Date of Approval: November 16, 2020

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-541

STELFONTA®

tigilanol tiglate injection

Injectable Solution

Dogs

STELFONTA® is indicated for use in dogs for the treatment of:

- non-metastatic cutaneous mast cell tumors
- non-metastatic subcutaneous mast cell tumors located at or distal to the elbow or the hock

Sponsored by:

QBiotics Group Ltd

Executive Summary

STELFONTA® (tigilanol tiglate injection) is an antineoplastic drug approved for use in dogs for the treatment of non-metastatic cutaneous mast cell tumors and for the treatment of non-metastatic subcutaneous mast cell tumors located at or distal to the elbow or hock.

STELFONTA® causes lysis of cells and activates a protein kinase C cascade that spreads throughout the treated tumor. This results in an acute inflammatory response with swelling and erythema extending to the tumor margins and the immediate surroundings. STELFONTA® also increases the permeability of the tumor vasculature, leading to tumor vascular destruction. The expected outcome is tumor destruction with a wound where the treated tumor was located. STELFONTA® does not specifically target cancer cells.

Proprietary Name	Established Name	Application Type and Number	Sponsor
STELFONTA®	tigilanol tiglate injection	New Animal Drug Application (NADA) 141-451	QBiotics Group Ltd

Safety and Effectiveness

The sponsor conducted a field effectiveness study comparing STELFONTA® to no treatment in client-owned dogs. The study included dogs of any breed and either gender diagnosed with measurable cutaneous or subcutaneous mast cell tumors (MCTs). If the MCT was subcutaneous, it had to be located at or distal to the elbow or hock. Enrolled dogs represented a range of weights and ages (all were at least 1 year of age). Twice as many dogs were enrolled in the STELFONTA® group than the untreated control group. On Day 0, dogs received either one intratumoral injection of STELFONTA® distributed evenly throughout the tumor or no treatment. All dogs in both groups received prednisone or prednisolone, famotidine, and diphenhydramine as concomitant medications to reduce the risk of systemic adverse reactions due to mast cell degranulation.

On Day 28, 60 of the 80 dogs (75%) in the STELFONTA® group had a complete response, meaning that there was complete disappearance of the treated tumor. Only 2 of the 38 dogs (5.3%) in the untreated control group had a complete response on Day 28. On Day 30, 18 dogs in the STELFONTA® group that did not achieve a complete response were retreated with STELFONTA®. On Day 58 (28 days after the second injection), a complete response was seen in 8 of the 18 dogs (44.4%). On Day 30, 33 dogs in the untreated control group were treated with STELFONTA® for the first time, and on Day 58, a complete response was seen in 20 of 32 dogs (62.5%) (1 dog was excluded from the analysis).

The sponsor conducted one margin of safety laboratory study and one pilot pharmacokinetic field study. The margin of safety study was conducted in 48 young, healthy, intact female and male Beagles. Intratumoral injection was not feasible in this study because the dogs did not have MCTs and subcutaneous injection was too toxic; therefore, the drug was administered intravenously (IV). The pilot study was conducted in 10 client-owned dogs, between the ages of 6 and 14 years and of any breed and either gender, with MCT; STELFONTA® was administered intratumorally under actual conditions of use. These studies

showed that IV dosing caused higher systemic drug exposure than intratumoral injection, and therefore, supported the evaluation of systemic safety by the IV route of administration. In the pilot study, the pharmacokinetic results were highly variable and showed that a lower dose did not result in lower systemic exposure or fewer adverse reactions.

The sponsor also conducted one cardiovascular laboratory study to assess the potential effects of STELFONTA® on the cardiovascular system following a single 15-min IV infusion of STELFONTA® in 4 healthy, intact male, 2 to 4-year-old Beagles. STELFONTA® caused a reversible increase in heart rate that was dose-dependent, but had no obvious effects on body temperature, blood pressure, or electrocardiograms.

In the laboratory studies where STELFONTA® was administered IV, the most common adverse reactions were vomiting; lameness in the treated leg; and wound formation, edema, and erythema at the injection site.

In the field effectiveness study the most common adverse reactions were wound formation at the tumor site; injection site reactions, such as pain, edema, erythema, bruising, thickening, fibrosis, and necrosis; lameness in the treated leg; vomiting; diarrhea; and hypoproteinemia and hypoalbuminemia, likely due to the wound healing process and the drug's effect on tumor vasculature. Most wounds gradually reduced in size or completely resolved; however, a small number of dogs developed extensive wounds with cellulitis and severe tissue sloughing that extended away from the treated tumor and led to a prolonged recovery time and additional wound care. STELFONTA® can only be administered into subcutaneous MCTs located at or below the hock or elbow. Giving the drug into a subcutaneous MCT located elsewhere (for example, on the body, head, or neck) may result in the wound draining internally and in necrotic tissue accumulating in the subcutaneous space, increasing the risk of systemic adverse reactions, including death, from mast cell degranulation.

Many of the adverse reactions were expected based on the drug's mechanisms of action and were manageable. Most of the adverse reactions were mild or moderate; however, some were more severe (see "Safety Warnings" section below). In the field effectiveness study, there were no obvious differences in the types of adverse reactions, or their frequency, between dogs with cutaneous MCTs and dogs with subcutaneous MCTs.

In the various safety studies, the relevant clinical pathology findings (hematology, serum chemistry, and urinalysis) included a trend toward decreasing hematocrit, monocytosis, elevated ALT, elevated fibrinogen, proteinuria, increased white blood cells in the urine without bacteriuria, and decreased urine specific gravities or isosthenuria without azotemia. Peripheral lymph node changes were also seen, including redness, enlargement, inflammation, lymphoid hypercellularity, and hemorrhage.

Safety Warnings
The labeling for STELFONTA® includes a boxed warning for human safety regarding the risk of severe wound formation from accidental self-injection or needle stick injuries. The boxed warning also includes several statements regarding the safe use of STELFONTA® in dogs: STELFONTA® should always be given with a corticosteroid, an H1 receptor blocking agent (for example, diphenhydramine), and an H2 receptor blocking agent (for example, famotidine) to decrease the risk of severe systemic adverse reactions, including death, from mast cell degranulation. STELFONTA® should not be used to treat subcutaneous MCTs located above the elbow or hock. STELFONTA® may cause extensive wound formation at the tumor site, including cellulitis and severe tissue sloughing. Wound formation and healing are related to the drug's mechanisms of action. The drug's package insert includes color photographs of the types of wounds that may occur in both people and dogs.

Limited data are available on the potential teratogenic effects of STELFONTA®. Therefore, women who are pregnant or who may become pregnant should not administer the drug.

Conclusions
Based on the data submitted by the sponsor for the approval of STELFONTA®, FDA determined that the drug is safe and effective when used according to the label.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-541

B. Sponsor

QBiotics Group Ltd.,
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165 Moggill Rd.,
Taringa,
Queensland 4068, AU

Drug Labeler Code: 086132

U.S. Agent Name and Address:
James H. Schafer, DVM
Schafer Veterinary Consultants, LLC
800 Helena Court
Fort Collins, CO 80524

C. Proprietary Name

STELFONTA®

D. Drug Product Established Name

Tigilanol tiglate injection

E. Pharmacological Category

Antineoplastic

F. Dosage Form

Injectable solution

G. Amount of Active Ingredient

1 mg/mL

H. How Supplied

Packaged in a carton containing one 2 mL, single-dose, glass vial.

I. Dispensing Status

Prescription (Rx)

J. Dosage Regimen

Administer STELFONTA® as an intratumoral injection at a dose of 0.5 mL per cm^3 of tumor volume, as determined by the following calculations:

- **Determine the Tumor Volume in cm^3:**
  0.5 x [length (cm) x width (cm) x height (cm)]
- Confirm the Tumor Volume does not exceed 10 cm^3. Do not use STELFONTA® if tumor volume is >10 cm^3.
- **Calculate the Dose volume (mL) of STELFONTA® to inject:**
  Tumor Volume x 0.5 mL
- Confirm the dose of STELFONTA® does not exceed 0.25 mL/kg body weight.
- Do not exceed 5 mL per dog, regardless of tumor volume or body weight.
- The minimum dose of STELFONTA® is 0.1 mL, regardless of tumor volume or body weight. If the calculated dose is < 0.1 mL, administer 0.1 mL.

K. Route of Administration

Intratumoral injection

L. Species/Class

Dogs

M. Indication

STELFONTA® is indicated for use in dogs for the treatment of:

- non-metastatic cutaneous mast cell tumors
- non-metastatic subcutaneous mast cell tumors located at or distal to the elbow or the hock

II. EFFECTIVENESS

STELFONTA® causes lysis of cells and activates a protein kinase C cascade that propagates throughout the treated tumor mass, resulting in an acute inflammatory response with swelling and erythema extending to the tumor margins and the immediate surroundings. STELFONTA® also increases the permeability of the tumor vasculature leading to tumor vascular destruction. The expected outcome is tumor destruction with a wound where the treated tumor mass was located. Many of the adverse reactions associated with STELFONTA® are directly related to these mechanisms of action.

The effectiveness of STELFONTA® was demonstrated in one adequate and well-controlled clinical field study. One hundred and twenty-three client-owned dogs with non-metastatic cutaneous mast cell tumors (MCT) and non-metastatic subcutaneous MCTs located at or distal to the elbow or the hock were randomly assigned to receive STELFONTA® or sham-treatment (untreated control). All dogs received prophylactic medications to address the potential for mast cell degranulation. Eighty-one dogs

were administered STELFONTA® intratumorally at a dose of 0.5 mg/cm^3 of tumor volume. Complete response (complete disappearance of the tumor) rates of the target MCT was greater in the STELFONTA® group compared to the untreated control group 28 days after treatment. STELFONTA® was found effective for the treatment of non-metastatic cutaneous MCTs and for the treatment of non-metastatic subcutaneous MCTs located at or distal to the elbow or the hock in dogs. The most common adverse reactions observed in the field study included wound formation, injection site pain, and lameness in the treated limb, which were related to the mechanisms of action, and vomiting, diarrhea, and hypoalbuminemia. Wound formation, vomiting, and diarrhea were mainly observed within the first 7 to 10 days after treatment. Injection site pain and lameness in the treated leg was mainly observed within the first 2 days after treatment. Hypoalbuminemia was mainly observed at Day 7 after treatment. Please refer to Section III. Target Animal Safety for a full assessment of the safety of STELFONTA®.

During development, tigilanol tiglate was also referred to as EBC-46.

A. Dosage Characterization

The targeted dose of STELFONTA® is 0.5 mg per cm^3 of tumor volume, distributed throughout the tumor mass, for the treatment of non-metastatic cutaneous MCTs and the treatment of non-metastatic subcutaneous MCTs located at or distal to the elbow or the hock and in dogs. The dose is based on the results of three pilot field studies. The maximum dose is 0.25 mg/kg body weight, the maximum total dose is 5.0 mg/dog, and the minimum total dose is 0.1 mg of tigilanol tiglate.

1. Pilot Field Study

   Title: Dose Determination Study of EBC-46 In Dogs For The Intratumoral Treatment of Cutaneous Mast Cell Tumours. (Study No. QB46C-C01)

   Twenty-seven client-owned dogs with cutaneous MCTs were enrolled in an unmasked, uncontrolled, non-randomized study evaluating three doses of tigilanol tiglate (non-commercial formulation). Dogs were treated once and were assigned to one of three cohorts (10, 10, and 7 dogs) with one of three concentrations (1, 0.5, and 0.2 mg/mL, respectively) of tigilanol tiglate injected intratumorally at 0.5 mL per cm^3 of tumor volume.

   Response to treatment of the target tumor was categorized using the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 guidelines, which define complete response (CR) as resolution of the target tumor; partial response (PR) as at least a 30% decrease in the longest diameter of target tumor; stable disease (SD) as a decrease of less than 30% or increase of less than 20% of the longest diameter of the target tumor; and progressive disease (PD) as greater than a 20% increase in the longest diameter of the target tumor.[1] The primary measure of effectiveness was determined as achieving CR at 21 days post-treatment.

   Of the 10 dogs treated at 1 mg/mL, 9 experienced CR and 1 experienced SD. Of the 10 dogs treated at 0.5 mg/mL, 5 experienced CR, 1 PR, 3 SD, and 1 PD. Of the 7 dogs treated at 0.2 mg/mL 2 experienced CR, 1 PR, and 4 SD.

A total of 64 adverse events (AEs) were reported during the study. All AEs were reported as Grade 1 (mild) or 2 (moderate) per the veterinary cooperative oncology group – common terminology criteria for adverse events v1.1 (VCOG-CTCAE) guidelines.[2] The most common AEs were local swelling at the treatment site (22 dogs), transient tachypnea (14 dogs), pain associated with the treatment site (10 dogs), and transient lethargy (5 dogs).

Based on the overall clinical response, the dose concentration selected was 1 mg tigilanol tiglate per mL, to be administered at a dose of 0.5 mL per cm^3 of tumor volume.

2. Pilot Field Study

Title: A Pilot Pharmacokinetic and Dose Confirmation Study of EBC-46 For The Intratumoural Treatment of Cutaneous or Subcutaneous Mast Cell Tumours in Dogs. (Study No. QB46C-C02)

Ten client-owned dogs with cutaneous or subcutaneous MCTs were enrolled in an unmasked, uncontrolled study evaluating tigilanol tiglate (non-commercial formulation). Dogs were administered an intratumoral injection of tigilanol tiglate (1 mg/mL) at a dose of 0.5 mL per cm^3 of tumor volume. One dog received treatment in two different tumors resulting in a total of 11 evaluable cases.

CR was defined as disappearance of the target lesion. By Day 14 of the study, 9 of 10 dogs (10 of 11 tumors) experienced CR and remained in CR at Day 28. One tumor continued to enlarge, and the dog was withdrawn from the study.

Twenty-one AEs considered possibly, probably, or definitely related to treatment were reported during the study. All of these AEs were reported as VCOG-CTCAE Grade 1 (mild) or 2 (moderate). The most common AEs included injection site reactions (pain or restlessness; 6 dogs), skin necrosis or dermatitis in a region nearby but distinct from the tumor injection site (3 dogs), and localized edema (3 dogs).

One of the dogs reported with skin necrosis and edema had been treated for a cutaneous MCT tumor on the left leg cranial to the tibia. Swelling of the left leg from the medial thigh to the hock was observed from 1 to 13 days after treatment with swelling extending to the inguinal area from 6 to 56 days after treatment. Skin necrosis consisting of two necrotic holes in the skin of the ventral abdomen was observed 13 days after treatment and eventually resolved with medical care.

3. Pilot Field Study

Title: Round Cell Tumour 40% Dose Volume Study In Dogs. (Study No. QB46C-C03)

Twelve client-owned dogs with cutaneous or subcutaneous MCT were enrolled in an unmasked, uncontrolled study evaluating tigilanol tiglate (non-commercial formulation). Dogs were administered an intratumoral injection of

tigilanol tiglate (1 mg/mL) at a dose of 0.4 mL per cm^3 of tumor volume. Effectiveness was evaluated in 11 dogs.

Response to treatment of the target tumor was assessed on Day 28 and was categorized using the RECIST classification schema. On Day 28, 6 dogs experienced CR, 3 dogs experienced PR, and 2 dogs experienced SD.

A total of 44 AEs were reported during the study. All of these AEs were reported as VCOG-CTCAE Grade 1 (mild) or 2 (moderate) with the exception of one dog that died likely due to degranulation of the mast cell tumor (see Adverse Reactions below) on Day 4 of the study. The most common Grade 1 and 2 AEs included local swelling at the treatment site (8 dogs), lethargy (7 dogs), pain associated with the treatment site (6 dogs), apathy (5 dogs), bruising at the treatment site (3 dogs), and antibiotic support (3 dogs).

Based on the results of the study, the 0.5 mL per cm^3 dose was selected for final clinical development.

B. Substantial Evidence

1. Clinical Field Study

Title: A Multicenter, Randomized, Sham-controlled, Investigator- and Owner-masked, Pivotal Safety and Efficacy Study of Intratumoral EBC-46 in the Treatment of Canine Cutaneous and Lower Limb Subcutaneous Mast Cell Tumors. (Study No. PN1894)

Study Dates: August 2016 to September 2018.

Study Locations:

Bristol, CT
Canandaigua, NY
Catonsville, MD
Franklin Lakes, NJ
Houston, TX
Liverpool, NY
Mantua, NJ
Ocala, FL
Quakertown, PA
Seminole, FL
Springfield, MO

Study Design: This was a multicenter, prospective, randomized, masked, sham-controlled (untreated controlled) field study.

Objective: To evaluate the effectiveness and field safety of STELFONTA® when administered via intratumoral injection into cutaneous MCTs and subcutaneous MCTs located at or distal to the elbow or the hock. The study was conducted in accordance with Good Clinical Practice.

Study Animals: The study enrolled 123 dogs of any breed or sex diagnosed with measurable cutaneous MCTs or subcutaneous MCTs located at or distal to the elbow or the hock. Dogs ranged in age from 3.5 to 15.9 years in the STELFONTA® group and 4.0 to 15.0 years in the untreated control group. Weight ranged from 3.2 to 54.4 kg in the STELFONTA® group and 5.1 to 63.5 kg in the untreated control group, at the time of treatment. There were 30 neutered males, 47 spayed females, 2 intact males, and 2 intact females enrolled in the STELFONTA® group (for a total of 81 dogs) and 16 neutered males and 26 spayed females in the untreated control group (for a total of 42 dogs). The most commonly enrolled breed was large mixed breed (16.2%). Tumor stage according to the World Health Organization (WHO) was Ia (one tumor confined to the dermis, without regional lymph node involvement) in 43 dogs, IIIa (multiple dermal tumors; large infiltrating tumors) without regional lymph node involvement in 10 dogs, and unknown/not provided for 28 dogs in the STELFONTA® group. Tumor stage according to WHO was Ia in 26 dogs, IIIa in 3 dogs, and not known/provided for 13 dogs in the untreated control group. Cutaneous MCTs were identified in 74 dogs in the STELFONTA® group and 35 dogs in the untreated control group and subcutaneous MCTs were identified in 7 dogs in each group. At Day 0, the average MCT volume was 1.7 cm^3 in the STELFONTA® group and 1.6 cm^3 in the untreated control group.

The tumor grades, based on the Kiupel system[3], for each group are listed in Table II.1 below.

Table II.1. Tumor Grades

Tumor Grade	STELFONTA® Group (n=81)	Untreated Control Group (n=42)
High Grade MCT	5 (6.2%)	4 (9.5%)
High Grade MCT Suspected	2 (2.5%)	2 (4.8%)
Low Grade MCT	45 (55.6%)	23 (54.8%)
Low Grade MCT Suspected	13 (16.0%)	6 (14.3%)
MCT Grade Not confirmed	16 (19.8%)	7 (16.7%)

Experimental Design:

Table II.2. Treatment Groups

Treatment Group	Number of Dogs in Group	Dose
STELFONTA®	81	50% tumor volume (0.5 mg tigilanol tiglate per cm^3 tumor volume)
Untreated control	42	Not applicable

Housing: Following treatment on Day 0 (and Day 30 if applicable), dogs remained in the hospital for 24 to 48 hours. Afterwards, dogs remained in the care of the owner and in their normal home environment for the duration of the study.

Randomization and Masking: Dogs were randomized using a block of size 6 in a 2:1 tigilanol tiglate-to-sham ratio based on the order of enrollment at each site. Owners were masked to their dog's treatment assignment. Lesion evaluators and other masked personnel recording observational data had no access to treatment group assignment. Each site had at least one Treatment Administrator that was unmasked and had the responsibility of following the randomization plan for all dogs and administering treatment.

Inclusion Criteria:

a. Signed owner consent
b. ≥1 year of age
c. Cytological diagnosis of cutaneous or subcutaneous MCT – WHO stages Ia or IIIa without regional lymph node involvement based on palpation of regional lymph node and cytology if lymph node is palpable
d. If the MCT is subcutaneous, the tumor is located at or distal to the elbow or hock;
e. In the Investigator's opinion, the dog has a life expectancy of at least 3 months without treatment;
f. At least 1 measurable lesion with a longest diameter ≥1 cm and a tumor volume ≤10 cm^3 without significant ulceration (an excoriated surface or abraded surface)
g. The target MCT is not a local recurrence from a previous surgical site or following radiation; recurrence following systemic therapy is acceptable
h. Performance Score of 0 or 1 [where 0=normal activity, able to perform at pre-disease level; 1=restricted activity: decreased activity from pre-disease status; 2=compromised activity, ambulatory only to the extent needed to eat, sleep and consistently urinate and defecate in acceptable areas; 3=disabled, completely disabled, unable to move such that must be hand fed or force fed, unable to urinate and defecate in acceptable areas; 4=dead or completely recumbent and imminent death].

Exclusion Criteria:

a. Presenting with clinical signs associated with MCT (substage b), including but not limited to vomiting, diarrhea or inappetence
b. Previously received biopsy, surgery, or radiotherapy for the target tumor
c. Systemic or local anticancer therapy in the 2 months prior to enrollment including but not limited to radiation therapy, chemotherapy, small molecule inhibitor, and/or other intratumoral therapy
d. Treatment with non-steroidal anti-inflammatory drugs (NSAIDs) ≤ 7 days prior to screening
e. Treatment with immunosuppressive therapy, such as corticosteroids or cyclosporines ≤14 days or long-acting corticosteroids (e.g. injectable methylprednisolone acetate) ≤ 21 days prior to Day 0 (with the exception of the study required pretreatment medication)
f. Treatment with anti-atopic medication such as oclacitinib or caninized anti-cIL-31 monoclonal antibody 14 days prior to screening
g. Prior treatment with tigilanol tiglate
h. Calculated dose of tigilanol tiglate is greater than 0.25 mg/kg or <0.1 mg (0.1 mL)

i. Presence of a concurrent medical disorder likely to result in death or euthanasia within 3 months or a condition that may be disruptive to the intent and objectives of the study
j. Lactating, pregnant, or intended for breeding
k. Participation in another clinical trial
l. Unavailable for the entire study duration
m. Considered unsuitable for study enrollment by the Investigator

Drug Administration: STELFONTA® was provided at a concentration of 1.0 mg/mL and dosed at 0.5 mg/cm^3 of tumor volume. The maximum dose was no greater than 0.25 mg/kg, the maximum total dose was 5.0 mg, and the minimum total dose was 0.1 mg. Using calipers, the tumor volume was determined using the modified ellipsoidal calculation: ½ (length (cm) x width (cm) x height (cm)).

The Treatment Administrator or designee wore a protective lab coat or gown, eye protection, and gloves during preparation and administration of tigilanol tiglate.

On Day 0, prior to treatment, the target tumor was shaved in both groups. Only one tumor was selected for treatment. For dogs in the STELFONTA® group, the dose was administered evenly by fanning the dosing solution (partial withdrawal and redirection and re-insertion of the administration needle) throughout the tumor mass from a single-entry injection point.

The actual dose volumes administered to the treated group on Day 0 ranged from 0.1 to 4.6 mL (mean 0.9 mL) and the calculated dose on a mg/kg basis ranged from 0.0* to 0.24 mg EBC-46/kg (mean 0.05 mg/kg) [*Small tumors on larger dogs could lead to a dose rate of <0.05 on a mg/kg basis].

STELFONTA® treated and untreated control dogs had the option to receive treatment with STELFONTA® on Day 30 based on Day 28 tumor response assessment. Eighteen previously STELFONTA® treated dogs and 36 untreated control dogs received STELFONTA® on Day 30 at the same dosing plan as Day 0.

For Day 30 treatments, the dose volume ranged from 0.1 to 4.6 mL (mean 0.7 mL). The dose administered on a mg/kg basis ranged from 0.0* to 0.30 mg EBC-46/kg (mean 0.04 mg/kg) [*Small tumors on larger dogs could lead to a dose rate of <0.05 on a mg/kg basis].

Concomitant Medications: The following concomitant medications were given regardless of treatment group allocation to decrease the potential for severe adverse reactions due to mast cell degranulation. Prednisone or prednisolone was initiated 2 days prior to study treatment at a dose of 0.5 mg/kg orally twice daily for 7 days (2 days before, on the day of treatment, and 4 days post treatment), then 0.5 mg/kg once daily for an additional 3 days. Famotidine (0.5 mg/kg orally twice daily) and diphenhydramine (2 mg/kg orally twice daily) were initiated on the day of study treatment and continued for 7 days. Pre-treatment medications were also administered prior to any Day 30 treatments following the same schedule.

Other commonly used concomitant medications prescribed based on veterinary discretion included antibiotics, analgesics, and sedatives. Antibiotics were used occasionally as a prophylaxis to treat injection site infections based on the treating veterinarian's clinical opinion, with only one case undergoing confirmatory culture and sensitivity diagnostics. Analgesics were used to treat tumor pain and were mainly initiated on the day of or day after treatment. Sedatives were occasionally used for treatment administration, conducting diagnostics, anxiety, and temperament issues.

Measurements and Observations: At the screening visit, fine needle aspirate samples were collected from the target tumor to confirm MCT and to cytologically determine the Kiupel grading.

Study observations included:
- physical examinations at screening and on Days 0, 1, 4, 7, 14, 28, 42, and 84,
- tumor measurements by caliper at screening and on Days 0, 28, 42, and 84,
- tumor images (photography) on Days 0, 1, 4, 7, 14, 28, 42, and 84,
- tumor observations on Day 0 at 2, 4, and 8 hours post treatment, on Day 1, and on Day 4,
- wound healing assessments on Days 7, 14, 28, 42, and 84,
- owner quality of life (QoL) assessments[4] at screening and on Days 0, 7, 14, 28, 42, and 84, and
- clinical pathology (hematology, clinical chemistry, and urinalyses) at screening and on Days 7, 28, and 42.

On Day 28 (and Day 58 if treated with STELFONTA® on Day 30), measurement of the longest diameter of the target tumor by caliper was performed by the two independent, masked evaluators for the interpretation of tumor response.

The primary effectiveness variable was the assessment for complete response (CR) of the target tumor at Day 28.

Response assessment was made according to the RECIST v1.1 guidelines as follows:
- Complete response (CR): Complete disappearance of the target lesion.
- Partial response (PR): At least a 30% decrease in the Longest Diameter (LD) of target lesion, taking as reference the baseline LD.
- Progressive disease (PD): At least a 20% increase in the LD of the target lesion, taking as reference the smallest LD recorded since treatment.
- Stable disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest LD since treatment.

Secondary effectiveness variables included:
- Objective tumor response (CR and PR) of the target tumor on Day 28.
- For dogs considered to be a CR at Day 28, the disease-free interval (DFI), assessed by target tumor measurement, on Days 42 and 84.
- Wound healing assessment.
- QoL assessments by the Owner.

Statistical Methods: The primary effectiveness variable was tested at an alpha level of 0.05, two-sided. The percentage of dogs with a CR on Day 28 was evaluated using a generalized mixed model (the GLIMMIX procedure in SAS), assuming a binomial distribution and a logit link. The model included a fixed effect for treatment group and random effects for site and treatment-by-site interaction. Treatment was considered effective if there was a significant difference (p=0.05, two-sided) in success rates and the success rate was greater in the treated group than the untreated control group.

For secondary variables, the number and percentage of dogs were presented by category for each study day and treatment group. Hypothesis testing was not applied to the secondary efficacy variables.

Safety variables collected prior to Day 30 were tested at an alpha level of 0.10, two-sided.

All statistical analyses were conducted using SAS for Windows (Version 9.3, or higher, Cary, NC).

Results: Effectiveness was evaluated in 118 dogs (80 dogs in the STELFONTA® group and 38 dogs in the untreated control group). At Day 28, a statistically significant difference (p<0.0001) was seen between the STELFONTA® and untreated control groups, with 75% of STELFONTA® treated dogs and 5.3% of untreated control dogs recorded as CR. The observed CR at Day 28 is summarized in Table II.3 below.

Table II.3. Tumor Response Following Day 0 Treatment

Group	Number of Dogs Treated at Day 0	CR at Day 28 (treatment success)	Not CR at Day 28
STELFONTA®	80	60 (75.0%)	20 (25.0%)
Untreated control	38	2 (5.3%)	36 (94.7%)

Objective tumor response (CR and PR) was observed in 64/80 (80.0%) of dogs in the STELFONTA® group and 2/38 (5.3%) of dogs in the untreated control group on Day 28.

Of the 60 dogs in the treated group that experienced CR at Day 28, the assessment of disease-free interval (DFI) occurred for 59 dogs at Day 42 and for 57 dogs at Day 84. At Day 42, 59/59 (100%) were disease-free at the injection site, and at Day 84, 55/57 (96.5%) were disease-free at the injection site.

At Day 30, 18 dogs in the STELFONTA® group that did not achieve CR were retreated with STELFONTA®. At Days 58 and 72, CR was observed in 8/18 (44.4%) and 7/18 (38.9%) of these retreated dogs, respectively. At Day 30, 33 dogs in the untreated control group were treated with STELFONTA® and

included in the analysis. At Day 58 and 72, CR was observed in 20/32 (62.5%) and 21/30 (70.0%) of these treated dogs, respectively.

Tumor Observations were conducted at 2, 4, 8, and 24 hours and Day 4 after treatment: The most frequently reported tumor observations in the 81 dogs treated with STELFONTA® on Day 0 were swelling, bruising, pain, and heat. These were reported at all tumor observation timepoints. The following were reported at 24 hours post treatment:

- Swelling: 97.5% (79/81 dogs)
- Bruising: 91.4% (74/81 dogs)
- Pain: 69.1% (56/81 dogs)
- Heat: 53.1% (43/81 dogs)

Intact skin at the tumor site was reported in 71.6% (58/81) of STELFONTA® treated dogs at 24 hours and in 17.3% (14/81) of STELFONTA® treated dogs on Day 4. On Day 4, the following tumor observations were reported with the highest frequency:

- Necrosis: 55.6% (45/81 dogs)
- Crater pockets: 37.0% (30/81 dogs)
- Exudate: 37.0% (30/81 dogs)
- Eschar: 28.4% (23/81 dogs)
- Ulceration: 11.1% (9/81 dogs)

Wound Healing (Days 7, 14, 28, 42, and 84): A wound healing assessment was performed on the dogs in the effectiveness dataset which included 80 dogs in the STELFONTA® group and 38 dogs in the untreated control group.

Wounds developed in 92.5% (74/80) of STELFONTA® treated dogs and 2.6% (1/38) of untreated control dogs by Day 7. On Day 28, the presence of wounds was 40% (32/80) in the STELFONTA® group and 2.6% (1/38) in the untreated control group. On Day 42 and Day 84, the presence of wounds was 27.1% (16/59) and 1.8% (1/57), respectively, in the STELFONTA® group. No wounds were present in the 2 dogs that remained in the untreated control group on Days 42 and 84.

The average wound size on Day 7 for a STELFONTA® treated dog was 3.3 cm x 2.4 cm (original average tumor size 1.9 x 1.6 x 0.9 cm). On Day 28, the average wound size was 2.0 x 1.4 cm.

The largest single wound from a dog treated with STELFONTA® on Day 0 was recorded on Day 7 and measured 15.5 x 5.1 cm (original tumor size 3.0 x 2.6 x 0.9 cm) and had reduced to 10.3 x 2.1 cm at Day 28 and 1.5 x 0.5 cm at Day 42.

The largest single wound from a dog treated with STELFONTA® on Day 30 was recorded on Day 39 (9 days after treatment) and measured 25.0 x 9.5 cm (original tumor size 2.5 x 1.9 x 1.3 cm located on the palmer aspect of the left metacarpal area) and had reduced to 4.3 x 1.1 cm at the end of the study (89 days after treatment). This dog required a wet to dry bandage for 10 days starting 4 days after treatment due to severe tissue slough. It is

important to note that this case had also been diagnosed with hypothyroidism at time of study screening. See the Adverse Reactions section below for a detailed description.

The largest total wound area for a STELFONTA® treated dog was reported seven days after treatment. The treated tumor was cutaneous and located on the left caudal stifle and the original tumor size measured 2.4 x 2.1 x 1.4 cm. The wound area consisted of three individual wounds recorded on the treated limb (both medial and lateral sides); 7.5 x 4.5 cm, 7.0 x 3.5 cm, and 11.5 x 7.0 cm. The wounds had reduced to 3.5 x 1.4 cm, 3.9 x 1.5 cm, and 9.7 x 4.3 cm 28 days after treatment. Two wounds, 0.5 x 0.7 cm and 2.5 x 2.9 cm, were present 42 days after treatment. All three wounds were no longer present at 84 days after treatment.

Exudate from the treated site, including serous, serosanguinous, sanguineous, seropurulent, and purulent discharges, were observed from Day 7 to Day 14, with decreasing frequency after Day 7. Necrotic eschar and sloughing of the treated site were observed from Day 7 to Day 14, with decreasing frequency after Day 7. Peripheral pitting or non-pitting edema and erythema of the surrounding area were observed from Day 7 to Day 28, with decreasing intensity and frequency after Day 7. Hyper-granulation of the treated site was observed from Day 7 to Day 14. Granulation of the treated site was observed from Day 7 to Day 84, with decreasing frequency after Day 14. Re-epithelialization of the treated site was observed from Day 7 to Day 84, with decreasing frequency after Day 14.

Abnormalities in skin color surrounding the treated site were observed from Day 7 to Day 28 with decreasing frequency after Day 7. Skin color was reported as, in decreasing order of frequency, bright red or blanches to touch, dark red-purple, grey pallor or hypo-pigmented, and blue-black or hyperpigmented.

Quality of Life: The mean scores for the QoL assessment by the Owners was similar between the STELFONTA® and untreated control groups at all time points.

Physical Examination: There was no significant difference in body weight, temperature, respiratory rate, and heart rate between groups during the study. Any abnormality occurring after treatment was recorded as an adverse event. See the Adverse Reactions section below.

Clinical Pathology: There was a statistically significant decrease in albumin and albumin/globulin ratios at Day 7 in the STELFONTA® group compared to the untreated control group. This finding was likely related to the wound that developed at the treated site. Mean values for both the STELFONTA® and untreated control groups were within the normal range specified by the laboratory.

The remainder of the statistically significant changes in clinical pathology values (hematology, serum chemistry, and urinalysis) between groups during the study were not clinically relevant. Abnormalities considered clinically significant by the Investigator were reported as adverse events. See the Adverse Reactions section below.

Adverse Reactions: Field safety was evaluated in 117 dogs treated with STELFONTA® and 42 dogs receiving sham treatment (untreated control). Eighty-one dogs were treated with STELFONTA® on Day 0, and 36 previously untreated control dogs were treated with STELFONTA® on Day 30. In addition, 18 dogs treated with STELFONTA® on Day 0 had the same tumor re-treated with STELFONTA® on Day 30 due to incomplete response. The most common adverse reactions included wound formation, injection site pain, lameness in the treated limb, vomiting, diarrhea, and hypoalbuminemia. Wound formation, vomiting, and diarrhea were mainly observed within the first 7 to 10 days after treatment. Injection site pain and lameness in the treated leg were mainly observed within the first 2 days after treatment. Hypoalbuminemia was mainly observed within the first 28 days after treatment. All dogs received concomitant medications as noted above under "Concomitant Medications" in the "Drug Administration" section.

The adverse reactions during the study are summarized in Table II.4 below.

Table II.4. Adverse Reactions During the Study

	STELFONTA® 1st Treatment (n = 117)	**STELFONTA® 2nd Treatment (n = 18)**	**Untreated Control (n = 42)**
Wound formation	110 (94.0%)	12 (66.7%)	3 (7.1%)
Injection site pain	61 (52.1%)	7 (38.9%)	1 (2.4%)
Lameness in treated limb	29 (24.8%)	2 (11.1%)	1 (2.4%)
Vomiting	24 (20.5%)	3 (16.7%)	4 (9.5%)
Diarrhea	24 (20.5%)	3 (16.7%)	2 (4.8%)
Hypoalbuminemia[a]	21 (18.0%)	2 (11.1%)	1 (2.4%)
Injection site bruising/erythema/edema/ irritation	20 (17.1%)	3 (16.7%)	1 (2.4%)
Anorexia	14 (12.0%)	2 (11.1%)	3 (7.1%)
Regional lymph node swelling/enlargement	13 (11.1%)	1 (5.6%)	1 (2.4%)
Tachycardia	12 (10.3%)	0 (0.0%)	1 (2.4%)
Weight loss	12 (10.3%)	3 (16.7%)	5 (11.9%)
Cystitis	10 (8.6%)	1 (5.6%)	2 (4.8%)
Dermatitis	9 (7.7%)	1 (5.6%)	1 (2.4%)
Personality/behavior change	8 (6.8%)	0 (0.0%)	2 (4.8%)

	STELFONTA® 1st Treatment (n = 117)	**STELFONTA® 2nd Treatment (n = 18)**	**Untreated Control (n = 42)**
Infection at injection site	8 (6.8%)	0 (0.0%)	0 (0.0%)
Tachypnea	7 (6.0%)	2 (11.1%)	1 (2.4%)
Pruritus	6 (5.1%)	3 (16.7%)	2 (4.8%)
Lethargy/Depression	6 (5.1%)	1 (5.6%)	1 (2.4%)
Pyrexia	3 (2.6%)	2 (11.1%)	0 (0.0%)

[a.] There was a statistically significant decrease in albumin and albumin/globulin ratios at Day 7 in the STELFONTA® group compared to the control group. The hypoalbuminemia ranged from 2.0 to 2.6 g/dL (reference range 2.7-3.9 g/dL).

Note: If an animal experienced the same adverse reaction more than once, only the highest grade was tabulated.

Adverse reactions were graded using the Veterinary Co-operative Oncology Group – Common Terminology Criteria for Adverse Events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1.[1] Most adverse reactions were Grade 1 (mild) or 2 (moderate). Grade 3 (severe) and 4 (life-threatening) adverse reactions in dogs treated with STELFONTA® included: lameness in the treated limb (6 dogs), injection site pain (4 dogs), wound formation (3 dogs), lethargy/depression (3 dogs), anorexia (2 dogs), infection at injection site (1 dog), pruritis (1 dog), and tachycardia (1 dog).

Adverse reactions associated with the administration of the corticosteroids were similarly reported in STELFONTA® and untreated control dogs and included elevated alkaline phosphatase, polyuria, and polydipsia.

There were no obvious differences in the types and incidences of adverse reactions between dogs with cutaneous MCTs and dogs with subcutaneous MCTs.

One dog from the untreated control group that was subsequently treated with STELFONTA® on Day 30 was reported with extensive wound formation (wound size 25.0 x 9.5 cm) with tissue slough (Adverse Event Grade 3) 7 days after treatment of a MCT on the left metacarpal area (original tumor size 2.5 x 1.9 x 1.3 cm treated at Day 30). The wound extended proximally up the leg to the shoulder and required bandaging of the leg and antibiotics. Scar contracture formed, requiring treatment under sedation to release the scar tissue. Clinical pathology abnormalities included elevated band neutrophils, anemia, and hypoalbuminemia. It is important to note that this dog was diagnosed with hypothyroidism at time of study screening. The wound had not fully healed by the end of the study 89 days after treatment but had reduced in size.

One dog treated with STELFONTA® on Day 0 was reported with a bacterial infection and cellulitis in the right rear leg 9 days after treatment of a MCT on the right rear paw. There was bruising of the upper thigh and necrotic skin on the caudal right thigh and cranial aspect of the hock. Bloody discharge under the necrotic tissue revealed rod bacteria and toxic neutrophils. The dog was treated with intravenous fluids and antibiotics and the cellulitis and swelling resolved by Day 14 post STELFONTA® treatment with a healthy granulation bed.

Human Exposure
There was one human exposure during the field effectiveness study where the veterinarian had a needle stick injury to the thumb at completion of tumor treatment and was injected with an unknown amount of STELFONTA®. The incident resulted in pain and necrosis of the center of the thumb at the point of needle stick. The wound healed over a period of three months. See Section V. User Safety for product labeling information regarding safety to humans handling, administering, or exposed to STELFONTA®.

A separate needle stick injury occurred to a Veterinarian/Research Scientist at QBiotics where a maximum dose of 0.1 mL tigilanol tiglate was administered into the distal extremity of the left index finger. Local inflammation, a localized burning sensation, blanching distally, bruising of the injection site, muscular pain up the left arm, and localized tissue necrosis occurred. The muscular pain resolved within 24 hours. The wound healed in 8 weeks.

Systemic mast cell degranulation and death
Two dogs from two separate pilot studies died from a suspected mast cell degranulation reaction. Both dogs were treated with STELFONTA® for a subcutaneous mast cell tumor located above the hock and did not receive the concomitant medications as prescribed which likely led to the severe reactions due to mast cell degranulation and deaths. See Section V. User Safety for product labeling regarding dog safety when administering STELFONTA®.

In a pilot field study, one dog with a large (10 cm^3) subcutaneous mast cell tumor on the right hip was treated with STELFONTA®. The dog had a partial response, based on RECIST[2] criteria, to the initial STELFONTA® injection and was retreated with STELFONTA® 30 days following the initial injection. The dog failed to receive any of the recommended concomitant medications of prednisolone, chlorpheniramine, and famotidine from 24 hours after the second STELFONTA® injection. On day 2 following the second STELFONTA® injection, the dog became anorexic, painful, and lethargic and had marked swelling of the right hind limb extending to the chest with hemorrhagic, ruptured blisters near the hock joint. Blood work showed anemia, hypoproteinemia, liver enzyme elevations, and white blood cell changes (leukocytosis, neutrophilia, monocytosis, and thrombocytopenia). The dog was hospitalized, received a blood transfusion, and was administered intravenous fluids, prednisolone, chlorpheniramine, and tramadol. Pitting edema progressed to the neck by four days following treatment. Despite supportive care, the patient died five days following treatment likely due to degranulation of the mast cell tumor caused by failure to receive the recommended concomitant medications and internal necrotic discharge of the tumor mass.

In a separate pilot study, one dog with a moderate (2.53 cm^3) subcutaneous mast cell tumor on the left caudal hind limb was treated with STELFONTA®. The dog was treated with chlorpheniramine and meloxicam on the treatment day (Day 0) and Day 1 only. The dog failed to receive further concomitant medication. On Day 3 the dog was lethargic and there was significant edema at the injection site. While intravenous fluid and antibiotic therapy was initiated on Day 3, the dog rapidly deteriorated and died on the following day likely due to degranulation of the mast cell tumor. Pathology findings included widespread cellulitis, panniculitis (likely of bacterial origin), and septic peritonitis.

Conclusion: The intratumoral injection of STELFONTA® at a dose of 0.5 mg per cm^3 of tumor volume is effective for the treatment of non-metastatic cutaneous MCTs and for the treatment of non-metastatic subcutaneous MCTs located at or distal to the elbow or the hock in dogs. See Section III. Target Animal Safety for a discussion of the safety of STELFONTA®.

III. TARGET ANIMAL SAFETY

Safety data was collected in the effectiveness studies (see Section II. Effectiveness), one intravenous margin of safety (dose escalation) laboratory study (#1014-1082), one cardiovascular intravenous laboratory safety study (#1013-1312) utilizing the final formulation, and one pilot pharmacokinetic field study in client-owned dogs (#QB46-C02) utilizing a non-commercial formulation. The most common adverse reactions were wound formation, injection site reactions (pain, edema, erythema, bruising, and necrosis), and lameness in the treated limb, and were related to STELFONTA®'s mechanisms of action. Other common adverse reactions included vomiting, diarrhea, and hypoalbuminemia. Most wound formations healed within 6 weeks, however, a small number of wounds had severe tissue sloughing extending away from the treated site resulting in extensive wounds that led to prolonged recovery times and additional wound care. The majority of adverse reactions were expected based on the mechanisms of action and were manageable.

The intravenous route of administration was chosen for the margin of safety study because of the low maximum tolerable dose, due to injection site reactions, following subcutaneous injection. In this study, STELFONTA® was administered once weekly for four weeks at doses of 0.025, 0.05, and 0.75 mg/kg, evaluating the systemic effects of the drug in healthy Beagle dogs. STELFONTA® related observations, when administered intravenously, included vomiting/retching, wound formation, decreased activity, loose feces, salivation, tremors, limited use of the leg that received the infusion, weakness, increased water consumption, erythema and edema at the infusion site, monocytosis, elevated alanine aminotransferase (ALT), and elevated fibrinogen. The cardiovascular laboratory study assessed the cardiovascular effects of the drug in healthy Beagle dogs. STELFTONA® related observations, when administered intravenously, included a reversible increase in heart rate, vomiting/retching, salivation, vocalization, slight incoordination, tremors, changes in activity levels, excessive panting, limited usage/swelling of leg or paw, decreased appetite, red feces, and decreased feces output. The pilot pharmacokinetic field study was conducted to help bridge the intravenous dosing of STELFONTA® to the intratumoral dosing and provide an understanding of the systemic exposure profile of tigilanol tiglate following intratumoral treatment. The intravenous route of administration resulted in higher drug exposure than intratumoral administration,

representing the worse-case scenario for systemic exposure to tigilanol tiglate. STELFONTA® related observations, when administered intratumorally, included hypoproteinemia, hypoalbuminemia, and injection site edema, inflammation, discoloration/bruising, and necrosis.

A. Margin of Safety Study (1014-1082)

Title: EBC-46 for the Treatment of Tumors: A Blinded 4-Week Repeat-Dose Intravenous Infusion Toxicity Study, Followed by a 14-Day Recovery Period in Beagle Dogs. (Study No. 1014-1082)

Study Date(s): May 2016 to April 2018

Study Location(s): Quebec, Canada

Study Design:

Objective: The objective of this study was to determine the toxicity and toxicokinetic profile of STELFONTA® following intravenous (IV) infusion to Beagle dogs once weekly on four occasions (total of four injections), and to assess the reversibility of any changes following a 14-day recovery period. The study was conducted in accordance with Organization of Economic Co-operation and Development (OECD) Good Laboratory Practice GLP.

Study Animals: Forty-eight (24 males, 24 females), intact, 6 to 8-month-old Beagle dogs, ranging in weight from 5.1-7.5 kg at study initiation were randomly assigned to four study groups consisting of 6 males and 6 females each.

Experimental Design: The IV route of administration was chosen for the study because of the low maximum tolerable dose following subcutaneous injection. Dogs were randomized by sex to one of 4 groups on the day prior to the first treatment. Each group included 6 males and 6 females. Data continued to be collected from 2 males and 2 females from each of the 4 groups during a two-week recovery period prior to euthanasia and necropsy. The dogs that continued on during the two-week recovery period are referred to as the 'recovery dogs' and the dogs that did not continue on were referred to as the 'main dogs'. Any personnel collecting or recording data, including necropsy personnel, were masked to treatment, as well as the Cardiologist, Ophthalmologist, Study Director, and Study Pathologist. Personnel administering treatment were not masked. Personnel administering treatment were not involved in the assessments or collection of data.

Drug Administration: Four dose groups were included in this study. Dogs either received a vehicle control or STELFONTA® at 0.025 mg/kg, 0.05 mg/kg, or 0.075 mg/kg (ranges between 0.02-0.036, 0.039-0.056, and 0.06-0.08 mg/kg, respectively due to dosing variability). The vehicle control formulation consisted of 40% propylene glycol in 30 mM sodium acetate buffer, pH 4.2, and was administered at the same dose volume and infusion rate as the 0.075 mg/kg group. The dose was administered as an IV infusion over a 15-minute period using an infusion pump in a temporary saphenous or cephalic vein catheter once weekly for a total of 4 doses on Days 1, 8, 15, and 22. Dogs in the vehicle control and 0.075 mg/kg groups were dosed at an infusion rate of 0.3 mL/kg/hour. Dogs

in the 0.025 mg/kg group were dosed at an infusion rate of 0.1 mL/kg/hour. Dogs in the 0.05 mg/kg group were dosed at an infusion rate of 0.2 mL/kg/hour.

Table III.1 Dose Groups

Group	No. Dogs	Test Article/ Dose (mg/kg)	Dose Volume (mL/kg)	IV Infusion Rate (mL/kg/h)	Dose Frequency	Post-Mortem
Vehicle control	6 M 6 F	Vehicle control / 0	0.075	0.3	Once weekly for 4 doses	Day 24 (4M, 4F) Day 36 (2M, 2F)
EBC-46 low dose	6 M 6 F	EBC-46 1 mg/mL 0.025	0.025	0.1	Once weekly for 4 doses	Day 24 (4M, 4F) Day 36 (2M, 2F)
EBC-46 mid dose	6 M 6 F	EBC-46 1 mg/mL 0.05	0.05	0.2	Once weekly for 4 doses	Day 24 (4M, 4F) Day 36 (2M, 2F)
EBC-46 high dose	6 M 6 F	EBC-46 1 mg/mL 0.075	0.075	0.3	Once weekly for 4 doses	Day 24 (4M, 4F) Day 36 (2M, 2F)

Measurements and Observations: Mortality checks and clinical observations occurred twice daily for at least one week prior to the initiation of treatment, and during treatment and recovery periods. On dosing days (Days 1, 8, 15, and 22), additional observations occurred prior to dosing, throughout the infusion period, and at 0.5, 1, 2, and 4 hours after the infusion was completed. Physical examinations occurred prior to animal assignment to the study, once during acclimation, one day prior to each dosing, daily on non-dosing days, and once prior to necropsy. Body weights were collected the week prior to initiation of treatment, up to one day prior to each dosing and twice weekly thereafter, including the day prior to necropsy. A fasted body weight was recorded on the day of necropsy. Food consumption was recorded daily throughout the study, starting one week prior to initiation of dosing. Rectal body temperature checks were recorded within 0 to 2 minutes before the end of infusion, at 0.5, 1, 2, and 4 hours after the end of the infusion and then once daily for an additional 4 days after each dose. Ophthalmology examinations were collected once during acclimation and prior to necropsy. Electrocardiography was collected once during acclimation, 2-3 minutes prior to infusion end, and 90 minutes and 3.5 hours after the end of the infusion on Days 1 and 22. Clinical pathology assessments (hematology, coagulation, clinical chemistry, and urinalysis) were collected once during acclimation and at 24 hours following the first and fourth doses. Recovery animals had additional clinical pathology assessments within 2 days prior to necropsy. Toxicokinetic sampling was obtained prior to dosing, immediately after the end of infusion, at 5, 15, and 30 minutes after the end of infusion, and at 1, 2, 3, and 6 hours after the end of infusion on treatment Days 1 and 22. The

infusion site was evaluated prior to and approximately 1 hour after each dosing and daily up to 3 days (until Day 24 in the main dogs and Day 25 in the recovery dogs) using a modified Draïze scoring system (refer to Tables III.2 and III.3).

Table III.2 Modified Draïze scoring system

ERYTHEMA/ESCHAR FORMATION (Maximum Score = 4)	SCORE
No erythema	0
Very slight erythema, barely perceptible (edges are not defined)	1
Well-defined erythema (pale red in color)	2
Moderate to severe erythema (definite red in color)	3
Severe erythema (beet or crimson red in color) and/or eschar formation (scab formation)	4

Table III.3 Modified Draïze scoring system

EDEMA FORMATION (Maximum Score = 4)	SCORE
No edema	0
Very slight edema, barely perceptible (edges are not defined)	1
Slight edema (edges are not definable, but the area is slightly raised)	2
Moderate edema (area well-defined and raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

Statistical Methods: A three-way repeated measure analysis of covariance (ANCOVA) was performed for the following study variables: post-treatment body weights, food consumption, body temperature, electrocardiographic measurements, respiratory rate, and numerical clinical pathology data. The fixed effects in both ANOVA and ANCOVA models were the group, the sex, the time, and their two-way and three-way interactions, with the animal as subject. The random effects in both models were the room, the replicates, and their interaction.

Results:

Mortality: All dogs survived the study until the scheduled termination dates.

Clinical Observations and Examinations: Vomiting only occurred in the STELFONTA® treatment groups, and not in the vehicle control group, during or immediately after dosing on all dosing days, affecting 21/36 of the dogs. Wound development at the infusion site was observed in one dog in the 0.025mg/kg group that started a few days after the first dose and a severe wound was observed in one dog in the 0.075 mg/kg group that started a few days after the

second dose. Decreased activity occurred in one dog in the 0.05 mg/kg group and 3 dogs in the 0.075 mg/kg group. Decreased appetite occurred in one dog in the 0.075 mg/kg group a few days after the second dose. Limited use of the limb that received the infusion was seen 13/48 dogs in all groups, including vehicle control, but was dose-dependent, occurring in 8/12 dogs in the 0.075 mg/kg group. Salivation occurred in 39/48 dogs and in all groups, including vehicle control. However, the incidence, severity, and duration increased in a dose-dependent manner. Excessive panting was observed in one dog in the 0.025 mg/kg group. Sneezing was observed in one dog in the 0.025 mg/kg group. A respiratory infection was observed in one dog in the 0.075 mg/kg group. The following occurred in all groups, including vehicle control, but in a dose-dependent manner in the treatment groups: loose feces, tremors and weakness. Tremors of a higher severity (moderate compared to slight) were observed in two dogs, one in the 0.05 mg/kg dose group and one in the 0.075 mg/kg dose group and occurred on dosing days. Weakness of a higher severity (moderate compared to slight) was observed in the STELFONTA® groups compared to the vehicle control after the first dose. However, weakness was not seen in any group after the second dose. Increased water consumption was observed in the vehicle control, 0.05 mg/kg, and 0.075 mg/kg dose groups but not in a dose-dependent manner.

Observations of vomiting, retching, or tremors were typically transient and resolved within 1 hour of dosing while salivation also typically resolved within 4 hours.

There were no clinically relevant effects on body weight or respiratory rate. Heart rates of greater than or equal to 160 beats per minute were observed in all dogs but more frequently in dogs in the 0.05 mg/kg and 0.075 mg/kg groups. On Day 4, the body temperatures in the 0.05 mg/kg dose group and 0.075 mg/kg dose groups were significantly different and higher than that in vehicle control; however, this finding is not clinically relevant. The mild increase in body temperature was stress related and not treatment related. Other than a trend in decreased food consumption from Days 22 - 29 in the STELFONTA® groups only, food consumption overall increased in all groups. This increase in food consumption is expected in growing puppies.

Draize Scoring: All dogs in all groups, including vehicle control, had slight edema and erythema at the infusion site (score of 1), starting typically 1 hour or 1-day post-infusion. Higher severity and incidence of erythema and edema occurred in the STELFONTA® groups compared to the vehicle control. Both erythema and edema scores of 3 and 4 occurred only in the STELFTONA® groups and appeared to be dose-dependent. Seventeen dogs (15 main dogs and 2 recovery dogs) in all four groups, including vehicle control, had an erythema score of 1 on the last day of observation (Day 24 and 25, respectively). One dog in the 0.075 mg/kg group had an erythema score of 4 on Day 24.

Electrocardiography: There were no clinically relevant effects on the electrocardiographic parameters.

Ophthalmology: There were no clinically relevant effects noted on ophthalmic examinations.

Clinical Pathology: Hematocrit (HCT) values trended down in all groups on Days 2 and 35 but were still within normal limits. Two dogs administered STELFONTA® had mildly low red blood cells on Day 2; however, these dogs were clinically normal and not anemic. One dog in the 0.05 mg/kg group was mildly anemic, with a HCT of 37% on Day 35 during recovery but did not have elevated reticulocytes and was clinically normal. Monocytosis occurred on Days 2 and 23 in all groups, including vehicle control, but in a dose-dependent manner in the treatment groups and was likely related to worsening inflammation as appreciated clinically with the Draize scoring.

Two dogs in the 0.075 mg/kg group had elevations in ALT on Day 23. Five dogs (one control, one 0.025 mg/kg, two 0.05, and one 0.075 mg/kg) had mildly low total protein levels on Day 2. Three of the dogs were recovery dogs and the total protein either resolved or improved during recovery.

Elevations in activated partial thromboplastin time occurred in 2 dogs during the dosing phase of the study in the 0.025 mg/kg group and occurred without clinical signs. Elevations in fibrinogen occurred in all groups during the dosing phase of the study in a dose-dependent manner and likely corresponded with inflammation, as was seen clinically with the Draize scoring.

Pharmacokinetics: Plasma drug analysis was performed on samples obtained on Days 1 and 22 at the following time points: pre-dose; immediately after the end of infusion (0 - 1 min post-EOI); at 5, 15, and 30 minutes and at 1, 2, 3, and 6 hours post-EOI. Plasma concentrations were measured using a Liquid chromatography–mass spectrometry (LC-MS/MS) bioanalytical method. There was a dose proportional increase in the maximum plasma concentration (C_{max}) and area under the plasma concentration-time curve from 0 to the last quantifiable concentration ($AUCl_{ast}$) with weekly doses of 0.025 mg/kg, 0.05 mg/kg and 0.075 mg/kg.

Mean AUCs and C_{max} were similar on Days 1 and 22 at each dose for males and females. The terminal elimination half-life (T1/2) was approximately half an hour (range 0.29 - 1.17 hours) on both Days 1 and 22. The time range for reaching maximum plasma concentration (T_{max}) was 0 - 1 minute post-EOI in all except one profile, for which T_{max} occurred at the subsequent time point, at 5 minutes post-EOI. The systemic exposure (mean C_{max} and AUC_{all}) were similar on Days 1 and 22 within each dose group, indicating there was no accumulation through repeat IV infusions. The mean [± standard deviation (SD)] pharmacokinetic parameters on Day 22 are shown in the table below.

Table III.4 The mean [± standard deviation (SD)] pharmacokinetic parameters on Day 22 for STELFONTA®

Parameter	0.025 mg/kg Group	0.05 mg/kg Group	0.075 mg/kg Group
C_{max} (ng/mL)	11.33±4.52	34.64±10.24	56.78±26.76
$AUCl_{ast}$ (hr*ng/mL)	4.92±1.20	10.18±2.92	16.96±5.96

C_{max}: maximum plasma concentration
AUC_{last}: area under the plasma concentration-time curve from zero and the last quantifiable concentration

Necropsy Examination: Infusion site findings of gelatinous material adhering to the subcutis or muscle, a dark, red area of the subcutis or muscle, and thickening of the skin or subcutis were observed in all groups in the main dogs only. A wound was observed in one 0.075 mg/kg dog. Lung adhesions were observed in 3 dogs administered STELFONTA® (two in the 0.025 mg/kg group and one in the 0.075 mg/kg group). The clinical relevance is unknown as none of the dogs experienced dyspnea or pyrexia during the study. Mediastinal, mesenteric, popliteal, and tracheobronchial lymph node findings of dark, red discoloration, mottled, and firm enlargement occurred in all groups (including recovery). Pituitary cysts occurred in 7 STELFONTA® group dogs only. One dog in the 0.075 mg/kg group had dilatation of the right hemisphere ventricle of the brain. The clinical significance is unknown.

Organ Weights: There were no clinically relevant effects noted on organ weights.

Histopathology: Infusion site findings of vascular/perivascular hemorrhage/edema, mixed cell infiltration, fibrosis, intima proliferation, perivascular chronic, active inflammation, chronic organizing thrombosis, and chronic inflammation occurred in all groups, mostly in the main group and one recovery dog. One 0.075 mg/kg dog had ulcerative inflammation, bacteria, and severe necrosis at the infusion site.

Lymph node findings included mandibular inflammation, lymphoid hypercellularity, and sinus histiocytosis; mediastinal erythrocytosis/hemorrhage, lymphoid hypercellularity, sinus histiocytosis, and inflammation; mesenteric erythrocytosis/hemorrhage, lymphoid hypercellularity, and sinus histiocytosis; popliteal erythrocytosis/hemorrhage and lymphoid hypercellularity; and tracheobronchial erythrocytosis/hemorrhage, sinus histiocytosis, and inflammation.

Adrenal gland vacuolation of the cortex, zona glomerulosa, and zona fasciculata occurred in 6 dogs (one control, one 0.025 mg/kg, one 0.05 mg/kg, and three 0.075 mg/kg). The adrenal gland findings may be associated with stress and inflammation. The dilatation of the ventricle of the brain and 7 pituitary cysts were confirmed on histopathology. Isolated findings included kidney tubular vacuolation in one dog in the 0.075 mg/kg group and chronic inflammation of the left thigh skeletal muscle and left sciatic nerve in one dog in the 0.075 mg/kg group.

Recovery Period: None of the recovery dogs in any group had gross necropsy findings at the infusion site. Only one of the recovery dogs in the 0.05 mg/kg group had histopathology changes, consisting of intima proliferation, at the infusion site.

Severe wound formation: One of the main dogs in the 0.075 mg/kg group developed a severe wound following the third dose on Day 15. Observations included moderate edema and erythema at the infusion site on the right pelvic limb and limited use of that limb. The erythema and edema worsened to severe with pain and discomfort suspected. Following the fourth dose on Day 22, the dog was observed with moderate tremors and weakness. The dog received antibiotics. Clinical pathology revealed an elevated ALT on Day 23. Gross necropsy revealed a wound, confirmed on histopathology as ulcerative inflammation, bacteria, and severe necrosis.

Conclusions: This study assessed systemic drug exposure when STELFONTA® was administered IV at increasing doses. Compared to the pharmacokinetic study (see Section III.C. Pilot Pharmacokinetic client-owned field study, QB46-C02), IV dosing resulted in higher systemic drug exposure compared to intratumoral dosing, under actual conditions of use. Test article-related findings included vomiting/retching, wound formation, decreased activity, loose feces, salivation, tremors, limited use of the leg that received the infusion, weakness, increased water consumption, and erythema and edema at the infusion site. Salivation, tremors, limited use of the leg that received the infusion, and weakness were dose-dependent observations. Clinically relevant clinical pathology findings included a trend toward decreasing HCT, monocytosis, elevated ALT, trend toward basic urine, proteinuria, and elevated fibrinogen. Gross and histopathology findings related to the drug included infusion site inflammation, redness, edema, thickening, and fibrosis; and peripheral lymph node changes including redness, enlargement, inflammation, lymphoid hypercellularity, and hemorrhage. Observations of vomiting, retching, or tremors were typically transient and resolved within 1 hour of dosing and salivation resolved within 4 hours.

B. Cardiovascular Study (1013-1812)

Title: EBC-46: A Cardiovascular Function Safety Pharmacology Study in Conscious Telemetered Male Beagle Dogs Following a Single Intravenous Infusion. (Study No. 1013-1802)

Study Dates: October 2013 to February 2017

Study Location: Laval (Quebec), Canada

Study Design:

Objective: The objective of this study was to assess the potential pharmacological effects of STELFONTA® on the cardiovascular system following a single 15-minute IV infusion of STELFONTA®. The study was conducted in accordance with Organization of Economic Co-operation and Development (OECD) GLP.

Study Animals: Four intact male, 2 to 4-year-old Beagle dogs, ranging in weight from 9 - 15 kg at start of treatment were enrolled. The dogs had previously undergone surgery for telemetry transmitter implantation for cardiovascular monitoring of arterial blood pressure, electrocardiogram, body temperature, and locomotor activity. The four dogs were acclimated 6 days prior to the start of treatment.

Experimental Design: During the acclimation period, the dogs were arbitrarily assigned to the study based on cardiovascular evaluation. This study was non-terminal.

Drug Administration: The study included 4 dose sessions: Control: vehicle, consisting of 40% propylene glycol in 30 mM sodium acetate, pH 4.2, STELFONTA® Low Dose: 0.01 mg/kg, STELFONTA® Mid Dose: 0.025 mg/kg, and STELFONTA® High Dose: 0.075 mg/kg. All four dogs received all treatments with at least a 3-day wash-out period between treatments (Refer to Table III.5).

Table III.5 Dose Sessions

Dose Session	Dose Dog 1001	Dose Dog 1002	Dose Dog 1003	Dose Dog 1004
1	Low Dose	Mid Dose	High Dose	Control Dose
2	High Dose	Low Dose	Control Dose	Mid Dose
3	Mid Dose	Control Dose	Low Dose	High Dose
4	Control Dose	High Dose	Mid Dose	Low Dose

The test and vehicle control were administered by an IV infusion over 15 minutes at a dose rate of 0.7 mL/kg/hour.

Measurements and Observations: Mortality checks and clinical observations occurred twice daily, starting with acclimation through the remainder of the study. These observations occurred prior to and after dosing on dosing days. Video recordings were reviewed and clinical signs were recorded for at least 6 hours after completion of the IV infusion. Physical examinations and body weights occurred at animal transfer to the study, prior to animal assignment and one day prior to each dosing. During each treatment session, cardiovascular function by electrocardiogram [PQ Interval, PR Interval, QRS Interval, QT Interval, RR Interval, QTcF (Fridericia's), and QTcV (Van de Water's)], heart rate, systolic arterial blood pressure, mean arterial pressure, diastolic arterial blood pressure, pulse arterial pressure, body temperature, and physical activity were recorded continuously for a period of at least 24 hours before each dosing and for at least 24 hours following each dosing relative to dosing completion. Cardiovascular function parameters were reported approximately every 15 minutes for a period of 1 hour prior to dosing, every 15 minutes for the 4 hours post-dosing and every hour for the remaining 24 hours post-dosing. Average values were calculated over at least 15 seconds, unless aberrant telemetry data were recorded or in absence of data.

Statistical Methods: Treatment effects (Dose Level) were assessed separately for each defined time interval. Each considered parameter was analyzed using a Latin-Square design ANOVA model. The ANOVA included the following variables as fixed effects: Animal (Sequence of Treatment), the Dose Session, and the

Dose Level. Pairwise comparisons between the Control (vehicle) and each test item dose level were done only when the Dose Level effect was significant ($p \leq 0.05$) at that time. A Dunnett's test was used to adjust for multiple pairwise comparisons.

Results:

Mortality: All dogs survived the study.

Body weight: All dogs lost weight (between 0.2 - 0.5 kg) over the course of the study.

Clinical observations: Retching/vomiting, changes in activity levels (both increases and decreases), and slight incoordination occurred only in the high dose group. Salivation, excessive vocalization, tremors, red feces, and decreased fecal output only occurred following administration of STELFONTA®. Clinically relevant findings that occurred in all dogs included limited usage and swelling of the leg that received the infusion, excessive panting, and decreased appetite. All clinical observations resolved 4 hours after dosing.

Cardiovascular Evaluation: There were no rhythm or conduction abnormalities, and no effects on arterial blood pressure, pulse arterial blood pressure, or body temperature noted for any of the dogs, including vehicle control. Following the mid dose, a minor increase in heart rate occurred up to 2 hours post-dose then returned to normal. Following the high dose, heart rate increased and remained elevated for 2.5 hours post-dose completion then returned to normal. Following the high dose, the QRS intervals trended towards mildly increased values at 15 minutes post-dosing completion, resolving by 45 minutes post-dosing completion. This observation was not statistically significant.

Conclusions: This study demonstrated that STELFONTA® causes a reversible increase in heart rate. Increased heart rate was dose-dependent and was observed for up to 2 hours following the mid dose (0.05 mg/kg) and for 2.5 hours post-dose completion following the high dose (0.075 mg/kg). STELFONTA® had no obvious effects on body temperatures, blood pressure, or electrocardiograms. Test article-related observations included vomiting, retching, salivation, vocalization, slight incoordination, tremors, changes in activity (increased and decreased), excessive panting, limited usage/swelling of leg or paw, decreased appetite, red feces, and decreased fecal output. Retching, emesis, incoordination, and changes in activity levels only occurred following the high dose (0.075 mg/kg). All clinical signs resolved within 4 hours post-dose completion.

C. Pilot Pharmacokinetic client-owned field study (QB46-C02)

Title: A Pilot Pharmacokinetic and Dose Confirmation Study of EBC-46 for the Intratumoral Treatment of Cutaneous or Subcutaneous Mast Cell Tumors in Dogs. (Study No. QB46C-C02)

Study Dates: September 2014 to May 2016

Study Location: Springfield, Missouri

Study Design:

Objective: The objectives of this pilot study were to (1) confirm the effectiveness and safety of a designated dose of tigilanol tiglate (EBC-46) in dogs for the intratumoral treatment of cutaneous or subcutaneous MCT, and (2) evaluate systemic concentration of EBC-46 after delivering the drug directly into the tumor. The study was conducted in accordance with Good Clinical Practice.

Study Animals: Ten client-owned domestic dogs between the ages of 6 - 14 years old and of any breed and either gender were enrolled. Females were non-pregnant and non-lactating. Males and females were not intended for breeding. At start of treatment, the dogs weighed between 6.9 - 44.5 kg.

Experimental Design: This non-randomized, non-masked field study involved a single treatment group of client-owned dogs receiving tigilanol tiglate at a single dose level. After a 7-day screening period, dogs were treated on Day 0. Regularly scheduled clinic visits occurred on Days 7, 14, and 28 (+2).

Drug Administration: All dogs received tigilanol tiglate at a single dose level of 0.5 mL per cm^3 tumor volume on Day 0. The dose did not exceed 0.25 mL per kg body weight and did not exceed a maximum dose of 5 mg (5 mL). The calculated dose was administered by intratumoral injection in a 360° fan-like manner. Six cutaneous and 5 subcutaneous tumors were treated in 10 dogs (one dog was enrolled a second time to treat a second mast cell tumor after successful treatment of the first tumor). Tumor volumes ranged from 0.1 to 6.8 cm^3 resulting in doses ranging from 0.002 mg/kg to 0.145 mg/kg and total doses ranging from 0.05 - 3.4 mg per dog.

Measurements and Observations: Physical examinations were performed at the screening visit, on Day 0 before the injection, on Day 0 at 4-hour post injection, and on Day 1, Day 7, and Day 14. The cutaneous or subcutaneous MCT was diagnosed at screening with a fine needle aspirate submitted to a veterinary pathologist for confirmation, as well as any abnormal (e.g., enlarged) lymph nodes. The tumor was measured with calipers at screening and Days 0, 7, 14, and 28, photographed, and its appearance described (e.g., erythema, swelling, pain, non-purulent discharge, bruising, tumor necrosis) at screening, on Day 0 before the injection, on Day 0 at 4-hour post-injection, and on Days 1, 7, and Day 14. If a complete resolution was observed on Day 14, the tumor site evaluations were also performed on Day 28. Clinical pathology assessments (hematology, coagulation, clinical chemistry, and urinalysis) occurred at the screening visit, Day 7, and Day 28. Adverse events were monitored during the study. Samples for pharmacokinetic analysis were collected within 2 hours before the tigilanol tiglate injection and at 5 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours following the completion of the injection.

Statistical Method: Summary statistics, as appropriate, were calculated for the variables. All calculations and summaries were conducted using appropriate procedures in SAS® (SAS Institute, Cary NC; version 9.4).

Results:

Clinical Observations and Examinations: There were no clinically significant abnormalities in body weight, heart rate, rectal temperature, or respiratory rate. Immediately post-injection, 3 dogs experienced pain and 3 dogs exhibited restlessness. One dog vomited after the injection and 3 days later. Three dogs required healing time beyond Day 28, ranging between an additional 1 - 5 months. Tumor site observations included necrosis, swelling (both localized edema and edema extending well beyond the tumor injection site), inflammation, bleeding ulcerations, bruising/discoloration, sloughing of tissue, open wound, mild drainage, malodor, and presence of granulation tissue. Three dogs developed dermatitis with or without skin necrosis in a region nearby but distinct from the tumor injection site. One dog developed a non-weight bearing lameness, muscle atrophy, and associated enlarged popliteal lymph node of the leg that was treated with tigilanol tiglate. One dog developed a mast cell tumor along the dorsal ridge of the injection site which was later removed surgically. Occasionally, in five cases, medical intervention such as bandaging, sutures, antibiotics, or pain medications were considered necessary by the treating veterinarian during the healing process.

One dog, an 8.5-year-old, neutered, male Doberman Pinscher, was withdrawn early from the study for lack of product effectiveness/inadequate tumor response. Based on the metastatic nature of the tumor and enlargement of the tumor, the investigator and owner elected to pursue chemotherapy.

Clinical Pathology: Hematology trends included a mild decrease in hematocrit, erythrocytes, and hemoglobin concentration and a mild increase in platelets and monocytes on Day 7. Clinically significant clinical chemistry changes included hypoalbuminemia in 5 dogs on Day 7 and hypoproteinemia in one of the dogs with hypoalbuminemia on Day 7. The low protein levels resolved by Day 28 and were likely related to the wound healing process that occurs secondary to the drug increasing the vascular permeability of the tumor site. Hyperglycemia, suspected to be related to a stress/pain response, was observed in 3 dogs. Urinalysis results included white blood cells in the urine without bacteriuria in 2 dogs, a trend toward decreased urine pH, decreased urine specific gravity in one dog on Day 28, and isosthenuria without azotemia in one dog on Day 7 that resolved by Day 28.

Pharmacokinetics: The following range of pharmacokinetic parameters were determined in this study: maximum plasma concentration (C_{max}) ranged from 0.356 ng/mL to 13.8 ng/mL, area under the plasma concentration time-curve to the last quantifiable plasma concentration (AUC_{last}) ranged from 2.25 h*ng/mL to 31.24 h*ng/mL, and elimination half-life ($T_{1/2}$) ranged from 2.85 to 36.87 hours.

There was no linear relationship between drug exposure (C_{max} and AUC_{last}) and tumor location (cutaneous or subcutaneous) or total dose (mg). The systemic drug exposure increased with the nominal dose (mg/kg) for cutaneous tumors only, not for subcutaneous tumors. For the 5 dogs with cutaneous tumors, the

doses ranged from 0.002 mg/kg to 0.145 mg/kg. The highest C_{max} was 11.1 ng/mL and the highest AUC_{last} was 31.24 h*ng/mL at a dose of 0.125 mg/kg. For the other 5 dogs with subcutaneous tumors, the doses ranged from 0.049 mg/kg to 0.094 mg/kg. The highest C_{max} was 13.8 ng/mL and the highest AUC_{last} was 30.81 h*ng/mL at a dose of 0.094 mg/kg.

Conclusions:

Six cutaneous and 5 subcutaneous tumors in 10 dogs were treated with tigilanol tiglate (one dog had two tumors treated consecutively). The variability of the PK parameters, C_{max}, T_{max}, and AUC, from intratumoral injection is high, and higher than the variability from IV infusion in the margin of safety study (Study #1014-1082). There is no linear relationship between systemic exposure (C_{max} and AUC) and nominal dose or total dose among the 10 dogs with two different types of tumors (cutaneous and subcutaneous). Therefore, a lower dose does not result in a lower systemic exposure or less adverse events. Plasma concentrations following intratumoral administration were much lower than IV administration, indicating that the drug exposure is lower with intratumoral administration compared to IV administration.

Clinically, this study demonstrated that tigilanol tiglate causes injection site reactions including substantial edema at the injection site and extending away from the injection site, inflammation, discoloration/bruising, and necrosis. These effects are considered to be related to the mechanisms of action of the drug. Clinical pathology findings related to the drug included hypoproteinemia, hypoalbuminemia, a trend toward acidic urine, increased white blood cells in the urine without bacteriuria, and decreased urine specific gravities or isosthenuria without azotemia.

IV. HUMAN FOOD SAFETY

This drug is intended for use in dogs. Because this new animal drug is not intended for use in food-producing animals, CVM did not require data pertaining to drug residues in food (i.e., human food safety) for approval of this NADA.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to STELFONTA®:

WARNING: SEVERE WOUND FORMATION IN HUMANS; EXTENSIVE WOUND FORMATION, MAST CELL DEGRANULATION, AND DEATH IN DOGS DUE TO MAST CELL DEGRANULATION

Human Safety

- **Accidental self-injection of STELFONTA® may cause severe wound formation. Sedation of the dog may be necessary to decrease the risk of accidental self-injection (See Dosage and Administration, Human Warnings, and Adverse Reactions).**

Dog Safety

- **Always administer a corticosteroid (e.g. prednisone or prednisolone), an H1 receptor blocking agent (e.g. diphenhydramine), and an H2 receptor blocking agent (e.g. famotidine) when treating with STELFONTA® to decrease the potential for severe systemic adverse reactions, including death, from mast cell degranulation (See Contraindications and Dosage and Administration).**

- **Do not inject STELFONTA® into subcutaneous mast cell tumors located above the elbow or hock (e.g. on the body, head, or neck). This may result in accumulation of necrotic debris in the subcutaneous space increasing the risk of systemic adverse reactions, including death, from mast cell degranulation (see Contraindications, Warnings and Adverse Events).**

- **Treatment with STELFONTA® has been associated with cellulitis and severe tissue sloughing extending away from the treated site resulting in extensive wounds that require additional treatment and prolonged recovery times (See Warnings, Precautions, and Adverse Events).**

WARNINGS:

NOT FOR USE IN HUMANS. KEEP THIS AND ALL MEDICATIONS OUT OF REACH OF CHILDREN.

Caution is required during treatment to avoid self-injection. Dogs undergoing treatment with STELFONTA® should be adequately restrained and sedation should be used if necessary. Use a Luer-lock syringe to administer STELFONTA®. Self-injection may result in local inflammatory reactions, including swelling, redness and severe wound formation. In case of self-injection, seek medical advice immediately and show the package insert to the physician.

Wear personal protective equipment consisting of disposable gloves, protective eye wear, and a lab coat or gown when handling STELFONTA®. STELFONTA® is an irritant and accidental exposure to skin, eye, or by ingestion should be avoided. In case of dermal or ocular exposure, repeatedly wash the exposed skin or eye with water. If wearing contacts, rinse the eyes first then remove contacts and continue to rinse with water. If symptoms such as local signs of redness and swelling occur, or if there has been ingestion, seek the advice of a physician and show them the package insert.

Limited data is available on the potential teratogenic effects of STELFONTA®. Therefore, STELFONTA® should not be administered by women who are pregnant or planning to become pregnant.

People with known hypersensitivity to tigilanol tiglate or to any of the excipients should avoid contact with STELFONTA®.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that STELFONTA®, when used according to the label, is safe and effective for use in dogs for the treatment of non-metastatic cutaneous mast cell tumors and for the treatment of non-metastatic subcutaneous mast cell tumors located at or distal to the elbow or the hock.

A. Marketing Status

This product may be dispensed only by or on the order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because professional expertise is required to diagnose mast cell tumors, to properly administer the injection, to provide adequate instructions for post treatment care, and to monitor the safe use of the product, including treatment of any adverse reactions.

B. Exclusivity

STELFONTA®, as approved in our approval letter, qualifies for FIVE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(i) of the FD&C Act because this is the first time we are approving this active ingredient in a new animal drug application submitted under section 512(b)(1) of the FD&C Act.

C. Patent Information

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.

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