STELFONTA®

(tigilanol tiglate injection)

TECHNICAL MONOGRAPH

STELFONTA®
(tigilanol tiglate injection)

Shaping the future of animal health

Virbac

CONTENTS

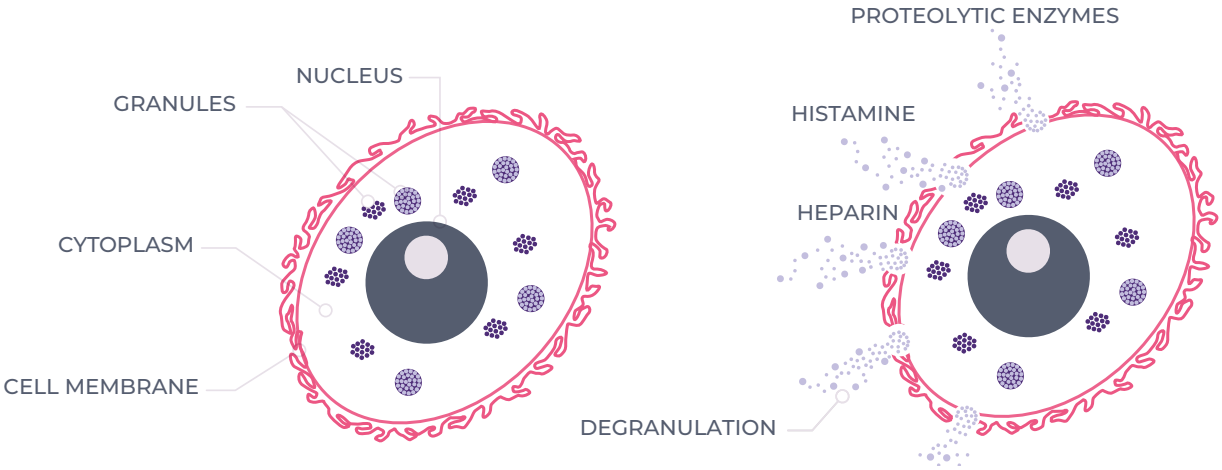
1 CANINE MAST CELL TUMORS

INTRODUCTION

What are mast cells?

Mast cells (MCs) are a special type of white blood cell that develops in the bone marrow and circulates to the peripheral tissues where they mature into functional mast cells.[1] MCs are involved in many biologic processes within the body ranging from innate and adaptive immune responses to hypersensitivity reactions and allergic responses. MCs also play an important role in wound healing, participating in leukocyte recruitment, angiogenesis, granulation tissue formation and epithelialization. MCs also contribute to persistent inflammation and inflammatory conditions.

MCs have cytoplasmic granules that contain histamine, heparin and proteolytic enzymes. Activation and release of the granule constituents is called degranulation.[2] MC activation and degranulation can occur in response to physiological and environmental factors.[1,2] Normal activation is a beneficial response leading to recruitment of inflammatory cells and stimulation of the adaptive or innate immune responses.[1]



What are mast cell tumors (MCTs)?

MCTs are the most common cutaneous tumor in the dog, accounting for up to 21% of all cutaneous tumors.[2] The average age of presentation is 8-9 years but MCTs have also been reported in younger dogs. The etiology or cause of mast cell tumors (MCTs) is largely unknown. A breed predisposition has been demonstrated in several breeds suggesting genetics may play a potential role in the development of MCTs.[3] These breeds include Beagles, Boxers, Boston Terrier, Pug, Labrador Retrievers, Weimaraners, Golden Retrievers, Staffordshire Bull Terriers and Shar-Peis.[3,4,5]

Canine MCTs most commonly develop as solitary nodules of the skin or subcutaneous tissues. A surgical biopsy is necessary to clearly differentiate between a cutaneous MCT and a subcutaneous MCT.[8] Cutaneous MCTs are located in the dermis and commonly extend into the epidermis causing ulceration. Cutaneous MCTs may also extend into the subcutaneous tissues.

WHERE ARE MCTS FOUND?

Canine MCTs are most commonly found on the dog's trunk, then the legs, followed by the head and neck.

CUTANEOUS MCTs originate in the dermis and may extend into the subcutis.

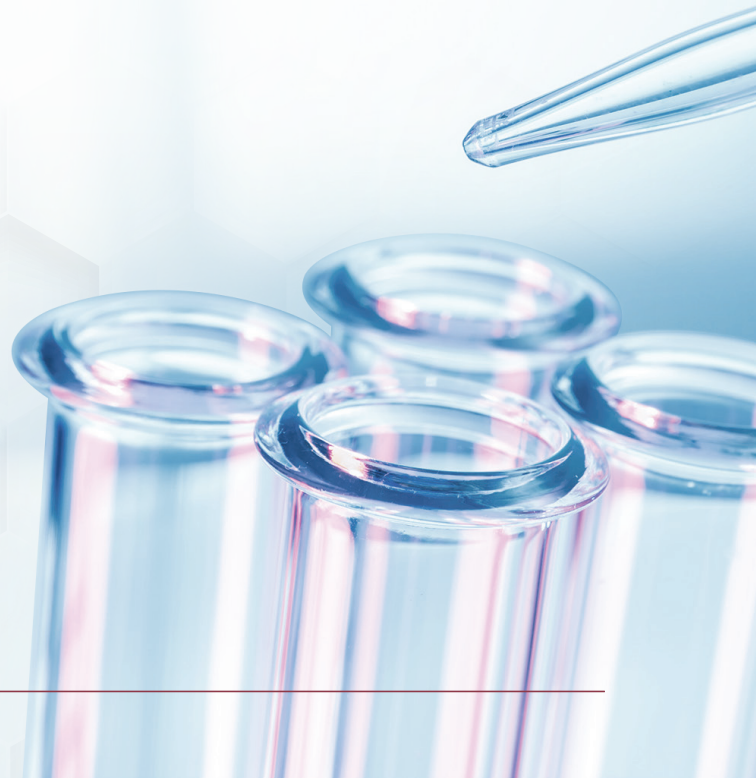
SUBCUTANEOUS MCTs have no dermal involvement.

Subcutaneous MCTs can occur anywhere in the body, including the gastrointestinal tract.

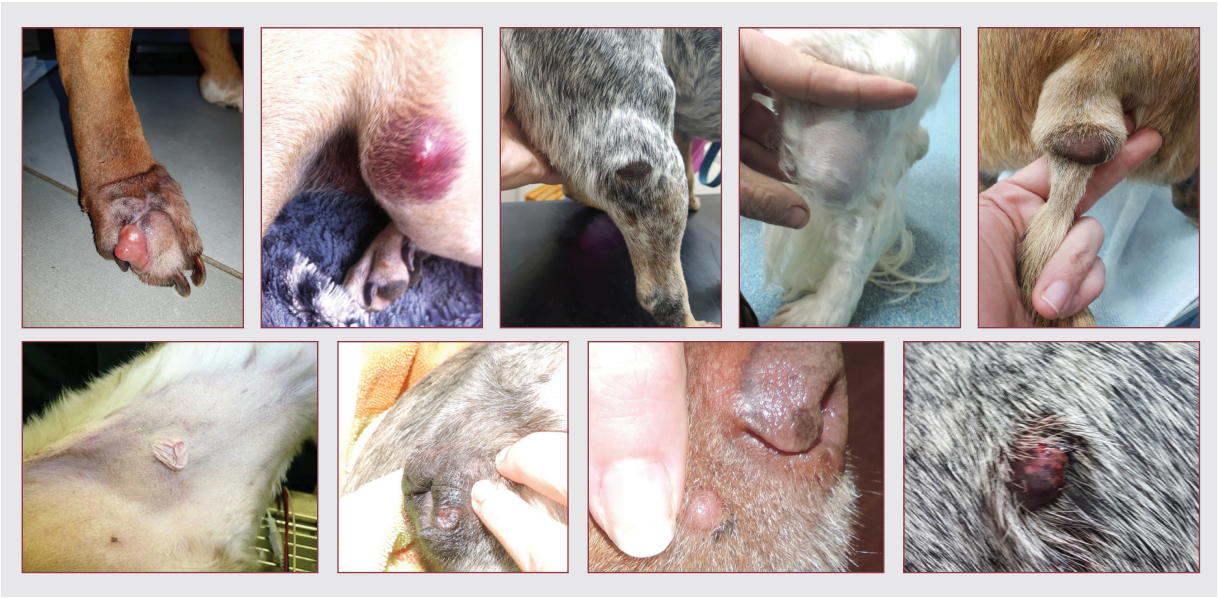
Conversely, subcutaneous MCTs are surrounded entirely by adipose tissue with no dermal involvement.[8,16]

Characteristics on physical examination may help differentiate between cutaneous and subcutaneous MCTs. If any part of the MCT palpates as adherent to the overlying skin, it is likely cutaneous in origin. Half of cutaneous MCTs occur on the trunk and perineal region, 40% on the limbs and less than 10% on the head and neck.[2] Subcutaneous MCTs can occur anywhere on the body. MCTs can also occur elsewhere with presentations of visceral form or systemic mastocytosis and gastrointestinal disease. These latter presentations often have a poor prognosis with documented short survival times.[9]

Although the most common presentation is a solitary nodule, MCTs have an extremely variable biological appearance. Low grade or well-differentiated MCTs are often solitary, small and slow-growing and can be mistaken for benign cutaneous masses. MCTs can be hairless or covered in hair. They can be red, ulcerated or swollen. They can also vary greatly in size and rate of growth.[2] Further, approximately 11 to 14% of dogs have been reported to develop multiple *de novo* MCTs.[6,7]



EXAMPLES OF MAST CELL TUMORS



Biological behavior of MCTs

MCTs spread *via* the lymphatic system to regional lymph nodes, abdominal viscera, and, less commonly, bone marrow. Although reported, spread of MCTs to the chest cavity and other body locations is rare.[2,10,15] Most dogs diagnosed with MCTs do not show obvious clinical signs; however, a subset of dogs can demonstrate tumor-associated signs locally or systemically secondary to the release of MCT granule substances (ie; histamine, heparin, other vasoactive amines) called degranulation (see following section).[2,11,12,13,14]

Clinical signs of degranulation

The vast majority of patients present with a solitary cutaneous or subcutaneous mass with no outward signs of illness.[2] However, some patients can present with clinical signs of degranulation associated with the release of histamine, heparin or other constituents from the mast cell granules. Any manipulation of a MCT can lead to a degranulation reaction. These reactions can also occur spontaneously. Signs of the local reaction include swelling, erythema, bruising and wheal formation. Owners may also report a fluctuation in the size of the mass with historical increasing and decreasing in size.

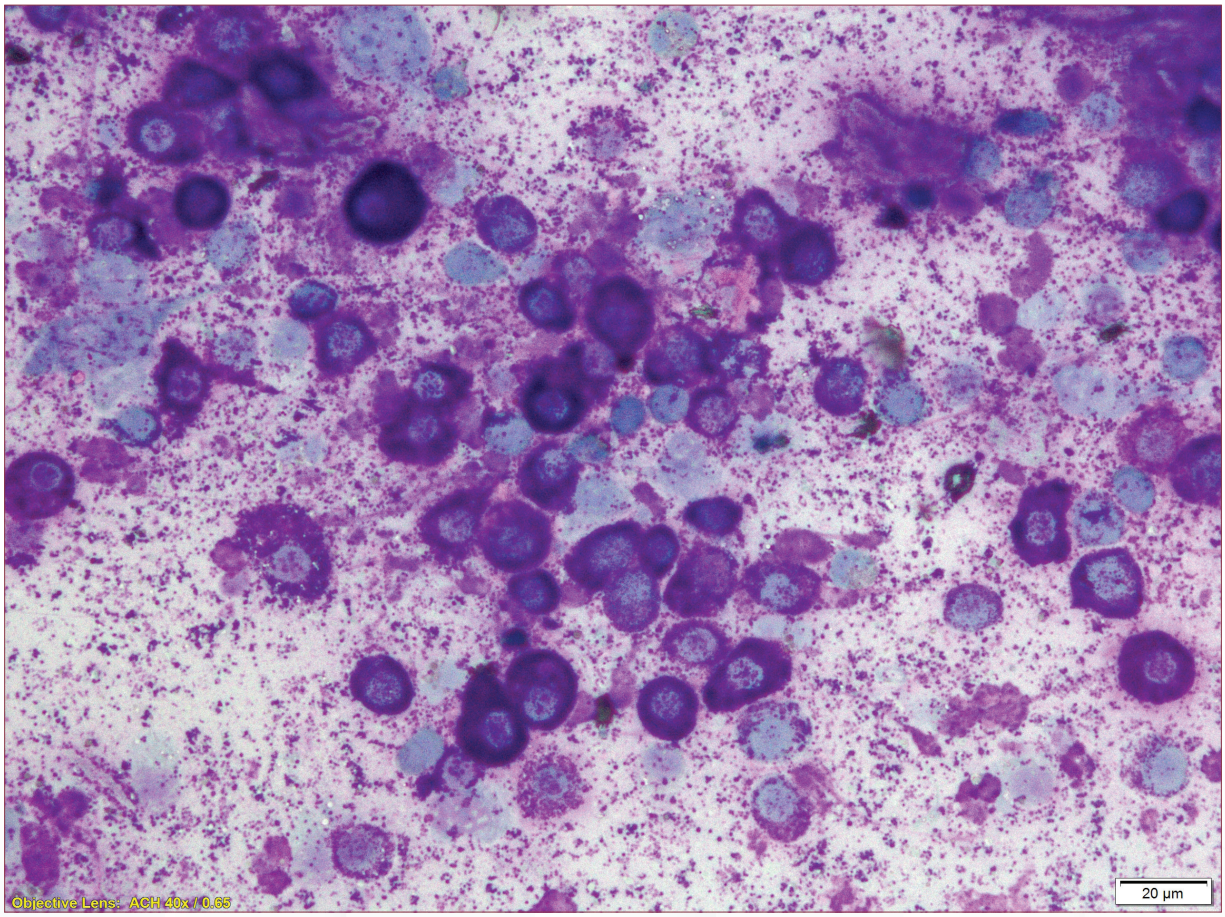
Histamine can act on H2 receptors on the parietal cells of the stomach to increase hydrochloric acid secretion leading to gastrointestinal upset with clinical signs manifesting as anorexia, nausea, vomiting, hematemesis, diarrhea, melena, lethargy and hypotension.[2]

Degranulation reactions can be life threatening and the importance of concomitant medications to mitigate the risks of degranulation cannot be overemphasized. Concomitant medications may include an H1 antagonist (diphenhydramine, chlorpheniramine) and an H2 antagonist (famotidine, cimetidine, ranitidine). Depending on the patient's clinical symptoms, supportive treatments may include but are not limited to intravenous fluids, gastrointestinal protectants and proton pump inhibitors.[2]

DIAGNOSIS, STAGING, PROGNOSIS

Diagnosis

Diagnosis of a mast cell tumor is generally straightforward in most cases with a fine needle aspiration cytology (FNAC). Cytology consists of individual small to medium-sized round cells with central to slightly eccentric round nucleus. The cytoplasm contains abundant, small, uniform cytoplasmic granules that stain purplish-red (metachromatic) making diagnosis easy.[8] These granules are often present in the background outside of the cells. However, poorly differentiated MCTs can have poorly staining characteristics and may lack granules. Prior degranulation can also affect the presence of granules within the cytoplasm. The lack of granules may make poorly differentiated MCTs a diagnostic challenge in surgical biopsy specimens as tissue biopsy with the addition of special stains and immunohistochemistry may fail to differentiate a poorly differentiated MCT from other poorly differentiated round cell tumors. It is important to note that the preferred method for diagnosis of such poorly differentiated MCTs is often cytology as some or few granules usually can be identified cytologically.[8]



Cytology (40X magnification) of a canine mast cell tumor with cells of varying size, containing many cytoplasmic granules.

Grading – histologic and cytologic

Histologic grade has historically been the method to predict biological behavior of MCTs. There have been two systems used in the classification of MCTs- Patnaik and Kiupel. Patnaik divided MCTs into one of three histological grades of well-, intermediate- and poorly-differentiated.[17,18] Although widely referenced and accepted as standard of care, this system has also been reported to have a high degree of inconsistency among pathologists and therefore the reproducibility of assigned grading has been poor amongst pathologists with interobserver variation reported as high as 63%.[8,19-21] Further, usage of this classification system typically yields a large percentage of intermediate tumors (72%), lending little guidance with regard to biological behavior and clinical applicability.[19] One of the histologic factors differentiating a low grade from an intermediate grade tumor is the depth of the MCT in the dermis. A subsequent study evaluating tumor depth as an independent prognostic factor found this variable to be of no significance.[7,8]

To more accurately predict tumors that are high risk for aggressive biological behavior, allowing for an increase in interobserver consistency, the research team led by Kiupel developed a two-tier histologic grading system (Kiupel)[19]. The Kiupel system involves dividing canine cutaneous MCTs into low- and high-grade based on four parameters. High-grade tumors exhibit any one of the following:

- at least 7 mitotic figures in 10 high-power fields (HPFs)
- at least 3 multi-nucleated cells in 10 HPFs
- at least 3 bizarre nuclei in 10 HPFs
- karyomegaly (specifically nuclear diameters of at least 10% of neoplastic cells vary by at least 2 times).

The Kiupel system has demonstrated statistical significance in predicting survival time; with high-grade MCTs associated with new tumor development and a shorter time to metastasis.[19] Since its inception, the two-tier system has consistently shown to be a better predictor of MCT-associated mortality and metastasis than the Patniak three-tier system.[22-25] These studies also demonstrate a high interobserver consistency rate that was far superior to that demonstrated by the Patnaik three-tier system of histologic grading. It is important to note that neither system has been applied in larger studies of subcutaneous MCTs; however, it is believed that the biologic behavior is similar based on other measurable histologic parameters.[8,16]

MAST CELL TUMOR GRADING [26,28]

HISTOLOGICAL GRADING
Evaluates tissue sample (biopsy).

PATNAIK SYSTEM

- **3-tier system**
- Grade 1 (well differentiated): Benign behavior
- Grade 2 (intermediate differentiation): Behavior can vary widely
- Grade 3 (poorly differentiated, often with infiltrative growth): Aggressive behavior

KIUPEL SYSTEM

- **2-tier system**
- Low-grade: Relatively benign
- High-grade: Behave aggressively
- Created in part to eliminate the poorly defined grade 2 tumors of the Patnaik system, therefore better dichotomizing MCTs

CYTOLOGICAL GRADING
Evaluates cell sample (FNA).

- Adapted from the Kiupel grading system (2-tier)
- Gaining momentum as a reliable grading protocol alternative
- Logistically easier and time efficient as the veterinarian is already obtaining an FNA for the diagnosis
- 94% accuracy

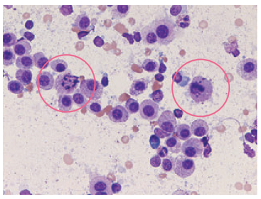
WHAT IS CYTOLOGICAL GRADING?[26,28]

Cytological grading is a relatively new technique that is gaining momentum as a reliable grading protocol alternative.

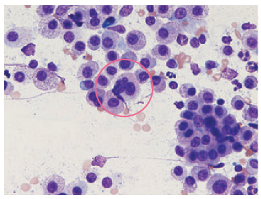
It is widely accepted that histologic grade provides a valuable assessment of MCTs in predicting biological behavior. It has also been recognized that using cytology as a less invasive method of grading would be valuable. In fact, there have been several attempts to create a standardized cytologic grading scheme for canine cutaneous MCTs.[26-28]

All criteria applied in the Kiupel two-tier grading system can be visualized on cytologic specimens. This made it easy to apply the two-tier system to cytologic samples directly and to use the criteria to develop a predictive algorithm for cytologic grading of cutaneous MCTs correlated to patient outcome. As a general rule, cytologic grading systems correlate well with histologic grading with a high sensitivity (84- 86%), specificity (97%) and a high agreement with histologic grade (94%).[8] However, the cytologic studies have varied greatly in methodology, with respect to the number of fields evaluated and type of stains, which has made the utilization of these methods challenging. It is also important to understand that due to different collection and processing methods between histology and cytology, samples are dissimilar with regard to the number of neoplastic cells. Another significant problem is the terminology defining the evaluation of a high-power field (HPF) is different between the two modalities – cytology is 100x objective and histology is 40x objective.[8]

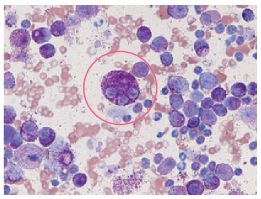
CHARACTERISTICS EVALUATED IN CYTOLOGICAL GRADING



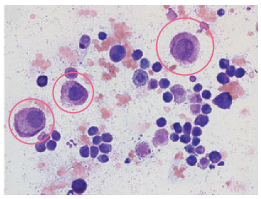
Mitotic figures
1 or more mitotic figures.



Bizarre nuclei
Nuclear pleomorphism.



Multinucleation
2 or more nuclei in 1 cell.



Anisokaryosis
>50% variation in nuclear size.

An extensive study evaluating cytologic grading criteria based on cytologically identifiable characteristics that correlated with histologic grade with a modified Wright stain has shown great promise in establishing an accepted cytologic grading system for canine cutaneous MCTs.[26] In this study, MCTs were classified as high-grade if they were poorly granulated or exhibited at least 2 of 4 of the following findings: (1) mitotic figures, (2) binucleated or multinucleated cells, (3) nuclear pleomorphism, or (4) greater than 50% anisokaryosis.[26] This system also correlated well with histologic grading with a high sensitivity (88%) and specificity (94%).[26] The authors of this study note that the algorithm tended to overestimate high-grade MCTs. Although not ideal, it is preferable for cytology as a screening test to yield low false negatives. Under this scenario, high grade tumors, which require more aggressive treatment, are less likely overlooked. This supports early, proactive treatment in patients with aggressive MCT disease.

One could argue that the consequences of diagnosing a low-grade tumor as high-grade could result in more aggressive diagnostic staging or more aggressive surgery.[8,26] However, it is still recommended to use caution when relying on any single diagnostic test for therapeutic decisions.[8]

Margin evaluation

The treatment of choice for low grade MCTs is surgical excision. For this reason, determining the cleanliness of surgical margins following excision is important in determining local tumor control. Unfortunately, MCTs are often surrounded by edema, reactive stromal cells, and inflammatory cells including non-neoplastic mast cells. In many cases the affected surrounding tissue can be several centimeters and often poses a challenge for surgical removal and subsequent pathologist's interpretation of the cleanliness of the surgical margin.[2,8] Numerous technical factors can also affect the assessment of surgical margins including retraction, shrinkage and distortion associated with the fixation and processing of the tissue.[2] Further, there is currently no way to differentiate neoplastic mast cells from non-neoplastic mast cells.[8] Arbitrarily, pathologists consider clusters of 3 or more mast cells as neoplastic and individual, well-granulated mast cells as non-neoplastic.[8] Finally, the method used to trim a MCT specimen for microscopic examination may have a large impact on the margin assessment.

Staging diagnostics

Grading of MCTs may be beneficial in determining prognosis and the need for further local and/or systemic therapy. It is perhaps equally important to determining the extent of disease in the patient or clinical stage of disease. As mentioned previously, MCTs spread via the lymphatic system to lymph nodes, liver, spleen and rarely the bone marrow. Despite the value clinical stage of disease provides, there is a lack of agreement amongst oncologists regarding the necessary staging to be performed on any given MCT patient.[2,8,29]

HOW ARE TUMORS **STAGED?**

Tumors are divided into 4 stages according to their clinical presentation:[1]

1 STAGE 1
One tumor confined to the dermis WITHOUT regional lymph node involvement.

2 STAGE 2
One tumor confined to the dermis WITH regional lymph node involvement.

3 STAGE 3
Multiple dermal tumors or one large infiltrating tumor with or without regional lymph node involvement.

4 STAGE 4
Any tumor with distant metastasis or a recurrence with metastasis (including blood and/or bone marrow involvement).

BETTER PROGNOSIS

POORER PROGNOSIS

1. Boston S. Canine Mast Cell Tumors. https://pdfs.semanticscholar.org/7bbc/df7e9e1e6a3edc978556eb94b314501074d2.pdf. Accessed May 22, 2019.

A recent study evaluated the value of full clinical staging in dogs with MCTs. In this study, nearly 31% of tumors exhibited metastasis to the local lymph node and 6.8% of dogs exhibited distant metastasis. However, more importantly, no dog had or developed distant metastasis in the absence of lymph node metastasis. This suggests that the utility of further staging is low in the absence of confirmed or suspected local lymph node metastasis.[29]

It is worth mentioning that lymph nodes normally contain low numbers of mast cells and neoplastic mast cells can recruit non-neoplastic mast cells via the lymphatic system. For this reason, cytologic evaluation of lymph nodes can prove challenging.[8] A standardized system for cytologic evaluation of lymph nodes has been proposed but is not widely accepted.[30,31] Clinical pathologists tend to subjectively evaluate the significance of mast cells in a lymph node sample based on pleomorphism, arrangement into aggregates and overall number.[30-32]

Other diagnostic tests may include thoracic and abdominal imaging (radiography or ultrasound) and FNAC of liver and/or spleen. Historically, examination of buffy coat smears for presence of mast cells in circulation was recommended however the specificity of this test is low and unreliable.[33] The incidence of bone marrow metastasis and infiltration in canine cutaneous MCTs is very low and therefore, most clinicians do not recommend bone marrow aspiration.[34] The current recommendation involves a more measured approach to clinical staging dictated by the presence of clinical signs and/or the presence of negative prognostic factors.[2]

Prognostic indicators, c-KIT[2]

The tyrosine kinase receptor KIT plays a key role in the survival, proliferation, differentiation and migration of mast cells. Aberrant expression of KIT protein has been shown to be a negative prognostic indicator for canine cutaneous MCTs. Three different KIT expression patterns have been detected with immunohistochemical staining of MCT tissue samples. These patterns have been correlated with aggressive biological behavior, decreased overall survival time and increased incidence of local recurrence. Mutations in exons 11, 8, and 9 of c-KIT have been identified and lead to constitutive phosphorylation or activation of c-KIT.

AgNOR, Ki67 and other proliferation parameters[2]

Several studies have evaluated markers of cellular proliferation as strong prognostic indicators. These include argyrophilic nucleolus organiser regions (AgNORs), Ki67 and mitotic count. Each of these have been evaluated as both a single prognostic factor and multivariable prognostic factor. Variability exists and standardization is lacking with regard to evaluation methods and selection of tumor area to be evaluated for each of these markers.

TREATMENT OPTIONS

Treatment decisions are largely based on the clinical stage of the disease and the presence or absence of negative prognostic factors. Clinicans must also consider individual patient history, clinical presentation and owner goals and concerns. Historically, there have been limited options for local tumor control, whereby the treatment of choice for solitary tumors amenable to wide excision was surgical management alone. Adjunctive systemic therapy is recommended for those tumors that are high grade or those that have confirmed metastasis at the time of diagnosis/presentation.

In the past, the recommended surgical margins for MCT excision included 3 cm of normal tissue. This recommendation is largely anecdotal and a study to demonstrate the patient benefit of this recommendation has not been published. More recent approaches to

surgical margins have been evaluated for the excision of cutaneous MCTs. Two separate studies found lateral margins of 2 cm and one uninvolved fascial plane deep to the tumor were likely to result in complete excision of low- and intermediate-grade cutaneous MCTs less than 5 cm diameter.[35,36] More recently, a study comparing a 'conservative' 2 cm margin with a more 'aggressive' 3 cm surgical margin in low grade cutaneous MCTs showed no advantage at achieving histologically tumor free margins with a wider approach.[61] High grade tumors were not evaluated in any of these studies.

Yet another study evaluated a proportional approach to surgical margins for the excision of cutaneous and subcutaneous MCTs.[37] In this study, tumors were resected with lateral margins equivalent to the widest measured diameter of the tumor to a maximum of 4 cm and a minimum depth of one well-defined fascial plane deep to the tumor. This later approach resulted in incomplete excision in 15% of cases.[37] Finally, a study evaluating histologically tumor free margins (HTFM) and local recurrence showed a significantly higher risk of local tumor recurrence for high grade tumors despite the achievement of HTFM.[38] Unfortunately, the grade of a MCT is usually not known while performing surgical excision, which complicates surgical planning.

When tumors are on a distal extremity and therefore, not amenable to a complete soft tissue excision, a limb amputation would be recommended to achieve complete surgical excision. This option is aggressive and while the likelihood of complete excision is high, it results in a less than ideal functional outcome. Radiation therapy as a primary therapy has shown promise in the treatment of MCTs with 1-year local control rates of approximately 50%.[2,8] For those tumors not amenable to complete surgical excision, a combination of cytoreductive surgery and radiation therapy has yielded the best long-term local control with 2-year control rates between 85-95%.[39-43]

Other local therapies that have been reported include hyperthermia, intralesional brachytherapy, photodynamic therapy, intralesional corticosteroids, cryotherapy and electrochemotherapy. None of these local therapies have been shown to be as clinically effective or as practical as surgery or radiation therapy alone or in combination. While surgery or the combination of surgery and radiation have shown the greatest local tumor control rates, these options may not be ideal for all patients. Foremost, 40-50% of MCTs occur on the limb, a location in which wide surgical margins are challenging to achieve. As discussed previously, a combination of surgery and radiation therapy may be effective for these patients; however, radiation therapy often comes with logistical (time and distance) constraints and financial concerns. Other factors that should be considered in any given situation include age of the patient, patient comorbidities and client goals. Clearly, there is opportunity for safe and effective options for local tumor control.

The treatment of anaplastic or undifferentiated high grade MCTs is often unrewarding as the regional and distant metastatic rate remains high with a less favorable prognosis.[2] Most canine patients diagnosed with poorly differentiated tumors and metastatic MCTs will succumb to the disease. Both combination and mono-chemotherapy have been investigated as adjuvant treatment to local tumor control in the treatment of patients with high-grade and/or clinically higher stages.[2] Numerous tyrosine kinase inhibitors (TKI), both human and veterinary-approved, have been investigated as well.[2]

2 STELFONTA® (tigilanol tiglate injection): REMOVAL OF A MCT WITH A SINGLE TREATMENT

WHAT IS STELFONTA?

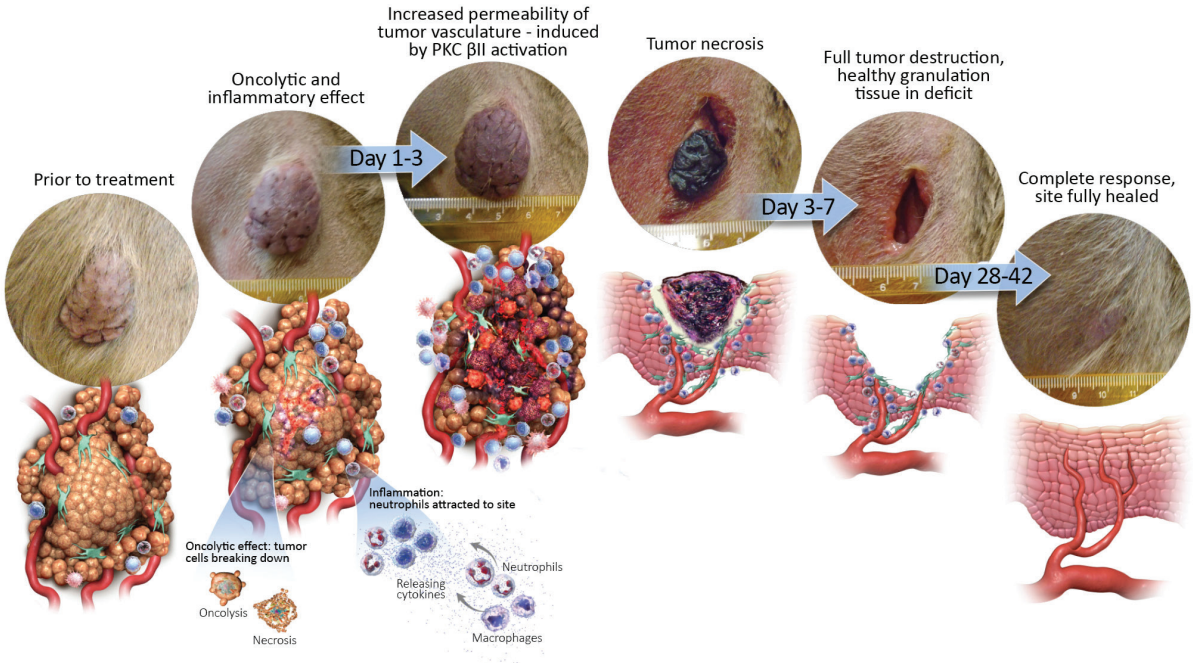
STELFONTA is approved by the United States Food and Drug Administration - Center for Veterinary Medicine as a prescription intratumoral injection indicated for the treatment of non-metastatic cutaneous MCTs and subcutaneous MCTs located at or below the elbow and hock.

Tigilanol tiglate is the active ingredient in STELFONTA and is produced by isolation of the compound from the seed of *Fontainea picrosperma* (blushwood).

Photos: Scott Hartvigsen

STELFONTA MODE OF ACTION

STELFONTA is a new type of anti-neoplastic agent with a unique mode of action. In non-clinical pharmacology studies, STELFONTA has been shown to elicit three interrelated effects which are responsible for its anti-tumor effectiveness. These effects occur concurrently soon after administration and result in tumor hemorrhagic necrosis and destruction of the tumor mass usually within 3 to 7 days.



The first effect is to cause induction of oncosis in tumor cells that are in direct contact with STELFONTA® (tigilanol tiglate injection). Oncosis is characterised by cellular swelling, blebbing, increased membrane permeability and release of ATP. In contrast, apoptosis is characterised by cellular shrinkage, pyknosis and karyorrhexis. Oncosis occurs within the first hours following treatment and is due to the drug-induced disruption of mitochondrial and endoplasmic reticulum function, leading to rapid ATP depletion and loss of osmotic balance, followed by terminal necrosis.

The second component of the drug's anti-tumor activity is associated with direct activation of the Protein Kinase C (PKC) βII isoforms in tumor vasculature endothelial cells. STELFONTA affinity for βII isoforms is highly specific and results in increased vasculature permeability and subsequent loss of tumor vascular integrity. This initially presents clinically as the development of a bruised appearance in the treated tumor within 15 minutes to 24 hours. The onset of hemorrhagic necrosis of the mass followed by tumor slough occurs within 3 to 14 days.

Thirdly, STELFONTA activates a PKC signaling cascade, which propagates throughout the tumor mass, resulting in an acute inflammatory response with swelling and erythema extending to the tumor margins and immediate surroundings. This inflammatory response, which generally resolves in 48 to 96 hours, is expected and contributes to the activity of STELFONTA by (a) restricting blood and oxygen supply to the tumor, and (b) recruiting and activating innate immune cells (principally neutrophils and macrophages). These cells then target the tumor mass and release reactive oxygen species, proteases, and cytokines.

This induction of an innate immune response also has an antimicrobial role and initiates downstream cytokine signaling that contributes to subsequent initiation of wound healing processes at the treatment site. STELFONTA has also been shown to directly effect keratinocyte and fibroblast function via production of cytokines and chemokines, which are associated with promotion of wound healing at the treatment site. Complete healing of the resulting wound following tumor destruction by STELFONTA is typically within 4 to 6 weeks.

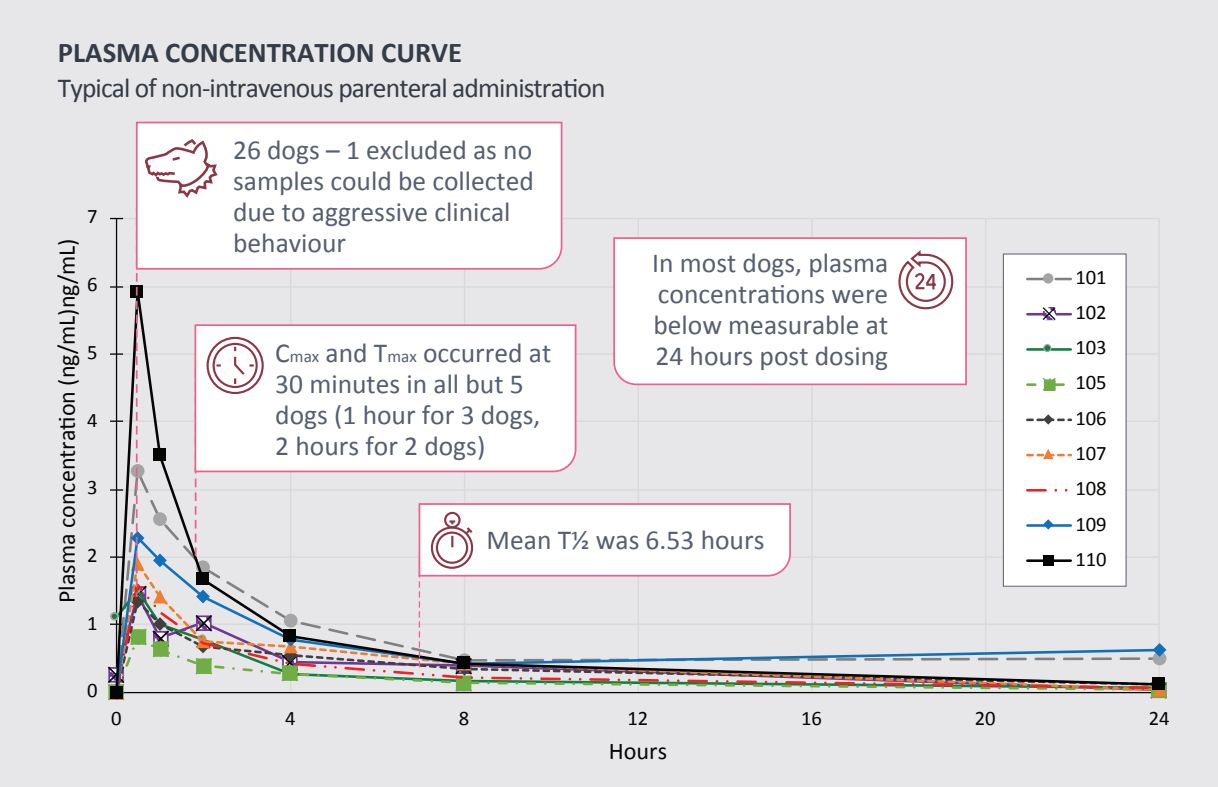
PRECLINICAL RESEARCH DATA

The margin of safety and toxicity of STELFONTA® (tigilanol tiglate injection) was evaluated in numerous preclinical rodent and dog studies that included single and repeat dose intravenous infusion and single-dose subcutaneous injection studies. Further safety and toxicity studies in the dog were undertaken, including a pharmacokinetic study, a laboratory target animal safety study and a laboratory cardiovascular study.

Pharmacokinetics/Pharmacodynamics [44, 45, 56]

The pharmacokinetics of STELFONTA post-intratumoral injection was measured in a dose determination study in dogs.[56] Three cohorts of dogs received intratumoral tigilanol tilgate at decreasing concentrations of drug. Patients with cutaneous MCTs had blood collected for measurement of plasma tigilonal tiglate concentrations at pretreatment, 0.5, 1, 2, 4, 8 and 24 hours post-treatment. The C_{max} occurred in the majority of dogs (21/26) within 30 minutes, with the remaining dogs reaching C_{max} by 2 hours (n=5 at 1 hr, n=2 at 2 hr).[56] (See figure below of dogs in Cohort 1 administered the 1.0 mg/mL formulation).

PHARMACOKINETICS OF TIGILANOL TIGLATE IN THE DOG



Individual plasma concentration curves for dogs in cohort 1 who received intratumoral administration of STELFONTA (tigilanol tiglate injection). Demonstrates C_{max} and T_{max} occurred at 30 minutes in the majority of dogs (21/26). Mean T 1/2 was 6.53 hours. In most dogs, plasma concentrations were below measurable at 24 hours post dosing.[56]

In vitro studies screening for tigilanol tiglate metabolites in canine liver microsomes demonstrated a half-life of tigilanol tiglate in hepatocytes of 21.8 minutes. A total of 13 metabolites were present that were more polar and oxygenated than the parent compound. Compounds with functional group substitutions of this nature result in reduced *in vitro* biological activity (>60X reduction of activity on PKC compared with parent compound, tigilanol tiglate).

The definitive route of excretion of tigilanol tiglate or its metabolites has not been determined. Analysis of urine, feces and saliva samples from dogs treated with STELFONTA® (tigilanol tiglate injection) show inconsistent amounts of low levels of tigilanol tiglate in isolated samples with no trend or consistency at range of 11–44 ng/g (ml). As mentioned previously, the amount of tigilanol tiglate that is dispersed systemically and subsequently excreted is thousands of times lower than the dose injected intratumorally.

Laboratory Target Animal Safety Study[44,45]

In a 4-week laboratory safety study, 48 healthy Beagle dogs 6 to 8 months old were administered STELFONTA intravenously. The dogs were distributed with 12 dogs per group consisting of 6 males and 6 females in each group. STELFONTA was administered intravenously over a 15-minute infusion once a week for 4 weeks at increasing doses ranging from 0 to 0.075 mg/kg body weight. Control dogs (0 mg/kg) received a vehicle control at a volume equal to the 0.075 mg/kg dose.

All dogs survived the study. There were no STELFONTA-related effects on body weight, body temperature, ophthalmic exam, electrocardiographic parameters, or organ weights.

The following were observed only in dogs in the groups administered STELFONTA and increased in a dose dependent manner:

- decreased food consumption from Days 22-29
- vomiting/retching during infusion or immediately post- infusion
- wound formation at the infusion site after the second or third dose
- decrease in activity sporadically throughout the study
- elevations in alanine aminotransferase on Day 23.

The following were observed in all groups (STELFONTA and control):

- limited use of the leg that received the infusion
- weakness after the first dose
- salivation
- infusion site edema and erythema (increased in frequency and severity throughout the study)
- tremors immediately post-infusion (increased in severity with dose).

Transient observations including vomiting, retching or tremors resolved within 1 hour of dosing. Salivation with resolution within 4 hours of dosing was observed in all groups. Additionally, all groups exhibited loose feces in a non-dose dependent manner.

Clinical changes at the infusion site included inflammation, erythema, and thickening of the skin. Correlative histopathology findings at the infusion site included hemorrhage, edema, inflammation, mixed cell infiltration, fibrosis, and chronic organizing thrombosis.

One dog in the highest dose cohort (0.075 mg/kg) had a wound, confirmed on histopathology as ulcerative inflammation and severe necrosis with bacteria present.

Clinical findings also included red, mottled, firm, and enlarged lymph nodes in all dose groups, including recovery dogs, confirmed on histopathology as inflammation, lymphoid hypercellularity, hemorrhage, and sinus histiocytosis.

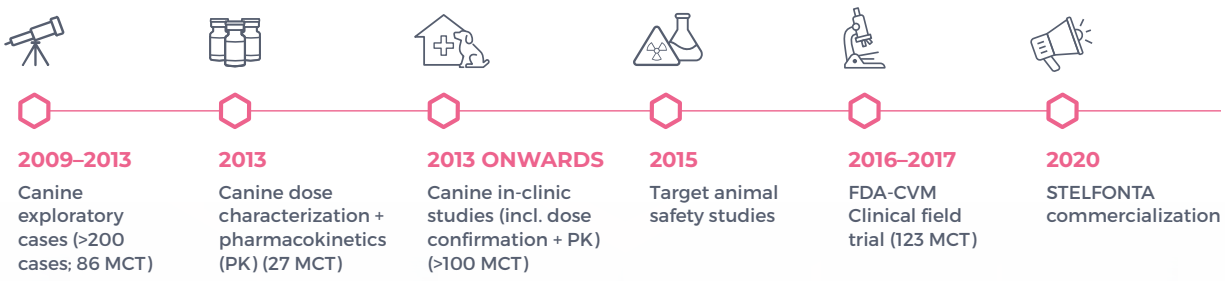
Histopathology findings unrelated to the infusion site included pituitary cysts in 7 dogs and 1 dog each from the highest dose cohort (0.075 mg/kg) was observed to have kidney tubular vacuolation, dilation of the ventricles of the brain, and chronic inflammation of both the left thigh skeletal muscle and left sciatic nerve.

Laboratory Cardiovascular Study[44, 45]

In a laboratory cardiovascular study, 4 healthy male telemetered Beagle dogs approximately 2-4 years old were administered STELFONTA® (tigilanol tiglate injection) as a single intravenous infusion. Treatment consisted of 4 treatment doses including a vehicle control and STELFONTA at doses of 0.01, 0.025 and 0.075 mg/kg body weight. All 4 dogs received all treatments with at least a minimum 3-day wash-out period.

There were no STELFONTA-related effects on body temperatures, blood pressure, or electrocardiograms. The following were observed after administration of the STELFONTA doses at all dose levels: salivation, vocalization, incoordination, tremors, red feces, and decreased feces output. Retching, emesis, incoordination, and changes in activity levels (increased and decreased) were seen after the highest dose (0.075 mg/kg) administration. Additionally, tachycardia was seen for the first 2.5 hours after the highest dose administration. The following was observed after all treatments (control and STELFONTA): excessive panting, decreased appetite and limited usage/swelling of leg or paw with resolution within 4 hours post dosing. All dogs exhibited mild weight loss during the study.

A SIGNIFICANT BODY OF EVIDENCE FOR STELFONTA



Pharmacokinetic Study[44, 45]

Numerous clinical field trials in the dog were performed in Australia to gain a better understanding of tumor efficacy, pharmacokinetics and safety of STELFONTA in spontaneously occurring disease (natural disease). In those early studies, STELFONTA treatment resulted in significant efficacy for MCTs at the prescribed label dose of 50% vol/vol. The efficacy (complete response) of a single STELFONTA treatment for all studies ranged between 70-90%.

Pharmacokinetic parameters of tigilanol tiglate were evaluated in an early field study in client-owned dogs. The systemic plasma levels of 10 dogs were measured following intratumoral injection of STELFONTA® (tigilanol tiglate injection) into 5 cutaneous and 5 subcutaneous MCTs with the recommended treatment dose of 0.5 mg/cm^3 (= 0.5 ml/cm^3) tumor volume, not exceeding 0.25 mg/kg body weight or a maximum dose of 5 mg. Tumor volumes in this small cohort of patients ranged from 0.1 to 6.8 cm^3, resulting in dose rates ranging from 0.002 to 0.145 mg/kg bodyweight (mean 0.071 mg/kg bodyweight).

Blood was collected from patients at time of completion of intratumoral injection, 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, and 24 hr. The most important information from this set of patients was a plasma T_{max} occurred in 6 of 10 dogs at the 5 minute time point and at the 15 minute time point in the remaining 4 dogs.

A reliable determination of C_{max} and AUC values could not be obtained in this cohort due to limitations on sampling timepoints and variable dose rates. However, measurements indicated a mean C_{max} of 5.86 ng/ml (range: 0.36–11.1 ng/ml) and a mean AUC_{last} of 14.59 h*ng/ml (range: 1.62–28.92 h*ng/ml). These levels are thousands of times lower than the dose of tigilanol tiglate injected intratumorally demonstrating that intratumoral injection of the drug does not result in high systemic levels.

There was also large inter-individual variability observed when determining half-life following intratumoral injection (range 1.24–10.8 hours). Tigilanol tiglate appears to exhibit flip-flop kinetics (sustained release rate) as a considerably shorter half-life of 0.54 hours was determined after intravenous infusion of 0.075 mg/kg in 12 dogs.

The common adverse events in this study were expected due to STELFONTA's mode of action at the treatment site. These events were injection site reactions including necrosis, swelling (localized edema and edema extending beyond the tumor injection site), pain, restlessness, inflammation, erythema, ulcerations, discoloration, sloughing of tissue, open wound, mild drainage, malodor, and presence of granulation tissue.

Three dogs experienced a more prominent reaction to treatment with dermatitis with or without skin necrosis in a region nearby but distinct from the tumor injection site. There were 3 dogs that required extended healing times beyond 28 days, with the longest requiring 5 months. The extended healing time in this single patient is unusual and the subsequent pivotal trial has shown the majority of STELFONTA treatment wounds heal within 4-6 weeks (78%) and most (96%) are healed by 12 weeks. Study investigators suggest that other unique aspects of this case, including the large tumor size and limb location, the enlarged reactive regional lymph node and wound intervention (wound debridement and periodic bandaging) may have played a role in delayed healing. Lastly, transient mild hypoalbuminemia was observed in 5 dogs with hypoproteinemia observed in 1 of these 5 dogs on Day 7 with resolution by Day 28.

3 STELFONTA PROVEN EFFICACY AND SAFETY IN FIELD CLINICAL STUDIES

PIVOTAL STUDY – EFFICACY AND SAFETY[44,45,58]

The effectiveness of STELFONTA® (tigilanol tiglate injection) was evaluated in a multi-center, randomized, untreated- controlled, investigator- and owner-masked field study involving client-owned dogs. Dogs were screened for the following MCT criteria:

- Non-metastatic World Health Organization
  - Stage Ia (one tumor confined to the dermis without regional lymph node involvement)
  - Stage IIIa (multiple dermal tumors; large infiltrating tumors without regional lymph node involvement)
- Cutaneous MCTs anywhere on the body
- Subcutaneous MCTs located at or distal to the elbow or the hock
- Tumor without significant ulceration

A total of 123 client-owned dogs met criteria for enrollment and were randomized in a 2:1 ratio to either the STELFONTA treatment group (n=81) or the untreated control (sham) group (n=42). Dogs in both groups received the same concomitant medication regimen (see table below) and underwent assessment for tumor response on day 28.

TREATMENT PLAN – CONCOMITANT MEDICATIONS

All dogs in both treatment groups received concomitant medications:

- **Prednisone (or Prednisolone) -** 0.5mg/kg q12h x 7 days then q24h x 3 days
- **Diphenhydramine -** 2mg/kg q12h
- **Famotidine -** 0.5mg/kg q12h

Drug	Day -2		Day -1		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
Prednisolone/ Prednisone	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●		●	
H1 blocker (i.e diphenhydramine)					●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
H2 blocker (i.e. famotidine)					●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

All dogs had hair shaved from the tumor site with minimal manipulation regardless of treatment group

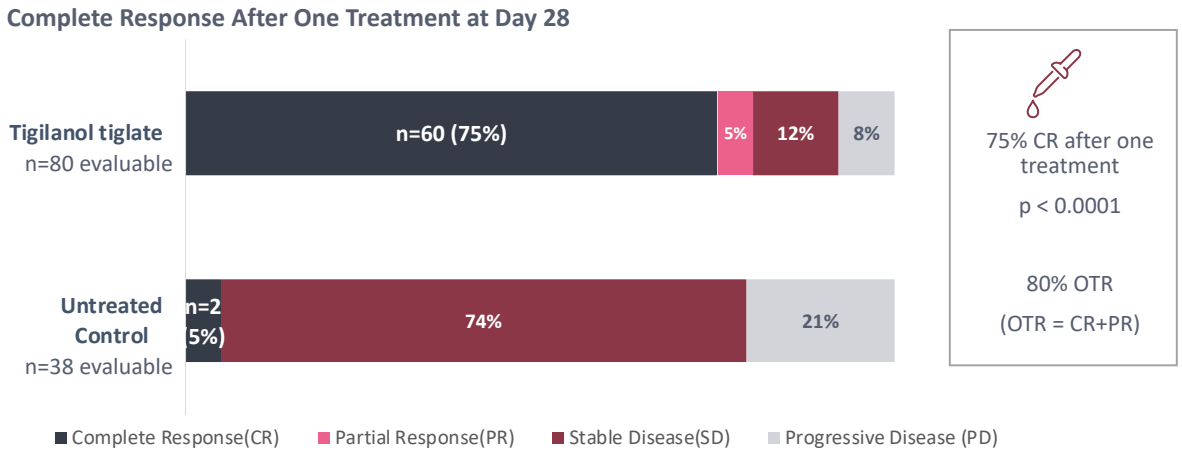
In Phase I, the treatment group received a single injection of STELFONTA. On the day of treatment, tumor volume ranged from 0.1 to 9.8 cm^3 with an average tumor volume of 1.7 cm^3.

A total of 118 dogs were evaluable in the effectiveness analysis; 80 dogs in the STELFONTA treatment group and 38 dogs in the sham treatment (untreated control) group. Response to treatment was evaluated using modified Response Evaluation Criteria in Solid Tumors (RECIST) criteria (Veterinary Cooperative Oncology Group (VCOG)),[59] where complete response (CR) is resolution of the target tumor, partial response (PR) is at least a 30% decrease in the longest diameter of target tumor, stable disease (SD) is a decrease of less than 30% or increase of less than 20% of the longest diameter of the target tumor, and progressive disease (PD) is greater than a 20% increase in the longest diameter of the target tumor.

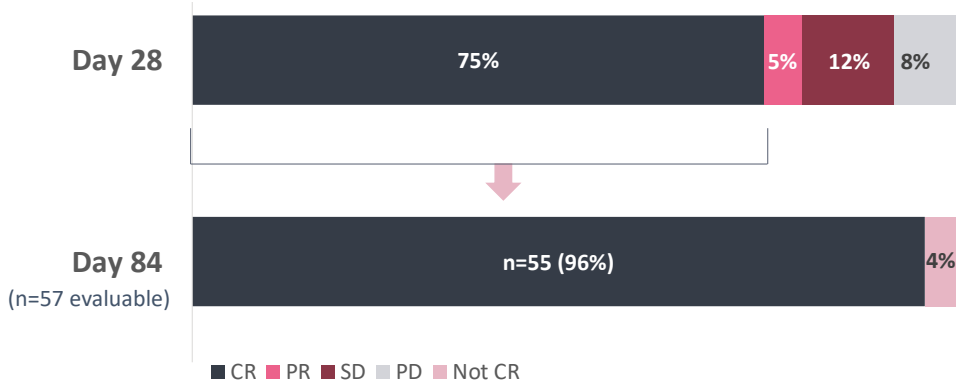
At 28 days after STELFONTA® (tigilanol tiglate injection) treatment, a statistically significant greater proportion of dogs in the STELFONTA treated group (60/80; 75%) achieved CR compared to dogs in the untreated control group (2/38; 5.3%) ($p<0.0001$). Further, an objective tumor response (CR + PR) was observed in 64/80 (80%) of the STELFONTA treated dogs.

The 60 dogs in the STELFONTA group that experienced CR at Day 28 underwent assessment at Day 42 (n=59) and Day 84 (n=57). At Day 42, 59/59 (100%) of dogs that had achieved CR with a single STELFONTA treatment were disease-free at the injection site, and at Day 84, 55/57 (96%) were disease-free at the injection site.

PRIMARY RESULTS

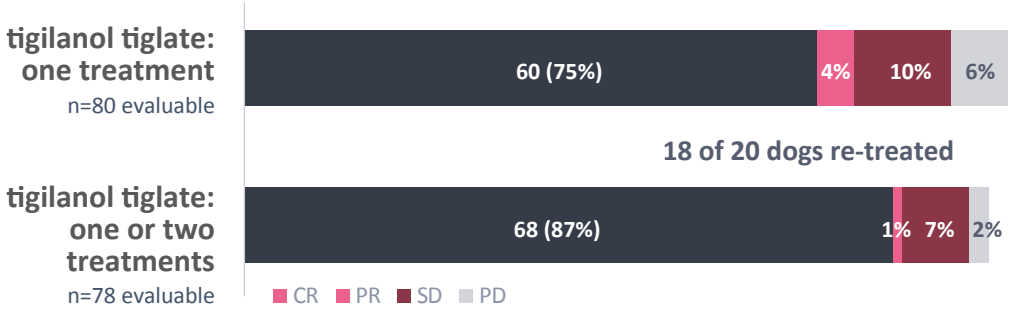


DISEASE FREE INTERVAL AT 84 DAYS



COMPLETE RESPONSE

After one treatment and cross over treatments

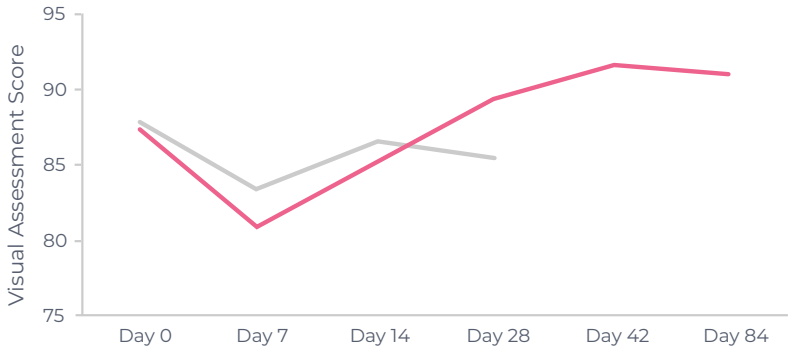


In Phase II, 18 of the 20 STELFONTA® (tigilanol tiglate injection) treated dogs that had not achieved CR received a second STELFONTA treatment. Additionally, 36 previously untreated control (sham) dogs were offered the option to cross-over and receive a single STELFONTA treatment. The resulting efficacy of STELFONTA in dogs receiving either 1 or 2 STELFONTA treatments was 87%.

Veterinary investigators prescribed antibiotics, analgesics, and sedatives at their discretion. No patients in either group received general anesthesia for evaluation, diagnostics or STELFONTA treatment. Prophylactic antibiotics were given in less than half (47/123, 38.2%) of patients with 14 of these cases prescribed to treat wounds. Amongst these, only a single case had confirmed bacterial infection with mixed non-resistant strains of aerobic and anaerobic bacteria. The majority of analgesics were used to manage discomfort and were mainly initiated on the day of or day after treatment. It is important to emphasize that pain management was at the discretion of the attending veterinarian investigators through prescription analgesics. A majority (63%) of patients received analgesia, with a median course length of 6 days and an average of 9 days.

Sedatives to treat patient anxiety and temperament during diagnostics and STELFONTA treatment were also left to the discretion of the attending veterinarian. As the clinicians became more experienced with STELFONTA, the use of sedatives decreased. During Phase I, sedatives were used in 35% (28/81) of patients, decreasing in Phase II, with only 20% (11/54) of patients receiving sedatives. Quality of Life (QoL) was assessed by owners throughout the study using a questionnaire that was developed for veterinary oncology patients.[60] The questionnaire asked owners to rate their dogs in the categories of happiness, mental status, pain, appetite, hygiene, water intake and mobility. As anticipated, owners from the treatment group assessed their pets as enjoying life slightly less and having slightly more pain at day 7, the time period during wound formation and tumor slough. During this period, the pet owners also rated their pets as slightly less active and less mobile. However, the QoL of the pets in the treatment group were equivalent or superior to the control group pets by day 14. Overall, owners of treated dogs considered their dog's health improved compared to owners of control group dogs since each previous visit and since the initial diagnosis.

QUALITY OF LIFE[65]



Quality of life significantly improves following treatment with tigilanol tiglate.

Tigilanol Tiglate
Untreated

PIVOTAL STUDY – SAFETY/ADVERSE EVENTS[44,45,58]

In the pivotal study, 117 dogs that received a single STELFONTA® (tigilanol tiglate injection) treatment (Phases 1 and 2) were monitored for adverse events (AEs) and side effects. In Phase 1, AEs were also recorded in the control group to allow comparison of possible side effects associated with the concomitant medications and/or the characteristics of the general patient population. AEs from physical examination, serum biochemistry, hemotology and urinalysis results were classified and recorded using the Veterinary Cooperative Oncology Group (VCOG) – common terminology criteria for adverse events.[46] Under VCOG definition, an AE is any "unfavorable and unintended sign (including an abnormal clinicopathological finding), clinical sign, or disease temporally associated with the use of a medical treatment that may or may not be considered related to the medical treatment."[46]

A total of 587 AEs in 21 VCOG categories were recorded by the investigators over the course of the study. It is important to note that the majority of AEs (94%; 549/587) were grade 1 or 2. Although the majority, 61% (356/587), were considered by the investigators to be definitely, probably or possibly related to STELFONTA treatment, these AEs were directly associated with the drug's mode of action. No or minimal veterinary intervention was required in these cases. The most common AE was wound formation which is directly related to STELFONTA mode of action and the deficit left following destruction of the tumor mass via tumor cell oncosis and tumor hemorrhagic necrosis followed by tumor slough.

There were 17 other types of frequent AEs (ie; those occurring in >5% of patients treated with STELFONTA). Four of these were also expected outcomes directly related to STELFONTA treatment including injection site pain, lameness in the treated limb, injection site bruising/erythema or edema and locoregional lymph node enlargement. These side effects are a direct result of the drug's mode of action; eliciting a rapid, localized inflammatory response leading to tumor destruction, slough and subsequent treatment site healing.

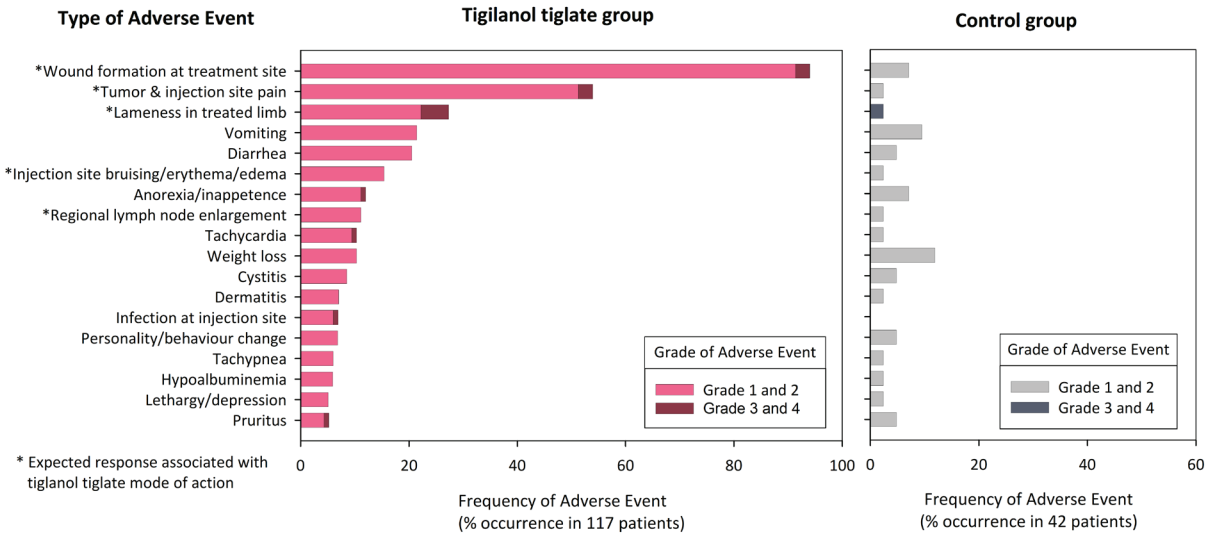
The remaining frequent AEs (13) occurred in <20% of patients. Investigators considered 9 of these possibly related to STELFONTA treatment.The majority of these (tachycardia, tachypnea, lethargy/depression, inappetence, weight loss) likely correlating with discomfort at the STELFONTA treatment site due to the inflammatory response at the tumor site and the tumor destruction process during the first 7 days following treatment. Analgesia was prescribed at the discretion of the attending veterinarian with a majority (63%) of patients receiving analgesia. Three of the remaining frequent AEs (cystitis, dermatitis and pruritis) were considered unlikely related to STELFONTA treatment.

The final frequent AE, low grade hypoalbuminemia, had a probable association with STELFONTA treatment in 2 dogs with the largest wounds post tumor slough. In these 2 dogs, low grade hypoalbuminemia (2.2 and 2.4 g/dL) compared to normal range (2.7 to 3.9 g/dL) was first recorded at 7 days after treatment; albumin levels in both dogs returned to normal after day 28 as the STELFONTA treatment wound healed.

There were a low number (6%, 38/587) of grade 3 and 4 AEs reported after STELFONTA treatment. Only 2 were considered likely related to STELFONTA treatment. One of these AEs involved the development of a bacterial infection which cultured positive for nonresistant strains of mixed aerobes and anaerobes and secondary localized cellulitis 7 days after treatment. This patient received 1 day of intravenous fluids and oral antibiotics. The cellulitis and swelling resolved by day 14 and the wound formed healthy granulation tissue by day 19. The second serious AE considered possibly related to STELFONTA treatment occurred in a 15-year old patient with significant co-morbidities. This patient was euthanized at the owner's request 82 days after treatment due to deteriorating quality of life due to discomfort and tumor recurrence.

Adverse reactions likely related to the required concomitant corticosteroids were similarly reported in STELFONTA® (tigilanol tiglate injection) and untreated control (sham) dogs and these included elevated alkaline phosphatase, polyuria, and polydipsia. The adverse reactions during the study are summarized below.

ADVERSE EVENTS



While localized bruising and swelling is an expected response, extensive reactions can be a sign of degranulation requiring urgent attention. Educate pet owners when and how to contact the veterinarian with concerns.

Possible side effects following STELFONTA administration

- Signs of degranulation (which can occur with any manipulation of a MCT):
  - vomiting
  - diarrhea
  - lethargy
  - anorexia/hyporexia
  - altered breathing/tachypnea
  - urticaria bruising and edema at or away from the treated site
  - tachycardia
  - hypotension
  - death
- Signs of localized inflammatory response including swelling and bruising at or away from the treated site
- Signs from concomitant medication use:
  - polyuria
  - polydispsia
  - increased panting
  - increased appetite
  - elevated alkaline phosphatase.
- Other signs included lameness in treated limb, tachycardia and weight loss

Expected adverse events seen following STELFONTA® (tigilanol tiglate injection) treatment

Signs due to mode of action include:

- Wound formation following tumor necrosis and slough
- Localized swelling, erythema, bruising at the tumor site
- Pain at the treated site
- Lameness in a treated limb
- Regional lymph node enlargement

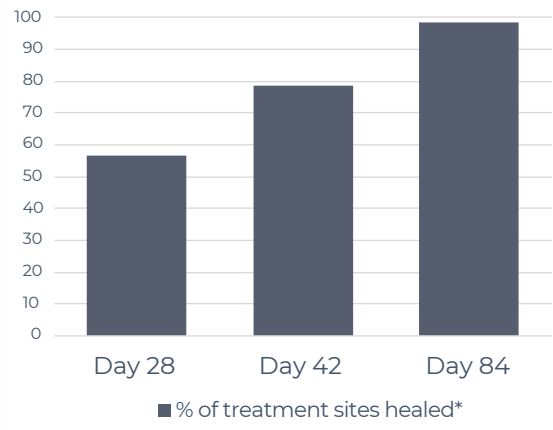
PIVOTAL STUDY – WOUND MANAGEMENT AND HEALING[44,45,58]

Formation of a wound at the treatment site is a process directly related to the efficacy of the drug in full or partial tumor destruction and the deficit remaining after tumor slough. In the pivotal study, 95% of the 117 dogs that received a single treatment of STELFONTA in either Phase 1 (n=81) or Phase 2 (n=36) developed a wound at the treatment site by 14 days.

Wounds resulting from slough of the target tumor most commonly formed 3-7 days after STELFONTA treatment, with maximum wound areas in 89% of cases (99/111) by 7 days post treatment. The remaining 11% (12/111) formed maximum wound size by 14 days following STELFONTA treatment. Wounds were less likely to form with small tumor volumes. Small tumors with volumes <0.5 cm^3, formed no wound in 12% (5/42) of cases; whereas tumors with volumes of 0.5 to <2.0 cm^3 failed to form a wound in 2% (1/43) of cases and none of the tumors with volume measuring ≥2.0 cm^3 failed to form a wound (0/32).

A secondary objective of this study was to identify elements related to wound healing. There was a direct relationship between wound healing and size and location of the wound. As expected, larger wounds healed more slowly than smaller wounds. Interestingly, wounds on the lower limbs healed more slowly than wounds on the body and upper limbs. This may correspond to wound closure relying predominantly on re-epithelialization in lower limb wounds versus regions such as the body and upper limbs where wound contraction contributes more to closure.

HEALING AT TREATMENT SITE



- For dogs with CR over **96% of treatment sites healed after 84 days**
- **Systemic antibiotics** in 12% of cases for wound management
- **Bandaging** in one treated cases and one flushed with saline to reduce odor
- **Elizabethan collar** in two treated cases

*Healing defined as: complete re-epithelialisation of treatment site

The majority of wounds were managed uneventfully by the investigators and owners without bandaging or other interventions. The wounds were left to heal by second intention with more than half of the wounds (56.5%) healed by 4 weeks following STELFONTA® (tigilanol tiglate injection) treatment, more than 3 out of 4 wounds (76.5%) healed within 6 weeks and 96.5% healed by 12 weeks post-treatment. As mentioned previously, 47 cases received prophylactic antibiotics; however only 5 dogs received active wound management during the study.

One case was treated with antibiotics for a positively cultured bacterial infection and secondary cellulitis (the serious AE case outlined in the Safety/Adverse Event section), one bandaged, two wore Elizabethan collars to prevent self-trauma of the wound, and one was flushed with saline to reduce odor.

The necessity of dressing or bandaging is variable and dependent upon patient characteristics, tumor location, response to therapy and treatment site drainage. Dressing and bandaging are not necessary nor recommended in the majority of cases. In the pivotal study, dressings or bandages at any stage after STELFONTA treatment were discouraged for the following reasons:

- Dressings may interfere with resolution of local edema resulting from the drug's mode of action in initiating an acute, transient and localized inflammatory response at the treatment site.
- Dressings may impair drainage and resolution of edema leading to increased wound size due to damage to normal surrounding tissue.
- Leaving wounds to heal in ambient oxygen may improve and enhance wound closure.[47-49]

Some discharge from the site is expected. The site can be cleaned with warm water as necessary. Wear disposable gloves when cleaning the area. Thoroughly wash any skin that comes in contact with the wound, wound discharge, or material contaminated with wound discharge (e.g. bedding).

In cases of prolonged healing, wound management measures may be required at the discretion of the attending veterinarian.

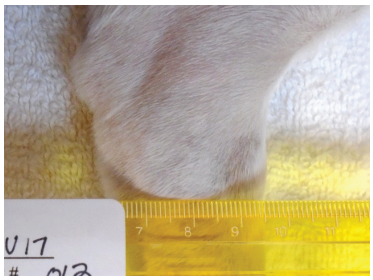
Clinical field trials have consistently shown the presence of well-developed granulation tissue in the exposed wound bed following tumor slough and rapid healing following STELFONTA treatment. This study demonstrated the same wound appearance and healing at the STELFONTA treatment site as previous studies. From these observations, it is apparent that wound healing processes are already underway prior to tumor slough. This is likely related to direct and specific effects of STELFONTA in promoting healing. *In vitro* studies with adult human fibroblasts have shown that tigilanol tiglate modifies fibroblast gene expression, differentiation and functioning[50,51], especially in relation to growth factor signaling and the composition of the extracellular matrix. *In vitro* studies have shown that tigilanol tiglate stimulates keratinocyte migration, a process related to effective re-epithelialization necessary in lower limb wound healing.[52]

The ability to accurately predict characteristics of wounds following STELFONTA® (tigilanol tiglate injection) treatment such as size of wound and healing time would be clinically useful for practicing veterinarians. Many characteristics and healing time following STELFONTA treatment have been evaluated, including the size of wound in relation to volume of tumor, tumor location and time to resolution of wound. In these studies, several variables associated with wound area have been identified following tumor slough. These include volume of the target tumor, locoregional lymph node enlargement and possibly tumor cytological grade.[62]

The question remains whether STELFONTA treatment results in smaller wounds with less loss of healthy tissue surrounding the tumor than surgical excision. Historically, recommended surgical approaches for MCT excision have involved, where feasible, 3.0 cm margins of normal tissue surrounding the tumor and one tissue plane deep.[35-38,53,54] However, there are a number of published studies specifically examining approaches to surgical margins for the excision of cutaneous MCT which have involved tighter (e.g. 2 cm)[36] or proportional margins based on tumor size.[37] More recently, a study comparing a 'conservative' 2 cm margin with a more 'aggressive' 3 cm surgical margin in low grade cutaneous MCTs showed no advantage in achieving histologically tumor free margins with a wider approach.[61] In order to make a comparison between STELFONTA treatment wounds and potential surgical wound size, we evaluated two dimensional measurements of theoretical margins of 1.5 cm and 3 cm in the pivotal study patients. The intention was to mimic both conservative and aggressive approaches to excision of cutaneous and subcutaneous MCTs regardless of tumor grade. It must be recognized that such a comparison is likely to oversimplify multiple aspects of surgical margin planning, clinical execution, and wound closure.

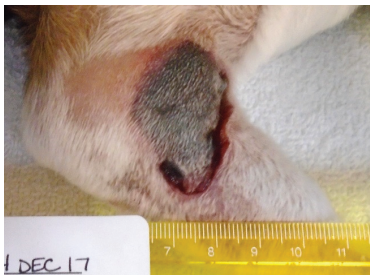
However, the comparison between STELFONTA treatment and theoretical surgical excision margins suggested that the wounds formed for dogs that achieved CR following a single STELFONTA treatment trended as substantially smaller and involved less loss of healthy tissue surrounding the tumor than would have occurred with the theoretical surgical wounds. Using the conservative 1.5 cm margin, 88% of wounds following STELFONTA treatment were smaller than the theoretically estimated area of the surgical wound.[64] It is possible that STELFONTA more precisely targets the tumor due to both its direct intratumoral route of administration and its mode of action. Recognizing the value of this information, there is ongoing work evaluating more comprehensive models for surgical margins and wound healing following STELFONTA injection.

HEALING ASSESSMENT AT THE TREATMENT SITE



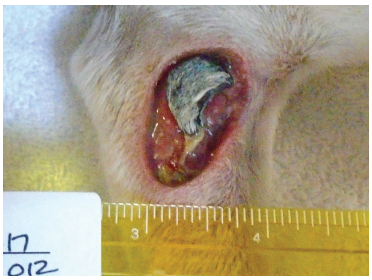
Day 0

Pre-treatment



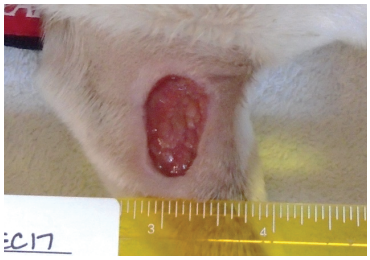
Day 4

Tumor necrosis evident



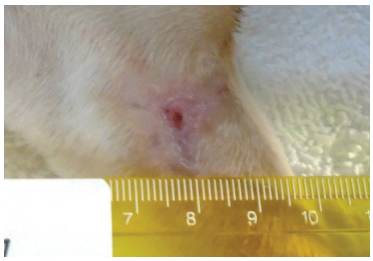
Day 7

Starting to granulate



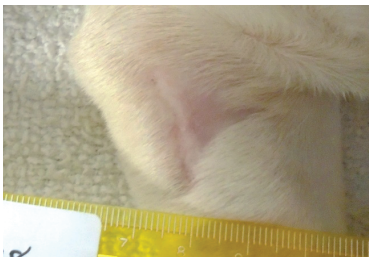
Day 14

Tumor destroyed
Granulation tissue present



Day 28

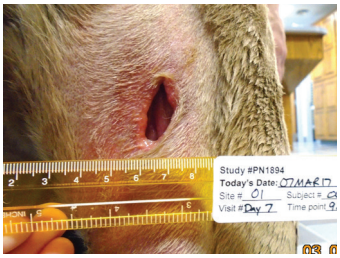
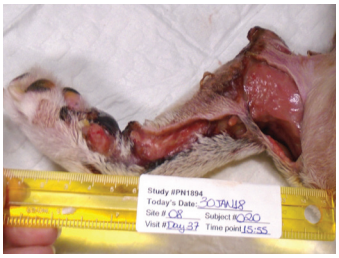
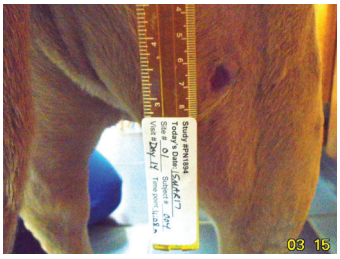
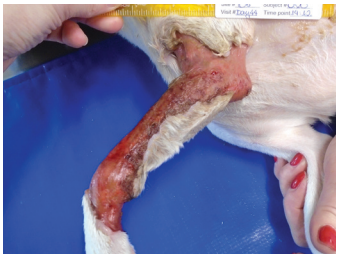
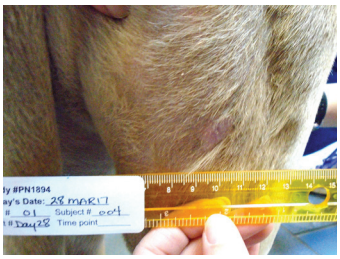
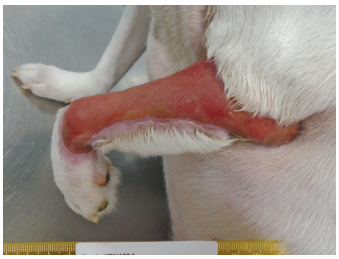
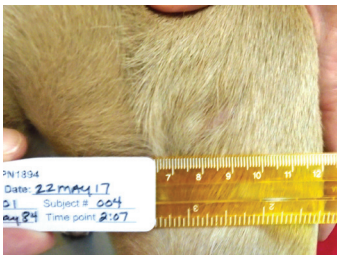
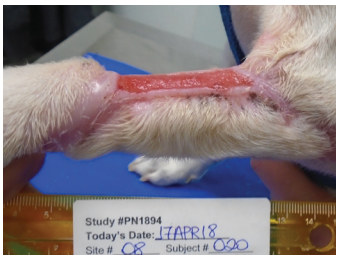
Tumor site healing



Day 42

Tumor site healed

- 11 yr old spayed female Jack Russell Terrier
- Subcutaneous MCT medial elbow
- Tumor volume 0.5 cm^3
- STELFONTA dose 0.3 ml
- CR at day 28

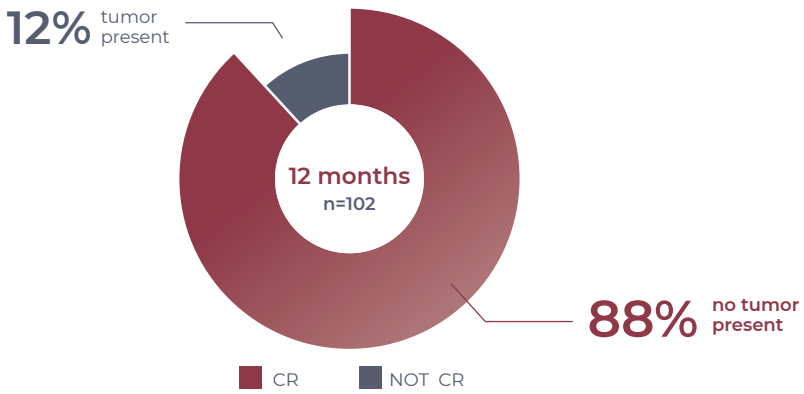
Patient ID	01-004	08-020
Age	12 yrs 3 mth	12 yrs 5 mth
Breed	Pit bull terrier	Small mixed breed
Sex	Male	Male
Weight	37.3 kg	9.8 kg
Location	Thigh	Metacarpus
Tumor Type	Cutaneous	Cutaneous
Cytological Grade	Low	High
Tumor Volume (cm^3)	2.7	3.1
Dose (mL)	1.4	1.6
Dose (mg/kg)	0.038	0.16
MaximumWound Surface Area (cm^2)	2.551	186.4
Time to heal	28 days	By day 84, wound size was $3.7cm^2$
	Typical Wound	Extensive Wound
Seven days after treatment		
Fourteen days after treatment		
Twenty-eight days after treatment		
Eighty-four days after treatment		

STELFONTA® (tigilanol tiglate injection) – DISEASE-FREE INTERVAL[57,63]

In an effort to establish long-term disease-free interval (DFI), dogs with cytologically confirmed MCT, enrolled in STELFONTA clinical trials (2013-2018) using a 50% vol/vol dose that achieved a complete response (CR, RECIST criteria)[59] at 28 days following a single STELFONTA treatment were evaluated.

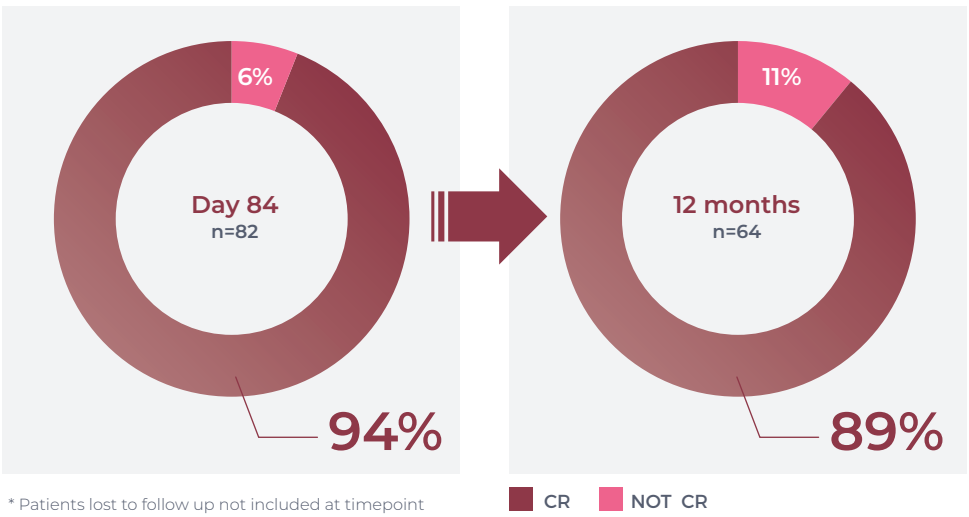
At 1 year following treatment, 74 dogs were available to be assessed at the treatment site with 65 dogs (88%) disease free and 9 dogs (12%) that had developed recurrence. Although data is still maturing in the many clinical trial patients, additional dogs are being followed for long-term DFI with some patients evaluable at 2-4 years following a single STELFONTA treatment.

DISEASE-FREE INTERVAL FROM AU AND USA CLINICAL TRIALS FOLLOWING A SINGLE STELFONTA INJECTION.



Dogs that participated in the pivotal study that achieved a CR at day 28 were assessed for disease-free interval. At the completion of the pivotal study, day 84, 94% (77/82) remained disease-free. To evaluate 12-month disease-free interval, patients' medical records were reviewed and telephone interviews were conducted with owners to determine presence or absence of MCT at the STELFONTA treatment site. At 12 months post STELFONTA treatment, 64 dogs were available for assessment. Of those available, 57 (89%) remained tumor free at the treatment site with 7 (11%) documented local tumor recurrences. Moreover, all of the recurrences occurred within the first 6 months, with the majority (5/7, 71%) within the first 12 weeks (day 84).

DISEASE-FREE INTERVAL – US PIVOTAL STUDY[63]

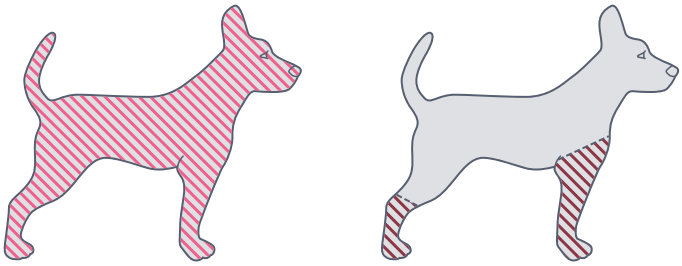


* Patients lost to follow up not included at timepoint

4 STELFONTA® (tigilanol tiglate injection). SEEING IS BELIEVING

INDICATIONS

STELFONTA® IS INDICATED FOR THE TREATMENT OF NOMETASTATIC CANINE MAST CELL TUMORS:



Cutaneous MCTs anywhere on the body

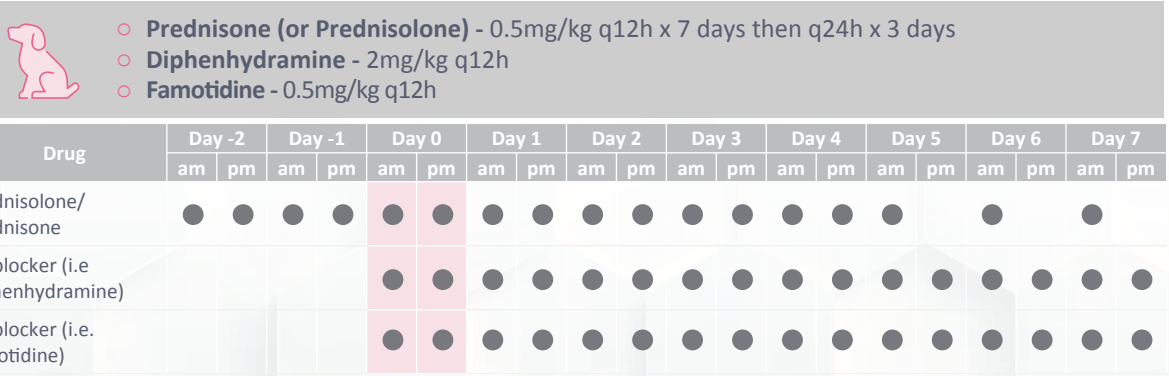
Subcutaneous MCTs located at or distal to the elbow or the hock

STELFONTA should not be injected into subcutaneous mast cell tumors located above the elbow or hock (e.g. on the body, head or neck) as 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.

CONCOMITANT MEDICATIONS

Administer the following medications to decrease the potential for severe systemic adverse reactions from mast cell degranulation:

- **Corticosteroid (e.g. oral prednisone or prednisolone at anti-inflammatory dose):** Start medication 2 days prior to STELFONTA treatment and continue for 8 days post-treatment (10 days total).
- **H1 receptor blocking agent (e.g. oral diphenhydramine):** Start medication on the day of STELFONTA treatment and continue for a total of 8 days.
- **H2 receptor blocking agent (e.g. oral famotidine):** Start medication on the day of STELFONTA treatment and continue for a total of 8 days.



Always administer a corticosteroid, an H1 receptor blocking agent and an H2 receptor blocking agent when treating with STELFONTA to decrease the potential for severe systemic adverse reactions, including death, from mast cell degranulation.

EASE OF ADMINISTRATION

Dosing Instructions:

Administer STELFONTA® (tigilanol tiglate injection) 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:

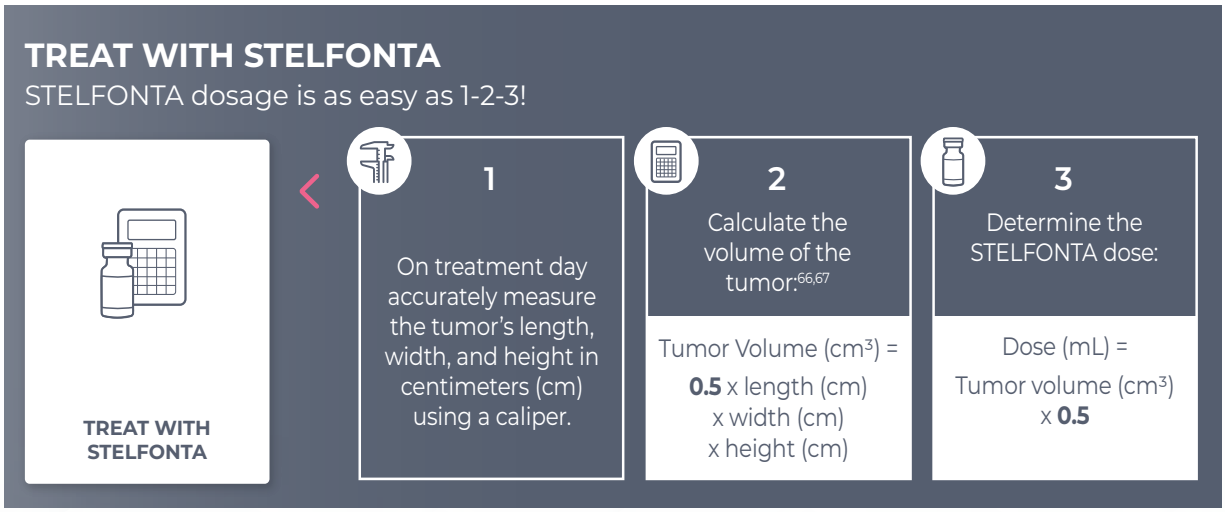
  0.5 x [length (cm) x width (cm) x height (cm)]

- Tumors must be less than or equal to 10 cm^3 in volume.
- 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.

Confirm owner has administered concomitant medications as instructed.
Confirm patient body weight in kilograms.



STELFONTA® (tigilanol tiglate injection) – DOSING EXAMPLE

- Tumor measures 1.51 x 1.51 x 1 cm
- Tumor volume = 1.14 cm^3
- Dose = 50% v/v
- Dose = (1.14 cm^3) x (0.5) = 0.57 mL

Tumor volume = 0.5 x (1.51 cm x 1.51 cm x 1 cm)
= 0.5 x (2.28 cm^3)
= 1.14 cm^3

CALCULATE THE DOSE OF STELFONTA:

Tumor volume x 0.5 mL
= 1.14 cm^3 x 0.5 mL
= 0.57 mL
Rounded to nearest 0.1 mL = 0.6 mL

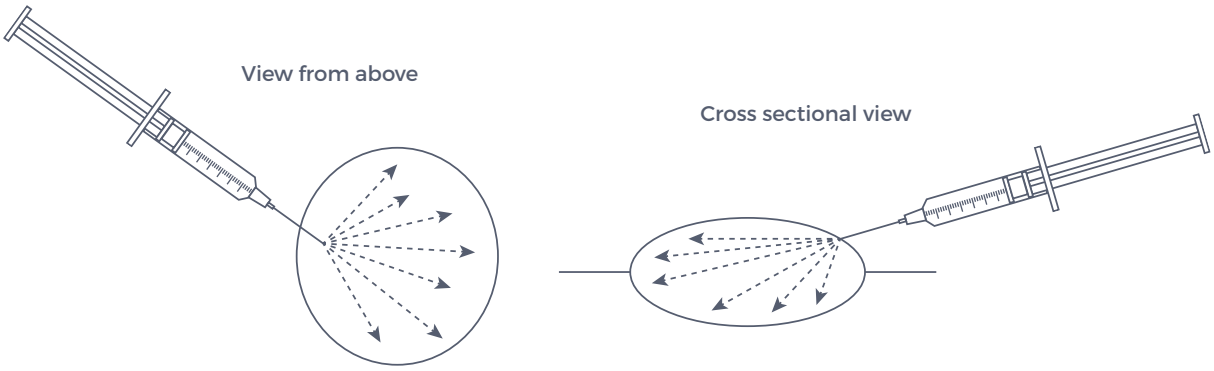
Administration of STELFONTA:

While general anesthesia is typically not indicated, tumor location and/or patient temperament may necessitate sedation to safely and accurately administer STELFONTA and decrease the chance of accidental self-injection. Wear gloves, eye protection and lab coat or gown in the preparation and administration of STELFONTA. Do not inject STELFONTA into normal subcutaneous tissue (e.g. beyond tumor margins) because severe edema, erythema, and necrosis of the injected tissue may occur. STELFONTA should not be injected into the margins, beyond the periphery, or deep to the tumor mass. Use STELFONTA with caution in tumors located within mucocutaneous regions (e.g., eyelids, vulva, prepuce, and anus) as tumor necrosis could cause a change in morphology of the mucocutaneous region resulting in loss of functional integrity.

- Shave the tumor site and surrounding area. Avoid manipulation of the tumor.
- Draw the calculated volume of STELFONTA into a sterile Luer lock syringe with a 23 gauge needle.
- Identify an appropriate injection point on the edge of the tumor mass inserting the needle according to the tumor's location, form, and appearance. Where a tumor protrudes above the surface of the skin, the needle should be inserted at an oblique angle of approximately 45°.

- Insert and embed the needle in the tumor mass through a single injection site and draw back slightly to ensure STELFONTA® (tigilanol tiglate injection) is not injected into a blood vessel. While applying even pressure on the syringe plunger, move the needle back and forth in a fanning manner to inject STELFONTA into the tumor. The drug should fully perfuse the entire tumor mass.
- When the total dose of STELFONTA has been administered, pause to allow tissue dispersion before removing the needle from the tumor. Pull back on the plunger to create a small negative pressure before removing the needle to minimize leakage from the injection site.
- After the needle is withdrawn, apply light pressure for 30 seconds over the needle exit hole using a gloved finger. If leakage does occur, rinse injection site with saline to wash STELFONTA from the skin surface. Do not re-administer.
- Dispose of the needle and syringe. To minimize risk of accidental self-injection, do not recap the needle.

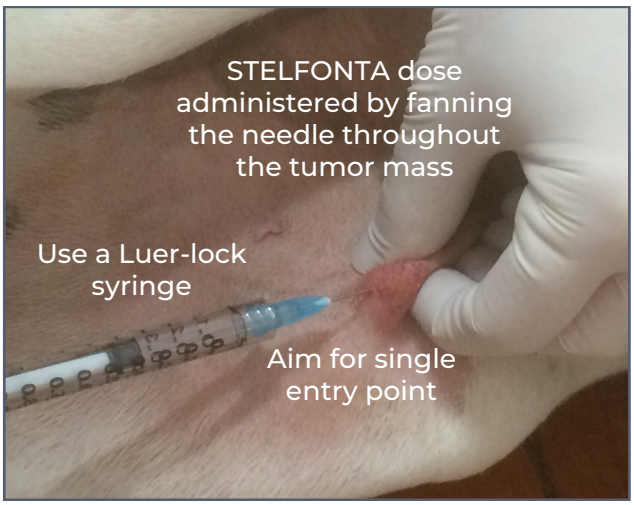
STELFONTA ADMINISTRATION

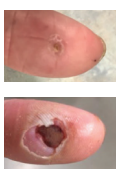


STELFONTA ADMINISTRATION

Single injection point into tumor

STELFONTA dose administered by fanning the needle throughout the tumor mass





Accidental self-injection of STELFONTA may cause severe wound formation. To decrease the risk of accidental self-injection, sedation of the dog may be necessary.

STELFONTA® (tigilanol tiglate injection) – PRACTICAL USE AND TIPS FOR THE 4 STAGES OF TREATMENT

Concomitant Medications – Pre-Treatment

- Please do not omit the concomitant medications from the treatment protocol. These medications are required. See insert for dosing instructions.
- An accurate tumor measurement is imperative for correct STELFONTA dosing.
- Use STELFONTA with caution in mast cell tumors with significant ulceration as leakage of the drug from the ulcerated area may occur following treatment, potentially reducing effectiveness.

Always administer a corticosteroid, an H1 receptor blocking agent and an H2 receptor blocking agent when treating with STELFONTA to decrease the potential for severe systemic adverse reactions, including death, from mast cell degranulation.

STELFONTA injection

- While sedation isn't generally required, it may be considered in cases in which proper administration may be difficult, e.g. in a nervous animal or with treatment of a sensitive area. As a general guideline, if you are able to obtain an FNA (fine needle aspirate) for diagnosis without difficulty, most animals will tolerate treatment without sedation.
- A Luer-lock syringe is essential to avoid leakage and protect the veterinarian from potential exposure due to separation of the needle from the syringe under pressure.
- Administer the treatment in a fanning motion to ensure the treatment reaches all aspects of the tumor and minimize the number of injection sites to prevent leakage from previous injection sites.

Accidental self-injection of STELFONTA may cause severe wound formation. To decrease the risk of accidental self-injection, sedation of the dog may be necessary.

Tumor Destruction

- Avoid bandaging the treatment site as this may restrict the blood flow and compromise healing.
- Some discharge from the site following treatment is expected. The site can be cleaned with warm water as necessary. Wear disposable gloves when cleaning the site to avoid exposure to residual drug.
- Swelling, bruising and redness are part of the process and a demonstration of efficacy. Wound formation is also a part of the mode of action.
- An odor may be present. This will be dependent on factors such as tumor size and the amount of necrotic tissue. This is typically noticed between days 3 and 6.
- Necrotic slough is typically grey or black, dependent upon depth of the tumor. This is an expected event in treatment.
- If a scab/necrotic tissue is present after day 7, it can be removed but removal is not necessary. Do not remove if still well attached to underlying tissue.

Treatment with STELFONTA has been associated with cellulitis and severe tissue sloughing resulting in extensive wounds that require additional treatment and prolonged recovery times.

Tumor Site Healing

- Wounds heal rapidly after necrotic tissue has sloughed away.
- Wounds typically heal by second intention with NO intervention required.
- In cases of excessive self-trauma, an Elizabethan collar or bandage can be used.
- The wound can be flushed with water in cases of excessive discharge or smell.
- No restriction on activity of the dog is required.
- Pet can be bathed or swim with extra care taken with the treatment site.

5 STELFONTA® (tigilanol tiglate injection) SUMMARY

STELFONTA IN ACTION

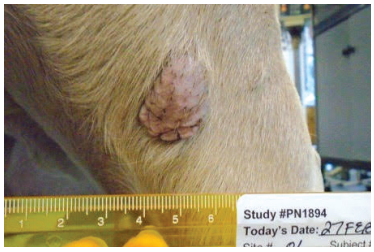
Second intention healing with minimal intervention is unique to STELFONTA and is an aspect that veterinarians often find most interesting. Generally, the tumor site is completely healed within 4 to 6 weeks.

From Day 1, cells in the tumor and blood vessels begin to break down, with visible swelling and changes in color of the tumor site.

Within 24 hours, necrosis causes transformation of the tumor color to black. Over the following days an open wound develops where the tumor has sloughed. The tumor breakdown and subsequent slough of the necrotic tissue is usually complete within 7 days.

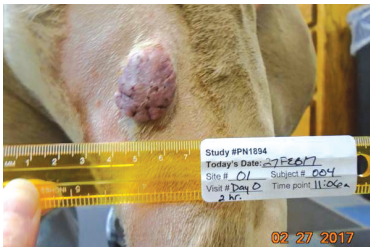
By Day 14, the tumor site begins to heal itself with accelerated closure, minimal scarring and healthy hair regrowth to follow – all without the need for intervention.

Within 4 weeks, the majority of tumor sites are fully healed.[58]



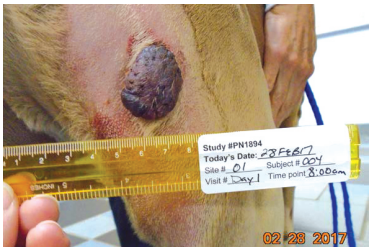
Day 0

Pre-treatment



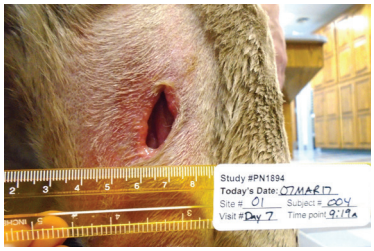
Day 0

2-4 hr post-treatment



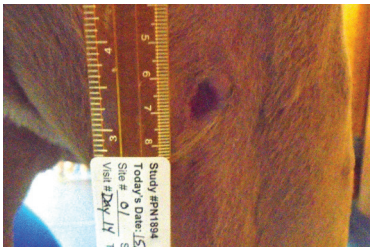
Day 1

24 hr post-treatment evident tumor necrosis



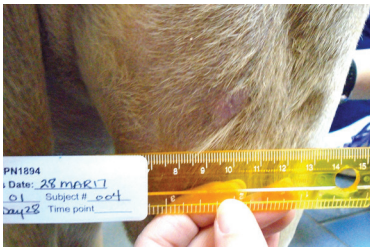
Day 7

Tumor destroyed Granulation of tumor site



Day 14

Tumor site healing



Day 28

Tumor site healed

- 12 yr old male Pit Bull Terrier
- Cutaneous MCT caudal lateral thigh
- Tumor volume 2.7 cm^3
- STELFONTA dose 1.4 mL
- CR at day 28

QUALITY OF LIFE

STELFONTA® (tigilanol tiglate injection) treatment has demonstrated no negative impact on dogs' quality of life in clinical trials. Importantly, in dogs whose health may be declining secondary to cancer diagnosis, owners reported their dog's general health was improved within 2 weeks of treatment.

TOLERABILITY

STELFONTA is generally well-tolerated. The majority of reported observations were transient, with no intervention required. Most commonly these observations were transient pain and swelling that were related to the mode of action of STELFONTA in the first few days. Prior to treatment, concurrent medications are required to help minimize potential side effects of tumor destruction by the body.

After treatment with STELFONTA, dogs may require additional care of the treated site to aid in the healing process. An Elizabethan collar or non-constricting dry gauze bandage may be needed to prevent the dog from self traumatizing the treatment site.

STELFONTA® (tigilanol tiglate injection). SEEING IS BELIEVING

The inherent characteristics of STELFONTA via its unique mode of action allow for ease of administration, typically without general anesthesia. The robust efficacy and generally autonomous healing process ultimately supports a rapid return to good quality of life in the vast majority of treated dogs.

1. STELFONTA removes 75% of mast cell tumors with a single treatment.
2. STELFONTA removes 87% of mast cell tumors after either one or two treatments combined.
3. STELFONTA starts to work within hours, with tumors typically destroyed by Day 7.
4. Tumor site heals via second intention, typically within 6 weeks following tumor destruction.
5. 89% of dogs had no tumor recurrence at the treated site at 12 months.

PACKAGE INSERT

STELFONTA® (tigilanol tiglate injection) 1 mg/mL

For intratumoral injection in dogs only
Antineoplastic
Single use vial

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. To decrease the risk of accidental self-injection, sedation of the dog may be necessary (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).**

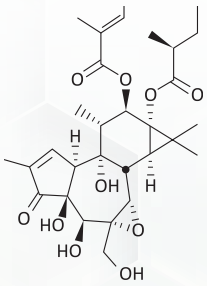
CAUTION

Federal law restricts this drug to use by or on the order of a licensed veterinarian.

DESCRIPTION

The active ingredient for tigilanol tiglate injection is a phorbol ester that activates alpha, beta I, beta II, and gamma isoforms of protein kinase C. The chemical name is (4S,5S,6R,7S,8R,9R,10S,11R,12R,13S,14R)-12-(2E)-2-methylbut-2-enoatyl-13-[(2S)-2-methylbutyroyl]-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tigliaen-3-one. The molecular formula is C30H42O10 and its molecular weight is 562.65 g mol^{-1}. Each mL of STELFONTA contains 1 mg tigilanol tiglate and sterile water for injection (60% v/v), propylene glycol (40% v/v), sodium acetate (<0.1% w/v), and glacial acetic acid (<0.1% w/v).

The chemical structure for tigilanol tiglate is:



INDICATION

STELFONTA injection 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

DOSAGE AND ADMINISTRATION

ALWAYS PROVIDE THE CLIENT INFORMATION SHEET TO THE DOG OWNER BEFORE DOSE ADMINISTRATION.

PACKAGE INSERT – CONTINUED

Concomitant medications

Administer the following medications to decrease the potential for severe systemic adverse reactions from mast cell degranulation:

- **Corticosteroid (e.g. oral prednisone or prednisolone at anti-inflammatory dose):** Start medication 2 days prior to STELFONTA treatment and continue for 8 days post-treatment (10 days total).
- **H1 receptor blocking agent (e.g. oral diphenhydramine):** Start medication on the day of STELFONTA treatment and continue for a total of 8 days.
- **H2 receptor blocking agent (e.g. oral famotidine):** Start medication on the day of STELFONTA treatment and continue for a total of 8 days.

Dosing Instructions

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.

Administration of STELFONTA:

Sedation may be necessary to safely and accurately administer STELFONTA to decrease the chance of accidental self-injection. Wear gloves, eye protection, and lab coat or gown in the preparation and administration of STELFONTA. Care should be taken to restrict injections to the tumor only. STELFONTA should not be injected into the margins, beyond the periphery, or deep to the tumor.

- Shave the tumor site. Avoid manipulation of the tumor.
- Draw the calculated volume of STELFONTA into a sterile Luer-lock syringe with a 23 gauge needle.
- Identify an appropriate injection point on the edge of the tumor. See Figure 1. Insertion of the needle depends on the tumor's location, form, and appearance. If a tumor protrudes above the surface of the skin, insert the needle at an oblique angle of approximately 45°.
- Insert and embed the needle in the tumor through a single injection site and draw the syringe plunger back slightly to ensure STELFONTA is not injected into a blood vessel. While applying even pressure on the syringe plunger, move the needle back and forth in a fanning manner to inject STELFONTA into the tumor. See Figure 1. The drug should fully perfuse the entire tumor.
- When the total dose of STELFONTA has been administered, pause to allow tissue dispersion before removing the needle from the tumor. Pull back on the syringe plunger to create a small negative pressure before removing the needle to minimize leakage from the injection site.
- After the needle is withdrawn, apply light pressure for 30 seconds over the needle exit hole using a gloved finger. If leakage does occur, rinse injection site with saline to wash STELFONTA from the skin surface. Do not re-administer.
- To minimize risk of accidental self-injection, do not recap the needle. Dispose of the needle and syringe.

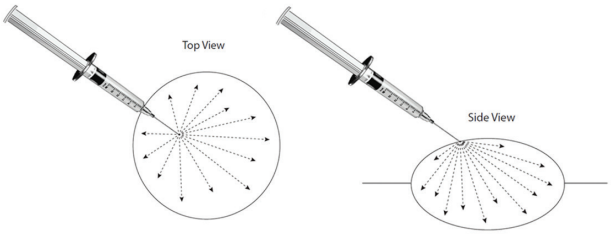


Figure 1: Dispersion of STELFONTA throughout the tumor.

PACKAGE INSERT – CONTINUED

CONTRAINDICATIONS

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 **Adverse Reactions**).

WARNINGS

Human Safety

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

Caution is required during treatment to avoid accidental self-injection. Dogs undergoing treatment with STELFONTA should be adequately restrained and sedation used if necessary. Use a Luer-lock syringe to administer STELFONTA. Do not recap the needle. Accidental self-injection may result in local inflammatory reactions, including swelling, redness and severe wound formation. In case of accidental self-injection, immediately rinse the area with water, 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.

Animal Safety

Dogs should be monitored during and for 5-7 days after intratumoral treatment with STELFONTA for signs of systemic mast cell degranulation such as vomiting, diarrhea, lethargy, anorexia/hyporexia, altered breathing, hypotension, urticaria, edema at or away from the treated site, or bruising at or away from the treated site. If signs are observed, appropriate treatment should be started immediately.

Always administer the recommended concomitant medications (corticosteroids, H1, and H2 receptor blocking agents) with STELFONTA. Death has occurred following mast cell degranulation when these concomitant medications were not administered according to this Package Insert (see **Dosage and Administration** and **Adverse Reactions**).

STELFONTA can induce a substantial local inflammatory reaction which may result in pain, bruising, and swelling. During this time, an analgesic may be needed in addition to the use of corticosteroids and both H1 and H2 receptor blocking agents.

Treatment with STELFONTA causes tumor necrosis which is part of the mechanism of action of the drug. Bruising, heat, pain, and swelling may begin at the site within 2 hours of treatment. By day 7 after treatment, wound formation including full thickness dermal necrosis with exudate, peripheral tissue edema, erythema, skin discoloration, tissue sloughing, and necrotic eschar may occur.

In addition to tumor necrosis, treatment with STELFONTA has been associated with cellulitis and severe tissue sloughing extending away from the treated site resulting in extensive wounds (see **Adverse Reactions**).

Do not inject STELFONTA into normal subcutaneous tissue or adjacent tissues (e.g. beyond tumor margins) because severe edema, erythema and necrosis of the injected tissue may occur.

PRECAUTIONS

STELFONTA has not been evaluated in dogs with signs of systemic disease due to the mast cell tumor(s).

STELFONTA is not intended for the treatment of metastatic mast cell tumors.

The safe and effective use of STELFONTA has not been evaluated for simultaneous treatment of more than one mast cell tumor.

The safe and effective use of STELFONTA has not been evaluated in dogs with a mast cell tumor volume >10 cm^3.

Use STELFONTA with caution in tumors located within mucocutaneous regions (e.g., eyelids, vulva, prepuce, and anus) as tumor necrosis could cause a change in morphology of the mucocutaneous region resulting in loss of functional integrity.

Use STELFONTA with caution in mast cell tumors with significant ulceration as leakage of the drug from the ulcerated area may occur following treatment potentially reducing effectiveness.

The safe use of STELFONTA has not been evaluated in dogs with concurrent diseases that may result in delayed wound healing.

After treatment with STELFONTA, dogs may require additional care of the treated site to aid in the healing process. An Elizabethan collar or a non-constricting dry gauze bandage may be needed to prevent the dog from self-traumatizing the treated site.

After treatment with STELFONTA, separation from other household animals may be necessary to prevent grooming and trauma to the treated site.

The safe use of STELFONTA under conditions of use has not been evaluated in dogs younger than 3.5 years old.

The safe use of STELFONTA has not been evaluated in dogs that are pregnant, lactating, or intended for breeding.

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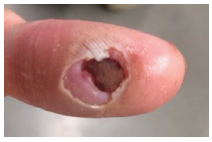
ADVERSE REACTIONS

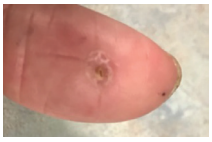
Human Exposure

There was one human exposure during the field 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 Pictures 1 and 2 below. A separate needle stick injury was reported with a maximum potential dose of 0.1 mL tigilanol tiglate into the distal extremity of the left index finger, resulting in a localized burning sensation, local inflammation, bruising, muscular pain up the left arm, and localized tissue necrosis. Muscular pain resolved in the first 12-24 hours and the wound healed in 8 weeks. There have been other needle stick injuries reported, with at least one injection into a thumb, with minimal (stinging, pain, and swelling) to no adverse events associated with these accidental self-injections.

Picture 1. Thirteen days after self-injection



Picture 2. Seventy-four days after self-injection



FIELD STUDY

In a well-controlled, multi-center, randomized, double-masked field study evaluating the effectiveness and safety of STELFONTA for the treatment of cutaneous and subcutaneous mast cell tumors in dogs, 117 dogs treated with STELFONTA and 42 dogs receiving sham treatment (untreated control) were evaluated for safety. Eighty-one dogs were treated with STELFONTA on Day 0. Thirty-six 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 in the Effectiveness section. The adverse reactions during the study are summarized in Table 2 below.

Table 2: Adverse Reactions During the Field Study

Adverse Reaction	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%)
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.

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Adverse reactions were graded using the Veterinary Co-operative Oncology Group – Common Terminology Criteria for Adverse Events (VCOG-CTCAE).[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 use of the required concomitant corticosteroids were similarly reported in STELFONTA and untreated control dogs and included elevated alkaline phosphatase, polyuria, and polydipsia.

Wound Formation

Tumor observations were conducted at 2, 4, 8, and 24 hours and 4 days after treatment. The 81 dogs treated with STELFONTA on Day 0 were reported most frequently with swelling, bruising, pain and heat 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)

At 24 hours post treatment, intact skin was reported in 71.6% (58/81 dogs) of STELFONTA® (tigilanol tiglate injection) treated dogs. On Day 4 intact skin was reported in 17.3% (14/81 dogs) of STELFONTA treated dogs. On Day 4, the following 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)

A wound healing assessment was performed on 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.

Exudate from the treated site including serous, serosanguinous, sanguineous, seropurulent, and purulent discharges were seen mainly on Day 7 and to a lesser extent on Day 14. Sloughing of the treated site was observed from Day 7 to Day 42, 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. Necrotic eschar and epithelialization of the treated site was observed from Day 7 to Day 84, with decreasing frequency after Day 14. Granulation or hyper-granulation of the treated site was observed from Day 7 to Day 84, with decreasing frequency after Day 14.

The average wound size at 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 total wound for a STELFONTA treated dog was reported seven days after treatment. The treated tumor was located on the left caudal stifle and the original tumor size measured 2.4 x 2.1 x 1.4 cm. The wound area initially 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, and 0.5 x 0.7 cm and 2.5 x 2.9 cm 42 days after treatment and were no longer present at 84 days after treatment.

One dog treated with STELFONTA was reported with an extensive wound formation (wound size 25.0 x 9.5 cm) with severe tissue slough (Grade 3) nine days after treatment of a mast cell tumor on the left metacarpal area (original tumor size 2.5 x 1.9 x 1.3 cm). 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. The wound had not fully healed by the end of the study 89 days after treatment. See pictures below comparing progression of this extensive wound formation versus commonly observed wound progression.

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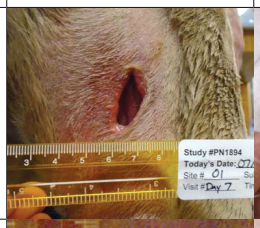
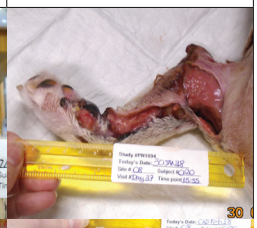
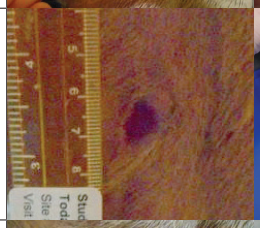
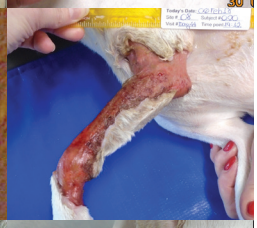
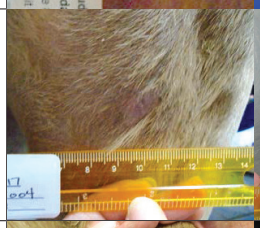
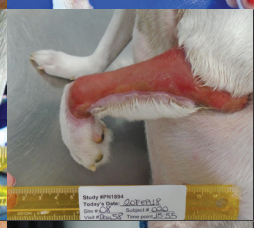
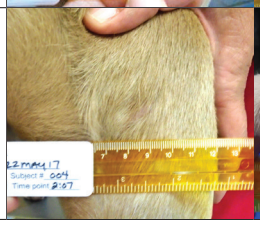
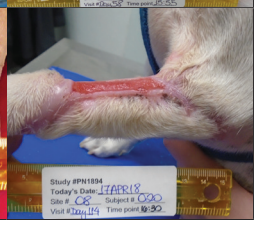
	Typical wound	Extensive wound
Seven Days after treatment		
Fourteen Days after treatment		
Twenty-Eight Days after treatment		
Eighty-Four Days after treatment		

One dog treated with STELFONTA was reported with a bacterial infection and cellulitis in the right rear leg 9 days after treatment of a mast cell tumor 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.

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.

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 Evaluation Criteria in Solid Tumors Guideline (RECIST)[2] response to the initial STELFONTA injection and was re-treated with STELFONTA, 30 days following the initial injection. The patient did not 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 dog died five days following treatment likely due to degranulation of the mast cell tumor and internal necrotic discharge of the tumor.

In a separate pilot field study, one dog with a moderate (2.53 cm^3) subcutaneous mast cell tumor on the left caudal hindlimb was treated with STELFONTA. The dog was treated with chlorpheniramine and meloxicam on treatment day (Day 0) and Day 1 only. The dog did not 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.

To report suspected adverse reactions, to obtain a Safety Data Sheet (SDS), or for technical assistance, call 800-338-3659. For additional information about adverse drug experience reporting for animal drugs, contact the FDA at 1-888-FDA-VETS or www.fda.gov/reportanimalae.

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INFORMATION FOR DOG OWNERS

Owners should be given the Client Information Sheet to read before STELFONTA is administered and should be advised to observe their dog for potential side effects, including signs of degranulation and excessive wound formation, as described in the sheet. Advise dog owners about possible adverse reactions, when to contact a veterinarian, and how to care for the treated tumor site.

Some discharge from the site following treatment is expected. The site can be cleaned with warm water as necessary. Advise owners to wear disposable gloves when cleaning the area.

CLINICAL PHARMACOLOGY

Mechanism of Action

In non-clinical pharmacology studies, tigilanol tiglate has been shown to have three inter-related effects that are responsible for its anti-tumor effectiveness. The first effect is to cause oncolysis of tumor cells that are in direct contact with tigilanol tiglate. The oncolysis occurs within the first hours following treatment and results from the disruption of mitochondrial functioning. Secondly, at the same time, tigilanol tiglate activates a protein kinase C (PKC) signaling cascade which propagates throughout the tumor, resulting in an acute inflammatory response with swelling and erythema extending to the tumor margins and immediate surroundings. This inflammatory response is normal and necessarily contributes to the activity of tigilanol tiglate by (a) restricting blood and oxygen supply to the tumor (causing localized hypoxia) and (b) recruiting and activating innate immune cells (principally neutrophils and macrophages), which then target the tumor and release reactive oxygen species, proteases, and cytokines that function in an antimicrobial role. This acute inflammatory response generally resolves within 48 to 96 hours. The third component of the antitumor activity of tigilanol tiglate is associated with direct effects of the drug in increased permeability of the tumor vasculature (via activation of the Beta-II isoform of PKC) leading to tumor vascular destruction. The resulting outcome is tumor destruction with a deficit or wound remaining where the tumor was located. Complete healing of the resulting wound following tumor destruction by STELFONTA is typically within 6 weeks.

Pharmacokinetics

Pharmacokinetic properties of STELFONTA were evaluated in a pilot study monitoring systemic levels following intratumoral injection, with a dose delivered according to the size of the mast cell tumor. A dose of 0.5 mg/cm^3 (0.5 mL/cm^3) was used in dogs with tumor volumes ranging 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 mg to 3.4 mg per dog. A total of 6 cutaneous and 5 subcutaneous mast cell tumors were treated in 10 dogs (one dog had two tumors treated consecutively). The following range of pharmacokinetic parameters were determined for STELFONTA in plasma: 1) elimination half-life ($t_{½}$): 2.85 to 36.87 hours; 2) maximum plasma concentration (C_{max}): 0.356 ng/mL to 13.8 ng/mL; and 3) area under the plasma concentration time-curve to the last quantifiable plasma concentration (AUC_{last}): 2.25 h*ng/mL to 31.24 h*ng/mL. There was no relationship between drug exposure (C_{max} and AUC_{last}) with tumor location (cutaneous or subcutaneous) or with total dose. In an evaluation of the pharmacokinetic data from the 5 dogs with cutaneous tumors, dose levels 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, doses ranged from 0.049 mg/kg to 0.094 mg/kg. The highest Cmax was 13.8 ng/mL and the highest AUC_{last} was 30.81 h*ng/mL at a dose of 0.094 mg/kg.

EFFECTIVENESS

The effectiveness of STELFONTA was evaluated in a well-controlled, multi-center, randomized, double-masked, field study in client-owned dogs. Enrolled dogs had non-metastatic World Health Organization stages Ia (one tumor confined to the dermis, without regional lymph node involvement) and IIIa (multiple dermal tumors; large infiltrating tumors without regional lymph node involvement) mast cell tumors that were (i) cutaneous, or (ii) subcutaneous and located at or distal to the elbow or the hock). A total of 123 client-owned dogs with a mast cell tumor measuring less than or equal to 10 cm^3 were randomized to treatment with a single injection of STELFONTA (n=81) or untreated control (n=42). On the day of treatment, the average tumor volume was 1.7 cm^3 (range 0.1 to 9.8 cm^3).

A total of 118 dogs were included in the effectiveness analysis; 80 dogs were in the STELFONTA group and 38 dogs were in the untreated control group. Response to treatment was evaluated using the RECIST[2], where complete response (CR) is resolution of the target tumor, partial response (PR) is at least a 30% decrease in the longest diameter of target tumor, stable disease (SD) is a decrease of less than 30% or increase of less than 20% of the longest diameter of the target tumor, and progressive disease (PD) is greater than a 20% increase in the longest diameter of the target tumor.

The primary effectiveness variable compared CR rates of the target tumor between groups 28 days after treatment. At 28 days after treatment, a statistically significantly greater proportion of dogs in the STELFONTA treated group (60/80; 75%) achieved CR compared to dogs in the untreated control group (2/38; 5.3%) ($p<0.0001$). An objective tumor response (CR + PR) was observed in 64/80 (80%) of the STELFONTA treated dogs. Of the 60 dogs in the STELFONTA group that experienced CR at Day 28, response assessment was conducted 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%) were disease-free at the injection site.

For all dogs, corticosteroids (prednisone or prednisolone) were initiated 2 days prior to treatment at a dose of 0.5 mg/kg orally twice daily and continued for 7 days total (2 days before, on the day of treatment and 4 days after treatment), then 0.5 mg/kg once daily for an additional 3 days. An H1 receptor blocking agent (diphenhydramine [2 mg/kg orally twice daily]) and H2 receptor blocking agent (famotidine [0.5 mg/kg orally twice daily]) were initiated on the day of treatment and continued for 7 days.

Other medications prescribed based on veterinary discretion included antibiotics, analgesics, and sedatives. The majority of antibiotics were used to treat injection site infections. The majority of analgesics were used to treat tumor pain and were mainly initiated on the day of or day after treatment. Sedatives were used for treatment administration, conducting diagnostics, anxiety, and temperament issues.

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Quality of Life (QoL)[3] was assessed by owners throughout the study and the mean scores for the QoL assessment was similar between the STELFONTA and untreated control groups at all time points.

Eighteen of the 20 STELFONTA treated dogs without CR received a second treatment. Twenty-eight days following the second treatment, CR was observed in 8/18 (44.4%) of these dogs. Forty-two days following the second treatment, CR was observed in 7/18 (38.9%) of treated dogs.

TARGET ANIMAL SAFETY

The margin of safety and toxicity of STELFONTA was evaluated in one laboratory safety study and one laboratory cardiovascular study utilizing final market formulation, and one pilot field study that used non-commercial formulation.

Laboratory Safety Study

In a 4-week laboratory safety study, 48 healthy Beagle dogs 6 to 8 months old were administered STELFONTA intravenously over a 15-minute infusion once a week for four weeks on Days 1, 8, 15, and 22, at doses of 0, 0.025, 0.05, or 0.075 mg/kg body weight (ranges between 0.02-0.036, 0.039-0.056, and 0.06-0.08 mg/kg, respectively due to dosing variability). Control dogs (0 mg/kg) received a vehicle control at a volume equal to the 0.075 mg/kg dose. The intravenous route was chosen for this study because subcutaneous injection was too toxic and intratumoral administration was not possible.

There were twelve dogs per group (6 male, 6 female). Four dogs/sex/group were necropsied two days following the last dose and two dogs/sex/group were necropsied following a 2-week recovery period.

All dogs survived the study, and there were no STELFONTA-related effects on body weight, body temperature, ophthalmic exam, electrocardiographic parameters, and organ weights.

The following were observed only in dogs in the groups administered STELFONTA: decreased food consumption from Days 22-29, vomiting/retching during infusion or immediately post-infusion, wound formation at the infusion site after the second or third dose, decrease in activity sporadically throughout the study, and elevations in alanine aminotransferase on Day 23.

The following were observed in all groups, including vehicle control and increased in a dose dependent manner: limited use of the leg that received the infusion occurred soon after dosing, weakness after the first dose, salivation and infusion site edema and erythema increased in frequency and severity throughout the study, and tremors occurred immediately post-infusion and increased in severity with dose.

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

Loose feces were observed in all groups in a non-dose dependent manner. Polydipsia occurred in the control, 0.05 and 0.075 mg/kg groups. Trending towards decreasing hematocrit (but still within reference intervals) was observed in all groups. One dog in the 0.05 mg/kg group was mildly anemic during recovery. Monocytosis and elevated fibrinogen were seen on Days 2 and 23 in a dose-dependent manner.

Gross pathology findings at the infusion site included inflammation, redness, and thickening of the skin. Correlative histopathology findings of the infusion site included hemorrhage, edema, inflammation, mixed cell infiltration, fibrosis, and chronic organizing thrombosis. Only one of the recovery dogs had changes at the infusion site consisting of proliferation of the intima. One dog in the 0.075 mg/kg group had a severe wound, confirmed on histopathology as ulcerative inflammation and severe necrosis with bacteria present. Gross pathology findings also included red, mottled, firm, and enlarged lymph nodes in all dose groups, including recovery dogs, confirmed on histopathology as inflammation, lymphoid hypercellularity, hemorrhage, and sinus histiocytosis. Pituitary cysts were observed in 7 dogs in all STELFONTA treated groups. One dog each from the 0.075 mg/kg group was observed to have kidney tubular vacuolation, dilation of the ventricles of the brain, and chronic inflammation of both the left thigh skeletal muscle and left sciatic nerve.

Laboratory Cardiovascular Study

In a 12-day laboratory cardiovascular study, 4 healthy male conscious telemeterized Beagle dogs approximately 2-4 years old were administered STELFONTA as a single intravenous infusion. Treatment consisted of four groups: vehicle control and STELFONTA at doses of 0.01, 0.025 and 0.075 mg/kg body weight. All four dogs received all treatments with at least a 3-day wash-out period.

All dogs survived the study and there were no STELFONTA-related effects on body temperatures, blood pressure, or electrocardiograms. The following were observed only after administration of STELFONTA in all dose groups: salivation, vocalization, incoordination, tremors, red feces, and decreased feces output. Retching, vomiting, incoordination, and changes in activity levels (increased and decreased) occurred in the 0.075 mg/kg group only. Tachycardia was seen for the first 2.5 hours after the 0.075 mg/kg dose only. The following were observed after administration of control or STELFONTA: excessive panting, decreased appetite, and limited usage/swelling of leg or paw. All dogs lost weight during the study. Clinical signs resolved around 4 hours post dosing.

Pilot Field Study

In a 28-day unmasked field study, 10 client-owned dogs, 6-14 years old were administered tigilanol tiglate (non-commercial formulation) once as an intratumoral injection at a dose of 0.5 mg tigilanol tiglate per cubic centimeter (cm^3) of tumor volume, not exceeding 0.25 mg/kg body weight (maximum dose of 5 mg). One dog was enrolled a second time to treat a second mast cell tumor after successful treatment of the first tumor. See pharmacokinetic results from this study under **Clinical Pharmacology**.

The most common observations after tigilanol tiglate administration were injection site reactions including necrosis, swelling (localized edema and edema extending well beyond the tumor injection site), pain, restlessness, inflammation, erythema, bleeding ulcerations, bruising/discoloration, sloughing of tissue, open wound, mild drainage, malodor, and presence of granulation tissue.

PACKAGE INSERT – CONTINUED

Three dogs experienced dermatitis with or without skin necrosis in a region nearby but distinct from the tumor injection site. One dog experienced non-weight bearing lameness, muscle atrophy and enlarged popliteal lymph node. One dog vomited after administration. Three dogs required longer healing times beyond 28 days, with the longest requiring 5 months. Hypoalbuminemia was observed in 5 dogs with hypoproteinemia observed in 1 of these 5 dogs on Day 7 and was resolved by Day 28.

STORAGE INFORMATION

Store STELFONTA vials refrigerated at 2°C to 8°C (35°F to 46°F).

Do not freeze.

Keep the vial in the carton at all times to protect the vial from light.

For single use only.

Dispose of any unused product in accordance with disposal for routine medical waste.

HOW SUPPLIED

STELFONTA is supplied as a sterile, colorless liquid in a 5 mL clear, single-use glass vial containing 2 mL of STELFONTA at a concentration of 1 mg/mL tigilanol tiglate in sterile water for injection.

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CLIENT INFORMATION SHEET

The Client Information Sheet contains important information about STELFONTA®. You should read this information before your dog is treated with STELFONTA. This sheet is provided only as a summary and does not take the place of instructions from your veterinarian. Talk with your veterinarian if you do not understand any of this information or if you want to know more about STELFONTA.

Your veterinarian has decided to include STELFONTA as a part of your dog's treatment plan for a mast cell tumor. Be sure to speak with your veterinarian about all parts of your dog's treatment plan.

What is STELFONTA?

- STELFONTA is a drug used to treat mast cell tumors, a common form of cancer that affects dogs.
- The active ingredient in STELFONTA is tigilanol tiglate, a substance that works by:
  - » Breaking down the tumor cell walls
  - » Disrupting blood vessels in the tumor
  - » Destroying the tumor forming a "pocket" or wound where the tumor was

What should I tell my veterinarian before my dog is treated with STELFONTA?

- Tell your veterinarian about all other medications your dog is taking, including prescription drugs, over the counter drugs, flea and tick medications, heartworm and deworming medications, and vitamins and supplements (including herbal or homeopathic products).
- Tell your veterinarian about your dog's previous or current medical conditions, including any infection.
- Tell your veterinarian if your dog is pregnant, is nursing puppies, or is intended for breeding purposes.

PACKAGE INSERT – CONTINUED

How is STELFONTA given to my dog?

- Your veterinarian will inject your dog's tumor with STELFONTA. The injection will be given at the veterinary clinic. Your dog may need to be sedated during the procedure.

How will STELFONTA affect my dog?

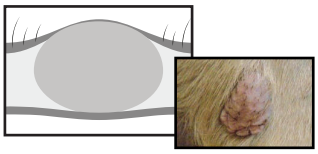
- STELFONTA is used to treat a mast cell tumor on your dog. It can be difficult to predict how your dog's tumor will respond to STELFONTA.
- A wound will form where STELFONTA was administered. It is difficult to predict the size and severity of the wound formed. See the diagrams below for more information.

What is the treatment and healing process?

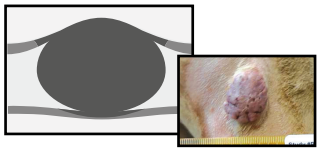
- Tumors treated with STELFONTA typically go through a 4- to 6-week treatment and healing process, as shown in the following diagrams. The healing process may take longer in some dogs.
- During the treatment and healing process, your dog may require additional care of the treated tumor site to aid in the healing process.

Less Than 4 Hours After Treatment:

DAY 1: PRE-TREATMENT



2-4 HRS POST-TREATMENT

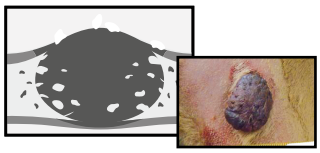


Start of Tumor Breakdown

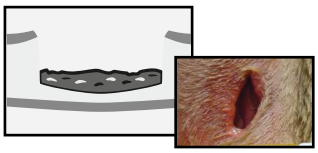
Within the first few hours following treatment with STELFONTA, the cells in the tumor and tumor blood vessels will begin to break down. You will be able to see a change in the color of the tumor. At the same time there is usually swelling at the treated tumor site.

1 – 7 Days After Treatment:

24 HRS POST-TREATMENT: Tumor Breakdown evident



Day 7: Tumor Breakdown with Formation of Healthy Wound Bed

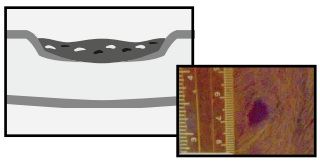


Continued Tumor Breakdown

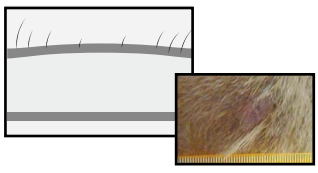
The treated tumor site will become blackened. The skin over the surface of the tumor may breakdown and fluid may leak from the tumor. Swelling of the treated tumor site may continue causing some discomfort to your dog through this stage. Your veterinarian can prescribe pain medication to help your dog through this period if required. As the tumor breaks down there will be a 'pocket' or wound where the tumor once was. A healthy wound bed will be seen, reddish in color, which will allow healthy new skin to grow.

7 – 42 Days After Treatment:

DAY 14: Treated Tumor Site Healing



DAY 28: Treated Tumor Site Healed



Wound Resolution

Healthy new skin will grow and close over the pocket or wound where the tumor once was. In many dogs, the hair will regrow and skin will return to its original color.

What are some possible side effects of STELFONTA® (tigilanol tiglate injection)?

- STELFONTA may cause side effects, even at the prescribed dose. These side effects include, but are not limited to:
  - » During the first days after treatment, you may see bruising or swelling around the treated tumor site. The swelling may cause your dog some discomfort and pain for several days after treatment. Your dog may seem tired during this time and may eat less.
  - » In some cases, a severe, larger than normal wound may develop, delaying wound healing. Your veterinarian will assess if your dog requires additional treatments during this time (e.g. bandages, Elizabethan collar).
- Other side effects may occur. For more information about side effects ask your veterinarian.

PACKAGE INSERT – CONTINUED

Contact your veterinarian if you notice any of the following changes in your dog:

- Excessive pain or lameness (limping)
- Tiredness or refusal to eat for more than 1 day
- Repeated vomiting or diarrhea
- Trouble breathing
- Changes to the treated tumor site, including increased or excessive swelling and bruising, extensive wound formation, or increased irritation
- Any other symptoms that your dog may show that concern you.

What do I need to know to safely care for my dog before and after treatment with STELFONTA?

- Your veterinarian will prescribe medications to decrease the potential for severe reactions that can occur during the treatment process. **It is essential that you give the medications as prescribed.**
- The treated tumor site is typically left uncovered. In some cases, your veterinarian may decide to cover the treated tumor site with a bandage.
- Some discharge from the treated tumor site following treatment is expected. The treated tumor site can be cleaned with warm water as necessary. Wear disposable gloves when cleaning the treated tumor site.
- If your dog is licking or rubbing the treated tumor site, contact your veterinarian. Your veterinarian may recommend an Elizabethan collar ("e-collar") or a bandage to cover the wound.
- If another animal in the household is licking or grooming the treated tumor site, the animals should be separated to prevent trauma to the area.

What precautions do I need to take when caring for my dog before and after treatment with STELFONTA?

- Thoroughly wash any skin that comes in contact with the treated tumor site, wound, wound discharge, or material contaminated with wound discharge (e.g. bedding).
- Do not wash any items soiled with wound discharge with other laundry.

Is there more information?

- This client information sheet gives the most important information about STELFONTA. For more information about STELFONTA, please talk with your veterinarian.

To report a suspected adverse reaction (side effect) call 1-800-338-3659. For additional information about adverse drug experience reporting for animal drugs, contact the FDA at 1-888-FDA-VETS or online at http://www.fda.gov/reportanimalae.

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