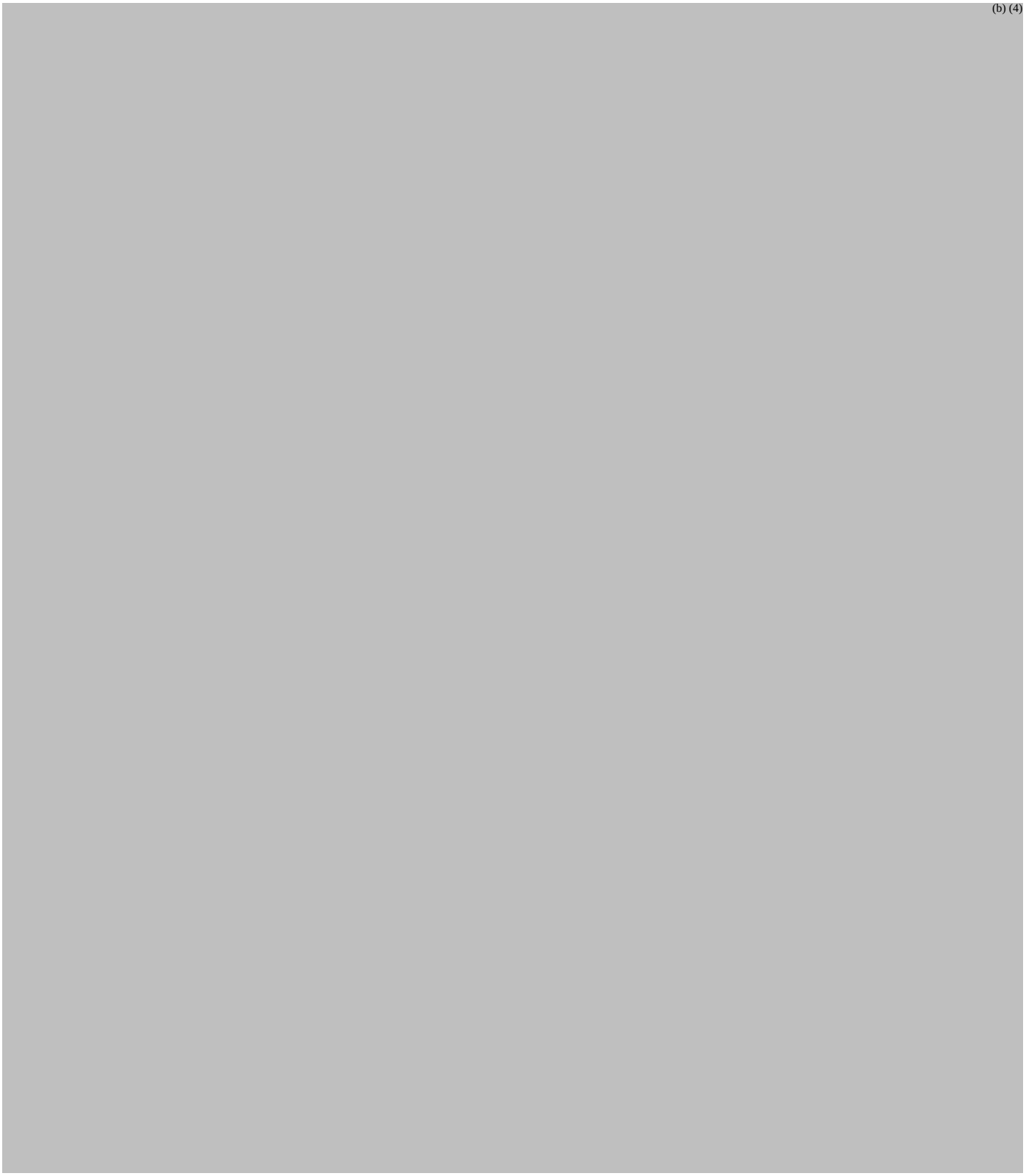
- Drug Product
  - No non-volatile leachables above the SCT (b) (4) mcg/vial) were observed.
  - No semi-volatile leachables above the SCT (b) (4) mcg/vial) were observed.

- Volatile leachables observed the reporting limit (b) (4) mcg/vial) include (b) (4) However, none of them were above the SCT (b) (4) mcg/vial).
- No metals or elements were observed above the ADI (ICH M7).

Leachable above the SCT (b) (4) mcg/day) in FX006 Powder for Injection

Temp		12 month	24 month	25 month	26 month	34 month
5 °C	Non-volatile			None	None	None
	Semi-volatile			None	None	None
	Volatile			None	None	None
	Metal/Element			None	None	None
25 °C	Non-volatile	None	None			
	Semi-volatile	None	None			
	Volatile	None	None			
	Metal/Element	None	None			

- Diluent
  - Non-volatile leachables observed above the reporting limit (b) (4) mcg/vial) (b) (4) but their levels were all below the SCT (b) (4) mcg/vial).
  - The classes of semi-volatile leachables observed the reporting limit (b) (4) mcg/vial) include (b) (4) Additionally, there were several unknown leachables observed in diluent batches; however, their levels were below the SCT ((b) (4) mcg/vial).
  - Volatile leachables observed the reporting limit (b) (4) mcg/vial) include (b) (4) . Among them, several semi-volatile and volatile leachables were observed above the SCT (b) (4) mcg/vial). (b) (4) noted, the proposed shelf life for the drug powder is 24 months at (b) (4) C and the proposed expiry of the diluent is (b) (4) months at (b) (4) °C (b) (4) We discussed this matter with the CMC team and the CMC team noted (b) (4) the proposed expiry in the label, which is at 2 to 8°C for 24 months or at temperatures not exceeding 25°C for up to 6 weeks (See the CMC review for more details). Therefore, we concluded that the safety assessments of leachables above the SCT (b) (4) mcg/day, see the summary table below) (b) (4)



10.3 In Vitro Release (Dissolution) Study

For detailed review, see the CMC review.

Title: In Vitro Release of Impurities from FX006
Study Number: RPT-054
Purpose: To examine the in vitro release rate of impurities from FX006 in relation to the release of the active ingredient, TCA.
Method: Two clinical batches (Lot 12-083-001 and Lot FL1100) were evaluated as described in the studies below.

Related Substances (TP70720)

The FX006 Assay and Related substances are quantified using a (b) (4) (b) (4) HPLC method (b) (4) (b) (4) Samples are prepared from a 5-vial composite.

In Vitro Release (TP71056)

The FX006 In-Vitro Release (IVR) method is a validated, accelerated method for Quality Control, employing 1000 mL of a 0.3% sodium dodecyl sulfate (SDS) surfactant based release media controlled to pH 7.2. The dissolution experiment is performed at 35 °C in a USP (b) (4) Dissolution apparatus operated at 75 rpm. Samples are withdrawn at 4, 24, 48, 72, 96, and 120 hours, with specifications established at the 4, 24 and 120 hour points. Samples are analyzed by a validated HPLC method, (b) (4) (b) (4) (b) (4)

Result: IVR data of only one impurity (b) (4) were included in this study report. The mean % AUC data of (b) (4)

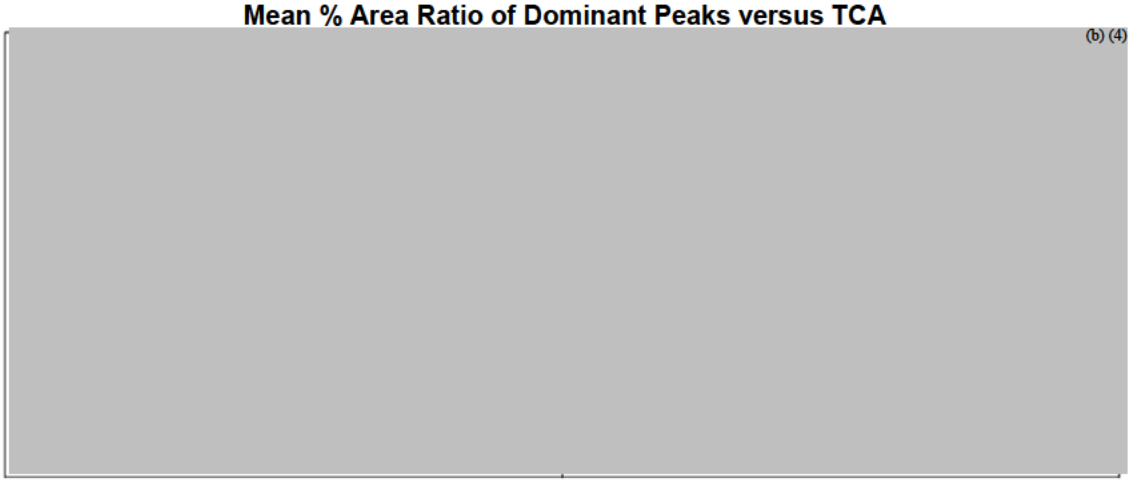
Purity Analysis of FX006 Batches Used in Impurity IVR Analysis (TP70720)

Impurity	12-083-001 (Analyzed July 8, 2015)	FL1100 (Analyzed July 8, 2015)
(b) (4)		

IVR data of FX006 Batches Used in Impurity IVR Analysis (TP71056)

IVR Time Point (Hours)	12-083-001 (Analyzed July 8, 2015)	FL1100 (Analyzed July 15, 2015)
4	(b) (4)	
24		
48		
72		
96		
120		

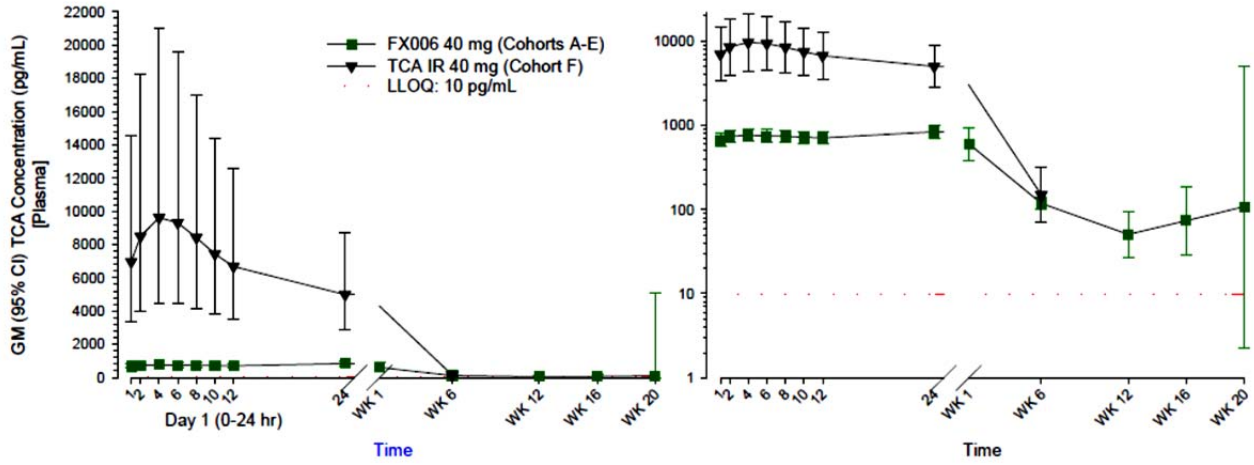
Mean % Area Ratio of Dominant Peaks versus TCA



11 Integrated Summary and Safety Evaluation

Flexion Therapeutics submitted NDA 208845 to seek marketing approval of Zilretta, which contains triamcinolone acetonide (TCA) formulated in 75:25 poly(lactic-co-glycolic acid) microspheres as an ER injectable suspension for intra-articular (IA) injection for osteoarthritis pain management of the knee(s). This NDA is a 505(b)(2) application referencing the approved TCA product, Kenalog-40 (TCA, injectable IR suspension, USP, NDA 14901), which is also approved for the IA route of administration. Compared to Kenalog-40, Zilretta appears to provide sustained TCA release to the synovial tissues resulting in lower systemic exposure (see the figure below) with the intention of providing better persistent pain relief with less hypercorticosteroid-related systemic effects, for approximately 3 months. Based on single-dose general toxicity data in dogs, FX006-treated (refer to Zilretta) animals showed slightly less hypercorticosteroid systemic effects (i.e., adrenal atrophy and lymphoid depletion) than animals treated with the same dose of TCA IR. However, in a repeat-dose study, systemic effects were not much different or slightly worse in FX006-treated animals (See **Section 6. General Toxicology** for details). See below for a more detailed summary of these studies.

Figure 5. Linear and Log Scale Geometric Mean (GM) with 95% CI for Plasma Drug Concentrations (pg/mL) Curve – FX006 and TCA IR (Plasma Drug Concentration Population)



Note: This is Figure 1 in the Clinical Study Report: FX006-2015-009

The extended-release properties of Zilretta are due to the incorporation of TCA in the PLGA microspheres. Note that PLGA has not been previously employed in an FDA-approved IA drug product and therefore its use is considered novel. The safety of PLGA was appropriately evaluated through the conduct of IA toxicity studies. All other excipients in Zilretta are considered qualified for safety as they are within levels found in FDA-approved products for the IA route.

Also of note, clinical comparative bioavailability studies demonstrated that the systemic exposure levels (AUC and C_{max}) to TCA following IA administration of Zilretta at the maximum recommended human dose (MRHD) in human subjects was within the approved reference product, Kenalog-40 (refer to clinical pharmacology review for detailed information). Therefore the systemic safety of TCA can be bridged to the reference product and the primary focus of the nonclinical evaluation is on the potential local toxicity of the PLGA microspheres and the Zilretta drug product.

General Toxicity Studies

The Applicant conducted two GLP studies to evaluate general toxicity studies in dogs: a single dose toxicity study with 3-, 4-, 6-, and 9-month recovery periods and a repeat-dose toxicity study with a 6-month recovery period (See the table below).

Table 9. Single- and Repeated-Dose General Toxicology Studies in Dogs

Study Number	FX006-TOX-2011-003	FX006-TOX-2012-001	
Dose regimen	A single IA injection	3 repeated injections with a 1-month interval (subset study)	3 repeated injections with a 3-month interval (main study)
Dosages of API (Note TCA:PLGA=1:4)	2.1, 6.25, and 18.75 mg/mL	6.25, and 18.75 mg/mL	

Controls	Diluent, Blank PLGA Microspheres (75 mg/mL, the same concentration in HD), and the Active Comparator (Kenalog-40, 18.75 mg TCA)		
Clinical Signs	• No mortality/morbidity was observed. • No test article-related adverse clinical symptoms were observed.		
Hematology/ Clinical Chemistry/ Urinalysis	No notable test article-related changes	TCA-related reduction in lymphocytes, eosinophil, and creatinine: reversible within a 6-month of recovery period	TCA-related reduction in lymphocytes, eosinophil, and creatinine: reversible within a 6-month of recovery period
Systemic Microscopic Finding	• Common hypercortisolism (atrophy in adrenal glands, lymphoid depletion) • Mostly reversible by the end of recovery period (6 or 9 months) • Severity/Incidence: Single IA injection of IR TCA (Kenalog-40) = Single IA injection **<** Main study (3 injections every 3 month) **<** Subset Study (3 injections every 1 month)		
Local Microscopic Finding	• Multinucleated macrophages, lymphocyte/plasma cell infiltration, and fibrosis – appears to be microsphere-related • Mankin/Safranin O staining joint scores: Slightly worse than the active comparator (Kenalog-40) o Single dose: mostly reversible by the end of the recovery period o Repeat dose (main and subset): not fully reversible • Severity/Incidence: Single IA injection of IR TCA (Kenalog-40) < Single IA injection **<** Subset Study (3 injections every 1 month) **≤** Main Study (3 injections every 3 month)		
Toxicokinetics at acceptable LOAELs	C_{max}: 4.32 ± 2.62 mg/mL $AUC_{0-\infty}$: 38.32 ± 12.58 ng●day/mL $T_{1/2}$: 32.3 ± 20.57 days	After the 3rd dose, C_{max}: 3.81 ± 0.88 mg/mL $AUC_{0-\infty}$: 48.1 ± 6.26 ng●day/mL $T_{1/2}$: 14.1 ± 3.26 days	After the 3rd dose, C_{max}: 4.94 ± 1.07 mg/mL $AUC_{0-\infty}$: 46.2 ± 8.70 ng●day/mL $T_{1/2}$: 20.9 ± 14.88 days
Acceptable LOAELs	Systemic: 18.75 mg/mL Local: 18.75 mg/mL	Systemic: 18.75 mg/mL Local: not determined	Systemic: 18.75 mg/mL Local: not determined
Safety Margin based on $AUC_{0-\infty}$*	1.5X	1.9X (at Dose 3)	1.8X (at Dose 3)
Safety Margin based on AUC_{0-24}*	3.7X	3.5X (at Dose 3)	4.1X (at Dose 3)
Safety Margin based on estimated synovial concentrations	2.9X	NA	NA

**Based on the human PK data (Study Number: FX006-2015-009)*
*** Based on synovial volumes of human (7 mL) (2) and dog (1.4 mL) (3)*

A Single-Dose Toxicity Study in Dogs (FX006TOX-2011-003)

Beagle dogs were treated with the vehicle control (diluent), blank microspheres, the active comparator (Kenalog-40 IR, 18.75 mg of TCA), LD (2.1 mg TCA), MD (6.25 mg TCA), and HD (18.75 mg TCA) via a single intraarticular injection. Note, the ratio of microsphere:TCA is 4:1 and the concentration of blank microspheres is the same as in the Zilretta HD group. Animals were euthanized at 3, 4, 6, and 9 month following dose administration. No mortality/morbidity and no test article-related clinical symptoms were observed. No notable test article-related changes in hematology, clinical chemistry, and urinalysis were observed.

Common findings of hypercortisolism such as slight atrophy of adrenal gland and lymphoid depletion were observed in animals treated with the test article in a dose-dependent manner. These systemic findings were comparable with the ones of animals treated with the active comparator, Kenalog-40 IR and Zilretta does not appear to produce toxicologically significant systemic adverse effects worse than Kenalog-40 IR. In addition, these findings were mostly reversible by the end of the 9-month recovery period.

From a local toxicity perspective, microscopic findings including multinucleated macrophages, lymphocyte/plasma cell infiltration, and fibrosis were observed in the injected right knees of animals treated with either blank microspheres or the test article. The level of severity and incidence increased in a dose-dependent manner and it appears that these local findings could be attributed to both the microspheres and the active ingredient (TCA). The severity was mostly minimal to slight and these findings were mostly reversible by the end of 9-month recovery period. Using Modified Mankin Scoring System, structural and cellular/histological changes of the knee joints were also evaluated. It appears that the joint scores got worse in a dose-dependent manner with Zilretta and the joint scores of animals treated at the HD were slightly worse than the observed in animals treated with the active comparator, Kenalog-40 IR at an equal dose of TCA. These changes in the joint appear to be reversible over time but they were not fully reversible in males dosed at HD by the end of the 9-month recovery period. Since these local effects have a trend of recovery over time and there were no irreversible microscopic findings such as necrosis, they appear to be acceptable local adverse effects. Therefore, the acceptable LOAEL for both systemic and local safety of Zilretta is 18.75 mg.

Systemic safety margin

At the acceptable LOAEL, C_{max}, $AUC_{0-\infty}$, and $T_{1/2}$ were 4.32 ± 2.62 ng/mL, 38.32 ± 12.58 ng*day/mL, and 32.3 ± 20.57 days, respectively. As comparison, C_{max}, $AUC_{0-\infty}$, and $T_{1/2}$ of Kenalog IR at the acceptable LOAEL (18.75 mg) were 41.7 ± 3.61 ng/mL, 69.34 ± 10.65 ng*day/mL, and 18.3 days, respectively. As noted above, the systemic exposure to TCA following Zilretta administration to human subjects did not exceed the exposure with the reference product, Kenalog-40 IR (see the Clinical Pharmacology review for more details); therefore, the systemic safety of TCA is not of concern. Nonetheless, the single-dose dog study provided adequate safety margins for systemic safety based on a comparison of AUC between dogs and humans (see the table below).

Table 10. Systemic Safety Margin of Zilretta

Dose	Based on AUC_{0-24}	Based on $AUC_{0-\infty}$
18.75 mg	3.7X	1.5X

Based on the human PK data (Study Number: FX006-2015-009) and the TK data of a single dose toxicity study in Beagle dogs (Study Number: FX006TOX-2011-003)

Local safety margin
The single dose IA dog study provides a 2.9X safety margin for local safety based on a comparison of the estimated synovial TCA concentrations between dog and human (see the calculation below).

Estimated synovial TCA concentration from Zilretta:
Human: 32 mg (TCA at MRHD) / 7 mL (human synovial volume) = 4.6 mg/mL
Dog: 18.75 mg (TCA at LOAEL) / 1.4 mL (dog synovial volume) = 13.4 mg/mL
Safety margin = 13.4 / 4.6 = 2.9X

A Repeat-Dose Toxicity Study in Dogs (FX006TOX-2012-001)
The proposed indication for Zilretta is for single use similarly to the reference product; therefore, this repeat-dose study is not required to assess the safety of Zilretta at this time. Nonetheless, a brief summary is included to provide potential concerns for a possible chronic use. The Applicant submitted a repeat-dose toxicity study in dogs: three repeated dosages of Zilretta with two different dosing intervals: 1-month (subset study) or 3-month (main study).

Main Study (three dosing with 3-month intervals, a 6-month recovery period):
The main study appears to be designed for a chronic use of Zilretta every 3 months. As note, the Applicant proposed that Zilretta delivers TCA to the synovial tissues for approximately 3 months. Beagle dogs were treated with blank microspheres, the active comparator (Kenalog-40, 18.75 mg of TCA), LD (6.25 mg TCA), and HD (18.75 mg TCA) via a single intraarticular injection. The ratio of microsphere:TCA is 4:1 and the concentration of blank microspheres is same as one in the HD. No mortality/morbidity and no test article-related clinical signs were observed in all groups. There were some changes (about 10%) in body weights but they do not appear to be test article-related. There were TCA-related reductions in lymphocytes, eosinophils, and creatinine but these changes were reversible by the end of the 6-month recovery period (Day 363/364). TCA-related liver weight increases were observed in animals treated with 18.75 mg TCA (Kenalog-40 and HD) but these changes were reversible by the end of the recovery period. The common hypercortisolism-related microscopic findings were observed: slight to severe atrophy of adrenal gland and lymphoid depleting. Minimal to moderate cell swelling in hepatocytes of the liver was also observed in a TCA dose-dependent manner. These changes were not fully reversible by the end of the recovery period. Systemic findings in animals treated with the test article were mostly comparable or slightly worse than animals treated with the active comparator (Kenalog-40). Local microscopic findings include minimal to mild macrophage, lymphocyte/plasma cell/neutrophil infiltration, neovascularization, granulation tissue, microsphere/debris, and fibrosis were observed and these findings appear to be mostly related to the PLGA microspheres and TCA attenuates these local adverse effects. Note that these local effects were not observed in animals treated with Kenalog-40. These local findings were partially reversible by the end of the 6-month recovery period. HD animals had comparable but slightly worse average joint scores (Modified Mankin Score based on surface structure and cellularity, Safranin O staining, and tidemark

integrity) compared to animals treated with Kenalog-40 and the joint changes did not reverse but rather worsened by the end of the recovery period. Therefore, a NOAEL/LOAEL for local safety could not be determined for this study.

Subset Study (three dosing with 1-month intervals, a 6-month recovery period):
Similar findings were observed in the subset study as described above in the main study. Adrenal gland atrophy and lipid depletion were observed at a slightly higher incidence and higher severity in the subset study groups compared to the main study groups. Similar to the main study, there were comparable but slightly worse average joint scores in Zilretta HD animals compared to animals treated with Kenalog-40.

In summary, a repeat-dose toxicity study showed that there appears to be a higher incidence and severity of local adverse effects in the joints of animals treated with the test article. These findings were not fully reversible within 6 months of the recovery period and these local findings observed in animals treated with the test article appears to be worse than the ones treated with Kanalog-40 IR at the same dose level. Lastly, local microscopic findings at the end of recovery period were worse in the main study than in the subset study. These findings may have to be considered more thoroughly if this NDA comes back for the chronic indication in future.

Impurity/Degradants
The Applicant proposed drug product specifications of NMT (b) (4) % for individual unspecified degradants and NMT (b) (4) % for three individual specified degradants (b) (4) which, if exposed to the body upon injection, exceed the qualification threshold (0.5% or 200 mcg TDI, whichever is lower) and identification threshold (0.2% or 2 mg TDI, whichever is lower), respectively, per the ICH Q3B(R2) guidance: *Impurities in New Drug Products*, which is available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073389.pdf.

The Applicant provided the following justifications:

1. (b) (4)
2. The rate of release: The Applicant demonstrated that one of impurities, (b) (4) using in vitro release (IVR) study.

Although the dose of TCA in Zilretta is 32 mg, it is formulated with PLGA microspheres to release TCA slowly over an extended period and, based on the human PK data, it is reasonable to consider the (b) (4) From the IVR study,

(b) (4) . (b) (4) of 32 mg, which is within the qualification threshold per the ICH Q3B(R) (See the calculation below).

(b) (4)

The Applicant only provided the IVR data for (b) (4) however, other impurities (b) (4) Taken together, the proposed specifications of these impurities appear to be acceptable.

From QSAR prediction studies (DEREK and Leadscope) submitted by the Sponsor, one of drug product impurities (b) (4) had a potential structural alert for bacterial mutagenicity. (b) (4) The Applicant conducted QSAR prediction studies (DEREK 3.0.1 Nexus 1.5. and Leadscope) for potential mutagenicity of TCA and several impurities of drug products. One of drug product impurities (b) (4) had a structural alert on bacterial mutagenicity, which possibly comes from (b) (4) from the DEREK analysis. (b) (4) was re-evaluated by CDER/OTS/OCP/DARS for bacterial mutagenicity using (Q)SAR models (DEREK Nexus 5.0.2 (DX), Leadscope Model Applier 2.2.1-1 (LMA), and CASE Ultra 1.6.2.1 (CU). Consistent with the Applicant's result, (b) (4) was predicted to be positive in Salmonella and *E. coli* TA100 and TA102 mutagenicity in DEREK analysis due to the presence of the (b) (4) (b) (4) the report concluded that overall the degradant was predicted to be negative for bacterial mutagenicity. (b) (4) the Applicant did not provide data to support that conclusion. Therefore, the structural alert cannot be dismissed based on the data submitted.

Bacterial Mutagenicity (Q)SAR Predictions[1]

(b) (4)

Software	*Salmonella* Mutagenicity	*E. coli*/ TA102 Mutagenicity
Derek Nexus	+	+
Leadscope Model Applier	-	-
CASE Ultra	-	-
Overall Software Prediction	+	+
Overall Expert Prediction	-	-

Positive Predictivity of Structural Alerts

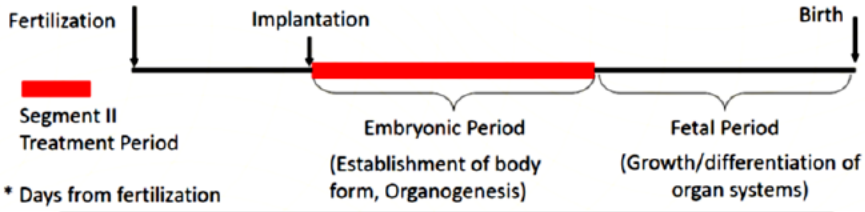
Alert	Positive Predictivity
[redacted]	(b) (4)

However, based on the proposed specification, the potential daily exposure to this degradant (b) (4) [redacted]. As noted, this NDA 208845 is seeking an approval of Zilretta for a single use. Therefore, the proposed specifications of impurities in the drug product are acceptable.

Reproductive Toxicity Studies

No reproductive Toxicology studies were conducted by the Applicant but a literature review consisting of studies in animals from the public domain to address the effects of TCA on reproduction and embryonic development was provided in accordance with the Pregnancy Lactation and Labeling Rule (PLLR). There was evidence in several species that demonstrated that TCA administration during the period of organogenesis (See the figure below for organogenesis periods for each species) at doses less than the MRHD based on body surface area comparisons caused resorptions, decreased fetal body weights, craniofacial abnormality, and prenatal deaths/still birth (4-13). Since these teratogenic effects were observed in several species, the information is considered important for the Zilretta product label to inform that there may be a potential risk for Zilretta to cause fetal harm if given to pregnant women.

Figure 6. Organogenesis Periods



	Implantation*	Organogenesis Ends*	Birth*
Mouse	5	15	19–20
Rat	5–6	16	21–22
Rabbit	6–7	19	30–32
Monkey	9**	44–45	166
Human	6–7	50–56	266

** Dosing typically starts on GD 20 due to the need to confirm pregnancy by ultrasound on GD 18–20

(Hoberman AM, Charles River Laboratory, Practical Reproductive and Developmental Toxicology, 2017)

The table below summarizes the key findings identified in the references provided with the PLLR literature review. Only TCA-related findings were noted in the table. For detailed information, refer to Section 9 (Reproductive and Developmental Toxicology). Based on these findings, appropriate language was included in the draft label that will undergo negotiation with the Applicant.

Table 11. PLLR Literature Summary

Reference	Species	TCA Dose	Dosing Regimen	Route of Admin.	Key Findings	HED tested in animal studies
Mice						
Walker 1965	A/J, 129/J, DBA/1J	0.012 or 0.05 mg/day	a single dose at GD 11, 12, 13, or 14	IM , SC	- cleft palate observed (all strains at above 0.0125 mg/day) - A/J strain was most sensitive to TCA compared to other strains	Up to 0.24 mg
	C57BL/6J, or C3H/HeJ	0.0005, 0.001, 0.003, 0.006, **0.0125**, 0.025, 0.05, 0.2, or 0.5 mg/day	four days from GD 11 to 14			Up to 2.4 mg
Kusinagi 1983	SWV and C57BL	**2.5** mg/kg	a single dose at GD 12	SC	- cleft palate observed in both strains but more sensitive in SWV mice - palatal slit observed only in C57BL/6 mice - TCA-induced teratogenic factors include uterus environment (cleft palate) and fetal genotype (palatal slit)	12 mg
Miyagi 2008	ICR-LSC	**15** or 30 mg/kg	A single dose at GD 7, 8, 9, or 10	IM	- the highest incidence of cleft palate was observed on E9	4.9 or 9.8 mg
Rats						
Rowland and Hendrickx 1983a	SD	**0.5** mg/kg	a single injection at GD 12, 13, or 14	IM	- cleft palate (the highest at GD 13) - body weight reduction - umbilical hernias, resorption, fetal growth retardation	4.8 mg
		0.5, 2.5, and 5 mg/kg	a single dose at GD 13			Up to 48.4 mg
Rowland and Hendrickx 1983b	SD	0, **0.125**, 0.25, or 0.5 mg/kg	3 consecutive days at GD 9-11, 12-14*, or 15-17	IM	- the most sensitive period to TCA exposure: GD 12-14 - dose-dependent fetal mortality/ malformation (cleft palate, umbilical hernias) - undescended testes at HD - a reduced degree of ossification (but no specific skeletal malformation), reduction in fetal weight	up to 4.8 mg

Watanabe 1995	SD	0, 0.25, **0.5**, 1, or 2 mg/kg/day	GD 14 and 15	SC	- cleft palate observed in all dosing groups - omphalocele, general edema, late resorption, and growth retardataion observed as well	up to 19 mg
Wise 1991	SD	0.05 or **0.1** mg/kg/day	once daily from GD 14 to 19	SC	- no maternal mortality observed but body weight reduction observed - fetal mortality, omphalocele, cleft palate, body weight reduction, growth retardation observed	up to 0.97 mg
		0.1 or 0.25 mg/kg/day				0.97 or 2.4 mg
Furukawa 2004	Wistar Hannover	**0.5** mg/kg	GD 12, 13 and 14	IM	- fetal mortality, cleft palate observed	4.8 mg
Rabbits						
Walker 1967	New Zealand White/ American Dutch	0.01, **0.1**, 0.25, 0.3, 0.5, 0.75, 1, 2, or 5 mg/day	4 days from 13.3 to 16.3 days post-conception	IM	- resorption and cleft palate Note: triamcinolone used, not TCA	up to 48 mg
NHPs						
Henrickx 1980	Rhesus monkey	**10** or 20 mg/kg/day	a single or multiple doses (2 or 3) between GD 21 and 43	IM	- increased prenatal death - severe craniofacial, CNS and skeletal/ visceral malformation - Reduced body and brain weights	194 or 387 mg
	Bonnet monkey	**10** or 15 mg/kg/day	a single or multiple doses (2 or 3) between GD 23 and 31			194 or 290 mg
	Baboons	**5** or 10 mg/kg/day	multiple doses (2 or 3) between GD 23 and 31			167 or 333 mg
Jerome and Henrickx 1988	Rhesus monkey	**0.5** or 2.5 mg/kg Kenalog-40	From GD 22 to 50 ± 3	IM	- increased prenatal death - severe craniofacial, CNS and skeletal/ visceral malformation	9.7 or 48 mg

*Human equivalent dose (HED): based on 60 kg average weight; Underlined dose (the lowest dose caused teratogenic malformations such as cleft palate and omphalocele); *the most sensitive dosing period when the lowest dose was determined*

12 Appendix/Attachments

References:

1. Fielder FG, Hoff EJ, Thomas GB, Tolksdorf S, Perlman PL, Cronin MT. A study of the subacute toxicity of prednisolone, methylprednisolone, and triamcinolone in dogs. Toxicol Appl Pharmacol. 1959;1(3):305-14. PubMed PMID: 13659538.
2. Heilmann HH, Lindenhayn K, Walther HU. [Synovial volume of healthy and arthrotic human knee joints]. Z Orthop Ihre Grenzgeb. 1996;134(2):144-8. doi: 10.1055/s-2008-1039786. PubMed PMID: 8779258.
3. Wehr B GA, Hudelmaier M, Kotyk J, Wachsmuth L, Eckstein F. Tibial cartilage surface area, thickness and volume in various animal species and in humans. Osteoarthritis Cartilage. 2007;15:C54-C5.
4. Hendrickx AG, Pellegrini M, Tarara R, Parker R, Silverman S, Steffek AJ. Craniofacial and central nervous system malformations induced by triamcinolone acetonide in nonhuman primates: I. General teratogenicity. Teratology. 1980;22(1):103-14. doi: 10.1002/tera.1420220113. PubMed PMID: 7444796.
5. Jerome CP, Hendrickx AG. Comparative teratogenicity of triamcinolone acetonide and dexamethasone in the rhesus monkey (Macaca mulatta). J Med Primatol. 1988;17(4):195-203. PubMed PMID: 3193449.
6. Kusanagi T. Occurrence of cleft palate, palatal slit, and fetal death in mice treated with a glucocorticoid: an embryo transfer experiment. Teratology. 1983;27(3):395-400. doi: 10.1002/tera.1420270313. PubMed PMID: 6879461.
7. Miyagi H, Kubota Y, Tsuda T, Sasaki Y, Ono S, Kimura O, Iwai N. Congenital anomalies induced by triamcinolone acetonide in murine embryos. Eur J Pediatr Surg. 2008;18(3):164-7. doi: 10.1055/s-2008-1038535. PubMed PMID: 18493890.
8. Rowland JM, Hendrickx AG. Comparative teratogenicity of triamcinolone acetonide, triamcinolone, and cortisol in the rat. Teratog Carcinog Mutagen. 1983;3(4):313-9. PubMed PMID: 6138866.
9. Rowland JM, Hendrickx AG. Teratogenicity of triamcinolone acetonide in rats. Teratology. 1983;27(1):13-8. doi: 10.1002/tera.1420270104. PubMed PMID: 6845214.
10. Walker BE. Cleft Palate Produced in Mice by Human-Equivalent Dosage with Triamcinolone. Science. 1965;149(3686):862-3. doi: 10.1126/science.149.3686.862. PubMed PMID: 17737386.
11. Walker BE. Induction of cleft palate in rabbits by several glucocorticoids. Proc Soc Exp Biol Med. 1967;125(4):1281-4. PubMed PMID: 6042444.
12. Watanabe CI, Yasuo; Nagao, Tetsuji. Palatal Slit and Cleft Palate in Rats Treated with Glucocorticoids II. Comparative Teratogenicity of Prednisolone, Triamcinolone Acetonide and Hydrocortisone. Congenital Anomalies. March 1995;35(1):133-40.
13. Wise LD, Vetter CM, Anderson CA, Antonello JM, Clark RL. Reversible effects of triamcinolone and lack of effects with aspirin or L-656,224 on external genitalia of male Sprague-Dawley rats exposed in utero. Teratology. 1991;44(5):507-20. doi: 10.1002/tera.1420440505. PubMed PMID: 1771593.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MISOL AHN
09/08/2017

JAY H CHANG
09/08/2017

RICHARD D MELLON
09/08/2017
I concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 208845

NDA Number: 208845 **Applicant:** Flexion Therapeutics, Inc. **Stamp Date:** 12/08/2016

Drug Name: Zilretta (triamcinolone acetonide extended release injectable suspension) **NDA Type:** 505 (b)(2)

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	N/A		The to-be-marketed formulation was used in the pivotal toxicology studies via the clinical route (intra-articular injection).
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		The NDA included an extractable/leachable evaluation. Based on a preliminary review, the data appear inadequate to support the safety of the drug product for approval.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 208845

	Content Parameter	Yes	No	Comment
				However, from a nonclinical perspective, it is not a filing issue. See potential review issue comments below.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		The proposed drug product specifications for specified and unspecified degradants exceed ICH Q3B(R2) thresholds. The Applicant has provided a justification for their proposed specifications, but it appears inadequate based on a preliminary review. See potential review issue comments below.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	N/A		
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?	X		The NDA is a 505(b)(2) application referencing the approved product, Kenalog-40 (NDA 14901). To bridge to the systemic safety of the referenced product, the Applicant has demonstrated that there is no systemic exposure to the API following intra-articular injection. To justify the local safety of the product, appropriate nonclinical intra-articular toxicology studies were submitted.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __YES__

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

N/A

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

1. The proposed drug product specifications of NMT (b) (4)% for individual specified degradants and NMT (b) (4)% for individual unspecified degradants exceed the qualification threshold (0.5% or 200 m TDI, whichever is lower) and identification threshold (0.2% or 2 mg TDI, whichever is lower), respectively, per the ICH Q3B(R2) guidance: *Impurities in New Drug Products*, which is available at:

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073389.pdf. We acknowledge that you have based your proposed specifications on the maximum daily exposure of triamcinolone acetonide over the intended prolonged release facilitated by the PLGA polymer ((b) (4)); however, we do not agree with this rationale for establishing degradant specifications. Unless the rate of exposure to individual degradants can be demonstrated to be similar to triamcinolone acetonide, you must assume that the total amount of individual impurities may be exposed on the first day post-injection and specifications must be based on the maximum daily dose of 40 mg. Therefore, you must either reduce the specifications for specified and unspecified degradants to be within the ICH Q3B(R2) thresholds outlined above, or provide data to justify the safety of the proposed specifications that exceed these thresholds. Based on submitted data for registration batches on stability testing, the levels of several impurities ((b) (4)) appear to remain within ICH thresholds and their specifications should be reduced. Another consideration may be to shorten the expiry of your drug product so that the impurity specifications are within the ICH Q3B(R2) qualification threshold. To adequately qualify impurities/degradants in accordance with ICH Q3B(R2), you must provide the following data:

a. You must complete a minimal genetic toxicology screen (two in vitro genetic toxicology studies, e.g., one point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.

b. In addition, you must conduct a repeat-dose toxicology study of appropriate duration via an adequate route to support the proposed indication. In this case, duration of 90 days is appropriate.

c. You may be able to justify the safety of a drug product degradant via comparative analytical studies demonstrating that the levels of the degradant in your drug product are equal to or below the levels found in the referenced drug product. If you elect to pursue this approach, refer to the FDA guidance for industry: *ANDAs: Impurities in Drug Products*, available at, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072861.pdf.

2. Based on a preliminary review of your NDA submission, it does not appear that an adequate extractables/leachables evaluation was performed. In pre-NDA written responses sent on 5/25/2016, we specifically stated that "although a toxicological risk assessment based on the results of the extraction study may be adequate to support the safety assessment during the development, *you should still evaluate at least three batches of your drug product over the course of your stability studies* and base the final safety assessment on the levels of leachables identified to determine the safe level of exposure via the label-specified route of administration." However, only two batches were evaluated (b) (4) and, only two batches were evaluated for the diluent of which only one batch (Lot 023C15) was evaluated in the inverted position at one time point (b) (4) As the container system consists of a glass vial and rubber stopper, our primary concern is the potential exposure to patients from leachables arising from the rubber stopper of the diluent. To address this issue, we strongly recommend that you provide leachables data, as soon as possible during this review cycle, from at least three batches of the FX006 Diluent placed on stability in the inverted position at multiple time points over the course of your stability studies, preferably at release, an intermediate time point, and towards the proposed expiry, in order to identify trends in leachable levels over time. Establish your

AET to be able to detect potentially carcinogenic or genotoxic compounds as per ICH M7 qualification thresholds (e.g., not more than 1.5 mcg/day or up to 120 mcg/day depending on the duration of treatment). However, from a general toxicology perspective, for parenteral products, the AET must be able to detect and identify any leachable that is present in the product at [redacted] (b) (4) in order, unless justified otherwise, to permit an adequate toxicological risk assessment.

For additional guidance on extractables and leachables testing, refer to the following documents:

- USP <1663>: Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems

- USP <1664>: Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems

- FDA guidance for industry: Container Closure Systems for Packaging Human Drugs and Biologics, available at, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070551.pdf

The extractable/leachable data must be accompanied by an adequate toxicological risk assessment. Although a toxicological risk assessment based on the results of the extraction studies may be adequate to support the safety assessment during development, evaluate at least three batches of your drug product that have been tested at multiple time-points over the course of your stability studies, as discussed above, and base the final safety assessment on the maximum predicted levels of leachables identified to determine the safe level of exposure via the label-specified route of administration. The approach for toxicological evaluation of the safety of leachables must be based on good scientific principles and take into account the specific container closure system or patch, drug product formulation, dosage form, route of administration, and dose regimen (chronic or short-term dosing). The safety assessment should be specifically discussed in Module 2.6.6.8 (Toxicology Written Summary/Other Toxicity) of the NDA submission. The risk assessment should be based on the maximum level of each leachable detected in long-term stability samples that include any intended secondary container closure system(s) unless otherwise justified. Include copies of all referenced studies upon which a safety assessment is based.

- If you employ a Permissible Daily Exposure (PDE) assessment as described in ICH Q3C, provide justification for all safety factors employed.

- Published literature to support the safety of any compound rarely provides adequate detail of the study design and study results to permit a thorough independent evaluation of the data. Summary reviews, (e.g., BIBRA, CIR, HERA), although they can be potentially useful to identify original source materials, are not acceptable as the source material is not provided and the conclusions cannot be independently verified. Submission of any published study reports must be accompanied by a detailed comparison to modern toxicology study endpoints and any shortcomings of the study must be discussed and justification must be provided to support your assertion that these data are adequate to support the safety of your drug product formulation.

- Safety justifications based on analogous compounds are also not acceptable unless you can provide adequate data to support your conclusions that a risk assessment based on one compound can be logically interpolated to represent an adequate safety evaluation for your extractables/leachables. This should include a detailed understanding of the absorption, distribution, metabolism, and elimination of the compounds and an adequate scientific bridge to interpolate a NOAEL for the novel leachable.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MISOL AHN
02/07/2017

JAY H CHANG
02/07/2017

RICHARD D MELLON
02/08/2017