SMALL ANIMAL

Using triamcinolone in combination with the investigational anticancer agent EBC-46 (tigilanol tiglate) in the local treatment of a canine subcutaneous mast cell tumour

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Case Report

Abstract

Here we describe the use of a combination of the corticosteroid triamcinolone and the novel anticancer agent EBC-46 (tigilanol tiglate) to effectively treat a large subcutaneous mast cell tumour (MCT) in an 8-year-old female desexed Staffordshire Bull terrier cross. The patient presented with a large focal swelling (399 cm^3) on the lateral aspect of the right hind leg proximal to the stifle joint. The mass was reported as having being present for approximately 4 years, but had rapidly increased in size in the preceding 6 months. A fine needle aspirate confirmed the lesion was a subcutaneous MCT. In our opinion, this tumour was not amenable to surgical removal in its enlarged state and was therefore administered intratumoural triamcinolone at 2-weekly intervals in an attempt to decrease the mass prior to tumour removal using EBC-46 (tigilanol tiglate) Injection. EBC-46 is an investigational drug purified from the seed of a native Australian rainforest plant which has shown significant potential as a local intratumoural treatment for a range of solid surface tumours in domestic animals. The MCT volume markedly decreased in size by more than 98% in response to 3 fortnightly triamcinolone injections. EBC-46 Injection was then delivered intratumourally into the lesion and effectively destroyed the tumour within 7 days of treatment. No sedation or anaesthesia was required, and EBC-46 Injection was well tolerated by the patient. The 'wound' remaining after tumour destruction healed rapidly. This case shows both the value of triamcinolone in substantially reducing the volume of a large MCT and illustrates the potential for a future low-intervention therapy combining triamcinolone with EBC-46 in treating MCT. EBC-46 Injection is currently being evaluated under an Investigational New Animal Drug (INAD) with the Food and Drug Administration - Centre for Veterinary Medicine (FDA-CVM) in the US where a Pivotal Field Efficacy study treating MCT in dogs is underway. The drug is also being evaluated under an Animal Testing Certificate (ATC) with the Veterinary Medicines Directorate (VMD) in the UK where a Pivotal Field Efficacy study treating soft tissue sarcomas (STS) in dogs is underway.

Background

Canine MCT is a common neoplastic disease in dogs accounting for 16-21% of all cutaneous neoplasms (Bostock, 1986; Rothwell *et al.* 1987). Canine cutaneous MCT originate in the dermis and may extend into the subcutis, but there is also a subset completely surrounded by adipose tissue with no follicular or epidermal involvement (Thompson *et al*, 2011). These are termed subcutaneous MCTs and histologically, have been described with 3 growth patterns: circumscribed, combined or infiltrative. Thompson *et al* (2011) describe the infiltrative growth pattern as that in which the tumour lacks demarcation, is non-encapsulated, and is infiltrative within the surrounding fat. In a study of 306 primary canine subcutaneous MCT, those that were histologically described as infiltrative represented 53% of tumours; with an associated mortality rate over 3 fold higher ($P = 0.02$) than those dogs identified with well-circumscribed tumours (Thompson *et al,* 2011). Although general consensus indicates that subcutaneous MCT are more effectively controlled by surgery than cutaneous tumours, the high likelihood of poor demarcation means that a wide reactive zone requires an aggressive surgical margin (Pratschke, 2015). When this

is not possible one, or a combination of, chemotherapy, radiotherapy or cytoreductive surgery is undertaken (Withrow and Vail, 2007). These therapeutic modalities are often costly, labour and time-intensive, and may not be readily available to dogs because of client preferences or lack of access to certain treatment modalities.

MCT can give rise to paraneoplastic disease associated with the release of bioactive substances from mast cell granules (degranulation) (Fox *et al,* 1990; Ishiguro *et al,* 2003, Harðardottir *et al,* 2015). These substances include histamine, heparin and proteolytic enzymes. In addition, a number of proinflammatory and tissue destructive enzymes may be released (Pimental *et al,* 2011). These bioactive substances can cause oedema, ulceration, and swelling at the tumour site, and possibly delayed wound healing and local coagulation abnormalities (Blackwood *et al,* 2012). In rare cases, a massive release of histamine from neoplastic mast cells can result in an acute anaphylactoid reaction (Blackwood *et al,* 2012). Neoadjuvant therapy using corticosteroids such as prednisolone (prednisone), triamcinolone and dexamethasone, have been shown to modulate and decrease tumour size (McCaw *et al,* 1994; Stanclift *et al,* 2008). It is widely reported that prior to removal, MCTs can be reduced in size by using intratumoural triamcinolone at a dose level of 1 mg per cm longest tumour diameter, repeated at 2-weekly intervals (Hilton, 2005). Some of this observed size reduction may be due to a decrease in tumour-associated oedema associated with mast cell degranulation (London, 2008). Corticosteroids also protect the body from histamine, heparin, and proteolytic enzymes that are released when mast cells degranulate. In addition, with a number of proinflammatory and tissue destructive 'mediators' in mast cell granules, corticosteroids may stabilize these granules and reduce mast cell mediator production (Pimental *et al,* 2011; London, 2008).

EBC-46 (tigilanol tiglate) is a small diterpene ester extracted from the seed of a native Australian rainforest plant, *Fontainea picrosperma* (family Euphorbiaceae). Preclinical studies in mice and case study treatment of dogs with surface tumours including MCT and other round cell tumours, skin and subcutis STS (as defined by Dennis *et al,* 2011), oral melanomas and squamous cell carcinomas, have demonstrated that EBC-46 has significant potential as a local antineoplastic treatment. EBC-46 is a protein kinase C (PKC) activator that primarily targets tumour vasculature and results in rapid tumour destruction through haemorrhagic necrosis followed by good functional and cosmetic healing (Boyle *et al,* 2014). Treatment of MCT with EBC-46 Injection does not usually require general anaesthesia or sedation (an important consideration as many MCT patients are aged and/or compromised).

Our aim was to investigate the intratumoural use of triamcinolone acetonide to modulate and decrease tumour size in a dog with a large subcutaneous MCT prior to tumour removal by local treatment with EBC-46 Injection. The dog's owner provided written consent for the treatment to proceed, and treatment was in accordance with the DAF (Queensland) Animal Ethics Committee (AEC) Project Authority CA 2015/09/904, the Australian Pesticides and Veterinary Medicines Authority (APVMA) small-scale trials permit PER7250 (experimental drug use), and standard veterinary care.

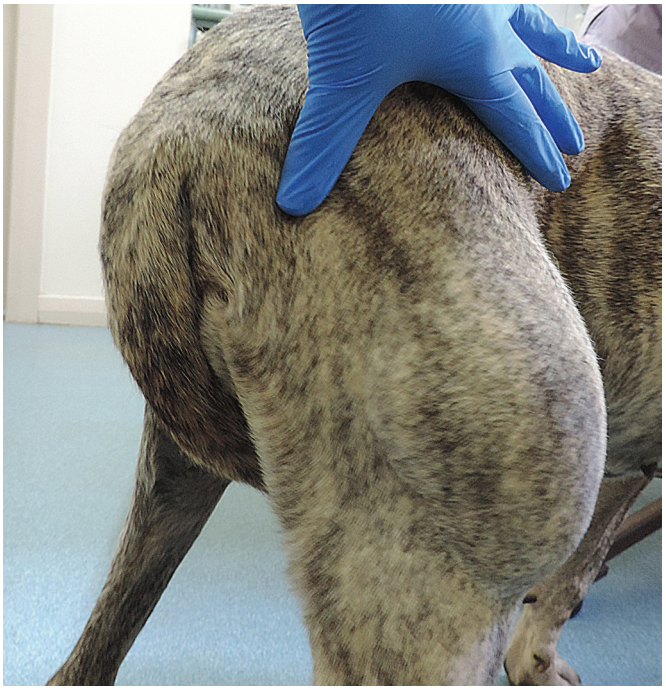

Figure 1. Day -43. Swelling of the lateral thigh region of an 8 year old female Staffordshire terrier cross at first presentation. Tumour volume = 399.4 cm³.

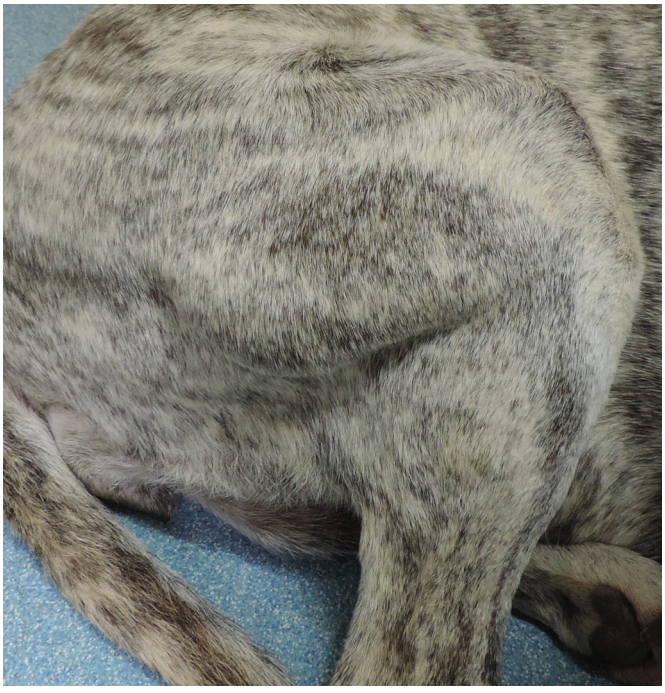

Figure 2. Day -14. The affected limb 14 days after two triamcinolone injections were delivered a fortnight apart. Note the flaccid appearance of the tumour. Tumour volume = 20.9 cm³.

Clinical investigation

An 8-year-old female desexed Staffordshire Bull terrier cross was presented with a focal swelling on the lateral aspect of the right hind leg proximal to the stifle joint (*Figure 1*, Day -43). The mass was reported as present for approximately 4 years, but had rapidly increased in size in the preceding 6 months. There was no lameness associated with the swelling and the dog was otherwise well.

The mass was firm and oedematous on palpation, but no heat or pain was associated with the swelling. Fine needle aspiration of the tumour mass confirmed a substantial population of well-differentiated mast cells, and a diagnosis of MCT was made. To examine for metastatic disease, a thorough palpation for regional (sentinel) lymph node (LN) presence and/or enlargement was conducted (Pratschke, 2015). No regional LNs were palpable and thus, were not amenable to fine needle aspiration for further metastatic investigation (Warland *et al*, 2012; Garrett, 2014). Evidence from a recent survey of 220 dogs with skin MCT indicated that no dog had, or developed, distant metastasis in the absence of LN involvement. This offered some reassurance for the absence of metastatic disease in this case. Cost prevented other traditional staging procedures such as abdominal ultrasound. However, it has also been stated recently that abdominal ultrasound rarely finds evidence of MCT metastasis (Garrett, 2014). In the opinion of the attending veterinarian, this tumour was not considered amenable to surgical removal in its enlarged state and was therefore administered intratumoural triamcinolone at 2-weekly intervals in an attempt to decrease the mass prior to tumour removal using EBC-46 Injection (Day 0).

Triamcinolone pre-therapy

The dog first presented for triamcinolone injection (triamcinolone acetonide 6 mg/mL; TRIAMOLONE FORTE; Jurox Pharmaceuticals; Batch #H0756) on Day -43. Intratumoural triamcinolone was dosed at 1 mg per cm longest tumour diameter (Hilton, 2005). To maximize the tumour perfusion of the drug, triamcinolone was injected in a fanning manner (Celikoglu *et al*, 2008) throughout the mass. No sedation or anaesthesia was required, and the dog tolerated the drug well. Tumour response was measured by assessment of tumour volume using the modified ellipsoidal calculation = ½ x length (cm) x width (cm) x depth (cm) (Monga *et al*, 2000; Celikoglu *et al*, 2008). Tumour dimensions were measured using digital calipers. Tumour length (cm) was designated as the longest measurable diameter; the width (cm) was the tumour at the widest point perpendicular to the length; gentle manipulation of the MCT to measure the depth (cm) was considered acceptable. A summary of tumour volume response to triamcinolone and clinical parameters recorded at the time of revisit is presented in *Table 1*.

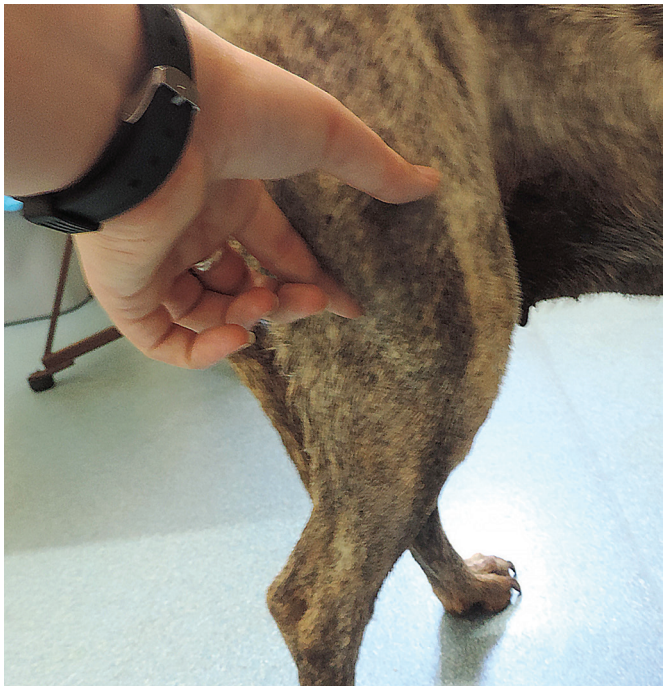

Figure 3. Day -2. The affected limb 12 days after a third triamcinolone injection. Tumour volume = 5.2 cm³.

On Day -43, the longest dimension (length) was recorded as 14.5 cm, which according to the recommended dose level (Hilton, 2005) would require a triamcinolone dose of 14.5 mg. In our opinion, this dose seemed excessive (>0.5 mg/kg Bwt) and it was not known how it would be tolerated by the dog. Therefore, a more conservative initial approach was adopted and a dose of 10.2 mg triamcinolone (1.7 mL)

Study Day	Triamcinolone acetonide dose level (mg)	Tumour dimensions			Tumour Volume (cm³)	Clinical Parameters			
		l (cm)	w (cm)	d (cm)		Bwt (kg)	Temp (°C)	HR/min	RR/min
-43	10.2	14.5	9.9	5.6	399.4	27.7	38.3	124	36
-28	10.2	10.3	5.4	3.2	89.0	26.3	38.0	120	36
-14	7.3	7.3	2.6	2.2	20.9	25.7	38.4	80	24
-2	Nil (pre-EBC -46 Injection)	2.4	2.2	2.0	5.2	26.5	38.1	88	32

EBC-46 Injection = EBC-46 (tigilanol tiglate) Injection; l = length, w = width, d = depth; Bwt = bodyweight; Temp = rectal temperature; HR = heart rate; RR = respiratory rate.

Table 1. Response to triamcinolone and clinical parameters on each clinic visit

was injected (0.36 mg/kg Bwt). The subsequent 2 doses approximated the recommended dose level (refer to *Table 1* for dose rates and timing). The owner reported improvement in general demeanour and mobility of the dog. At Day -14, the tumour had been reduced to a smaller two-textured mass compromising a more solid core surrounded by a flaccid oedematous pouch *(Figure 2,* Day -14). This 'pouch' formation may have been due to reduced oedema within the infiltrative section of a MCT displaying a combined growth pattern (Thompson *et al,* 2011); the solid core representing the well-circumscribed component of the tumour. The tumour volume decreased in size from 399.4 cm^3 to 5.2 cm^3 (98.7% reduction) over a 6 week period *(Figure 3,* Day -2).

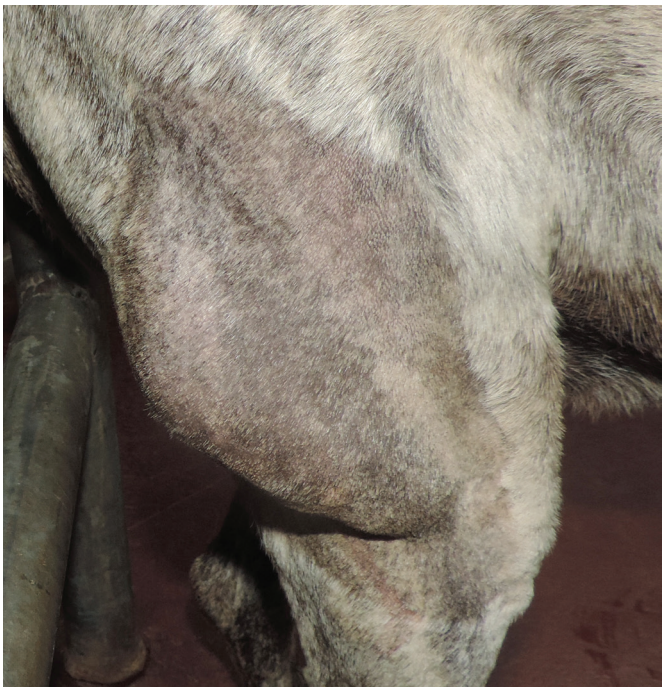

Figure 4. Day 1 post EBC-46 treatment. Localised swelling evident at the injection site.

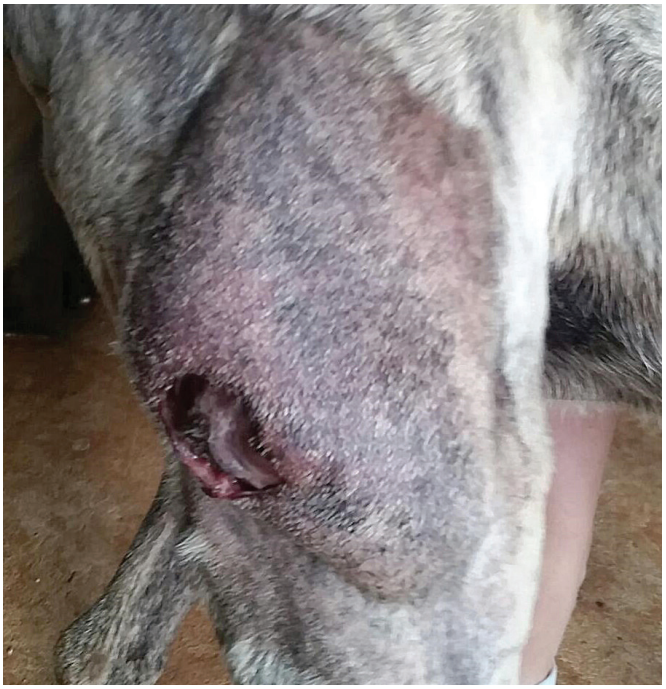

Figure 5. Day 4 post EBC-46 treatment. Tumour destruction evident with crater appearance and some remnant necrotic material visible.

EBC-46 Injection treatment

EBC-46 Injection dosing is calculated according to tumour size, and was delivered into the tumour at 0.4 mL per cm^3 of tumour volume (40% v/v tumour) as determined on the day of dosing (Day 0) equating to a dose of 4.1 mg EBC-46. Tumour volume was determined using the modified ellipsoidal calculation. On Day 0, the tumour had increased slightly in size from the Day -2 measure (now 10.3 cm^3). The tumour and a 5 cm border surrounding the tumour was shaved prior to treatment. EBC-46 Injection was delivered to the tumour in a single injection and fanning manner similar to that used for the triamcinolone treatment previously mentioned. No sedation or anaesthesia was required and the dog tolerated the injection well.

Determination of efficacy was based on objective tumour measurements made according to the Response Evaluation Criteria in Solid Tumours (RECIST) v.1.1 guideline (Eisenhauer *et al,* 2009) using the longest unidirectional tumour measurement (diameter). Measurements were recorded using digital calipers at pre-treatment and at 7, 14, 21, 28 and 42 days post treatment. Response to therapy was defined as complete response (CR, resolution of the target lesion), partial response (PR, at least 30% decrease in the longest diameter of target lesion), stable disease (SD, decrease in the target lesion of less than 30% or increase of the target lesion less than 20%) or progressive disease (PD, greater than 20% increase in the target lesion). Digital still colour images of the injection site were also collected and can be found in *Figures 4 to 9.* Check-ups for continued health and absence of tumour recurrence were conducted on Day 104 and lastly on Day 151 (14 and 21 weeks post treatment respectively) after which the dog was not available for follow up.

Clinical parameters such as bodyweight (kg), rectal temperature (°C), heart rate (HR) per minute, respiratory rate (RR) per minute, general demeanour and body function (e.g. eating, drinking, urine and stools), were recorded immediately pre-treatment, at approximately 2.5 and 24 hours post-treatment, and at 7, 14, 21, 28 and 42 days post treatment.

Prednisolone (MACROLONE 20; Mavlab Pty Ltd), an antihistamine (chlorpheniramine (IRAMINE 8mg; Mavlab Pty Ltd), and famotidine (FAMOTIDINE 20mg; Provet Pty Ltd) were considered mandatory co-therapy for managing potential paraneoplastic events resulting from mast cell degranulation *(see Table 2).* Prednisolone was dosed at 0.4 mg/ kg BW b.i.d. for 5 days (Day -1 to Day 3), then 0.4 mg/kg BW s.i.d. (am) for 3 days (Day 4 to Day 6). Chlorpheniramine and famotidine were dosed at 0.3 mg/kg BW and 0.4 mg/kg BW per os b.i.d. respectively for 7 days (Days 0 to Day 6). To manage anticipated and expected transient local pathology for tumour site necrosis following EBC-46 Injection treatment (localised

Drug	D-1		D0		D1		D2		D3		D4		D5		D6	
	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
Prednisolone	•	•	•	•	•	•	•	•	•	•	•	×	•	×	•	×
Chlorpheniramine	×	×	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Famotidine	×	×	•	•	•	•	•	•	•	•	•	•	•	•	•	•

D-1 = Day -1; D0 = EBC-46 Injection treatment day; • = Treatment; × = No treatment

Table 2. Mandatory co-therapy schedule.

Study Day	Activity / Observations	Tumour dimensions			Tumour Volume (cm^3)	Clinical Parameters			
		l (cm)	w (cm)	d (cm)		Bwt (kg)	Temp (°C)	HR/min	RR/min
0	Pre-treatment	–	–	–	–	25.9	38.4	104	24
0	EBC-46 Injection treatment (4.1 mg; 40%v/v)	4.3	2.67	1.8	10.3	–	–	–	–
0	2h post-treatment	–	–	–	–	25.8	38.2	96	32
1	24h post-treatment	–	–	–	–	26.1	38.2	104	Panting
7	7d post-treatment	0	0	0	0	25.5	40.2	120	36
14	14d post-treatment	0	0	0	0	25.4	38.6	88	24
21	21d post-treatment	0	0	0	0	25.5	38.2	96	24
28	28d post-treatment	0	0	0	0	25.5	38.5	128	36
42	42d post-treatment	0	0	0	0	25.4	38.7	104	36
104	Check-up	0	0	0	0	Site resolved – no tumour recurrence			
151	Check-up	0	0	0	0	Site resolved – no tumour recurrence			

l = length, w = width, d = depth; Bwt = bodyweight; Temp = rectal temperature; HR = heart rate; RR = respiratory rate.

Table 3: Response to EBC-46 Injection and clinical parameters on each clinic visit

bruising, swelling and pain), supportive analgesia is sometimes required. In this case, tramadol hydrochloride (TRAMAL SR Tablets 50 mg; bioCSL Pty Ltd) was given orally at a dose level of 1 mg/kg bodyweight b.i.d for 7 days (Days 0 to 6).

A summary of tumour response to EBC-46 Injection and clinical parameters recorded at the time of revisit are presented in *Table 3.*

Previous work with EBC-46 Injection has demonstrated exceptional *in vivo* wound healing following destruction of solid surface tumours in domestic animals (Campbell *et al,* 2014). Wounds are typically left open for exposure to ambient air over the course of healing (Sano *et al,* 2012; Sano and Ichioka, 2014) and healing is characterized by rapid granulation tissue development and enhanced re-epithelialisation. In this case, the wound remained uncovered throughout the healing process and an Elizabethan collar was not required. The dog resided on a North Queensland dairy property with unrestricted access to soil, pasture, ruminant faecal material, and outdoor water sources.

Through the first 24 hours, the dog presented with normal demeanour ('bright and happy') and clinical signs were unremarkable. In typical response to EBC-46 Injection action, localised bruising, swelling and pain were seen at the injection site from the resulting disruption of tumour vasculature and a localised inflammation *(Figure 4,* Day 1). The dog developed a slight limp on Day 1 due to the local inflammatory action of EBC-46. Reports from the owner indicated the dog remained in good health through Days 2 to 6 *(Figure 5,* Day 4). No viable tumour tissue remained on the Day 7 clinic visit. The lesion had a typical crater appearance with remnant necrotic material present. The wound crater also contained dirt, gravel and mucopurulent material as a result of unrestricted access by the patient to soil, pasture, ruminant faecal material, and outdoor water sources on its home dairy property. The dog was observed to be 'a bit quiet' with a slightly elevated temperature (40.2°C) and an enlarged popliteal LN resulting from an infected site due to foreign material. The wound was flushed and the dog given an amoxycillin/clavulanic acid injection (12.5 mg/kg BW) (NOROCLAV INJECTION; Norbrook), and sent home with a 5 day oral course of amoxycillin/clavulanic acid (12.5 mg/kg b.i.d.) (AMOXYCLAV

500mg; Apex Laboratories Pty Ltd) *(Figure 6,* Day 8). The dog recovered quickly, the wound continued to heal rapidly, lymph node swelling subsided, and the dog presented in normal ('bright and happy') demeanor on Day 14 clinic visit *(Figure 7,* Day 14). On Days 21, 28 and 42 clinic visits, the dog continued to improve with rapid wound closure. A complete response (CR) was recorded on Day 28, and the wound continued to resolve *(Figures 8 and 9,* Days 28 and 42 respectively).

Concluding remarks

This case provides a further example of the use of triamcinolone to significantly reduce the size of skin MCT on the dog (Hilton, 2005; London, 2008). The event was a large subcutaneous MCT on the lateral thigh and triamcinolone treatment made it more amenable to removal by surgery or other approaches. This case also demonstrates the ability of the investigational anticancer agent EBC-46 Injection to successfully achieve a local and precise removal of a subcutaneous MCT following reduction by triamcinolone.

This treatment had a low impact on the dog. General anaesthesia, sedation and/or local anaesthesia were not required. EBC-46 Injection effectively destroyed the tumour within 7 days, with rapid healing of the site, and no tumour recurrence when last assessed at 21 weeks post treatment.

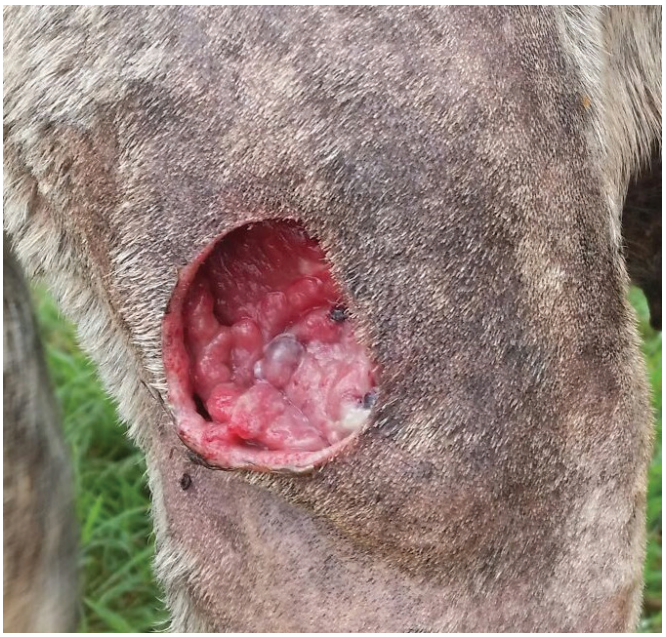

Figure 6. Day 8 post EBC-46 treatment. A clean, well demarcated wound, no necrotic material visible. The wound was flushed on Day 7 due to contamination with foreign material from unrestricted farmyard access.

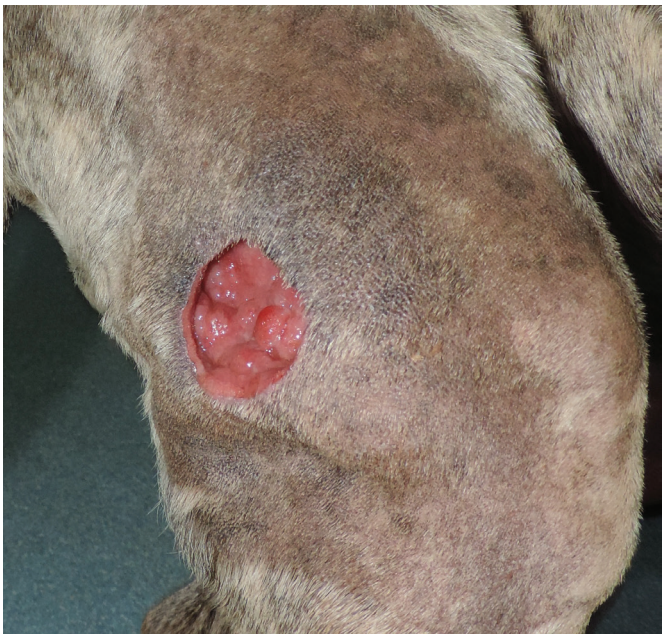

Figure 7. Day 14 post EBC-46 treatment. Good in-fill of wound crater with rapid granulation tissue development.

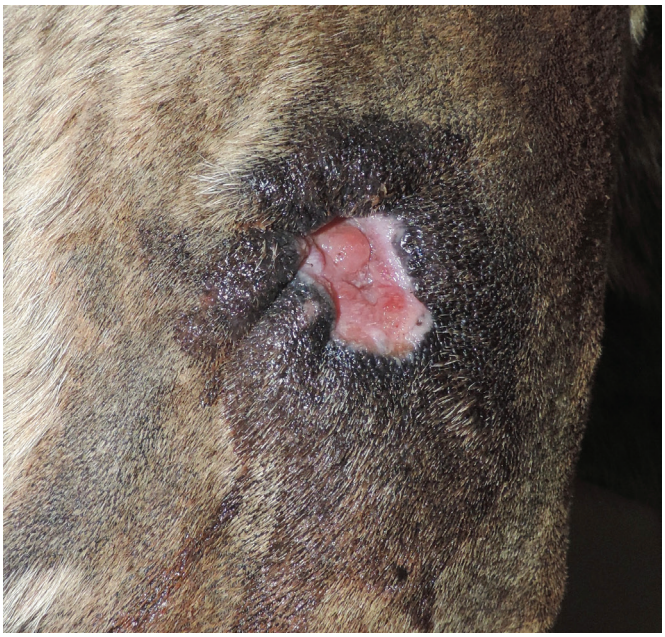

Figure 8. Day 28 post EBC-46 treatment. Wound resolving with enhanced re-epithelialisation over a granulation tissue bed. Confirmation of tumour removal - complete response (CR; RECIST).

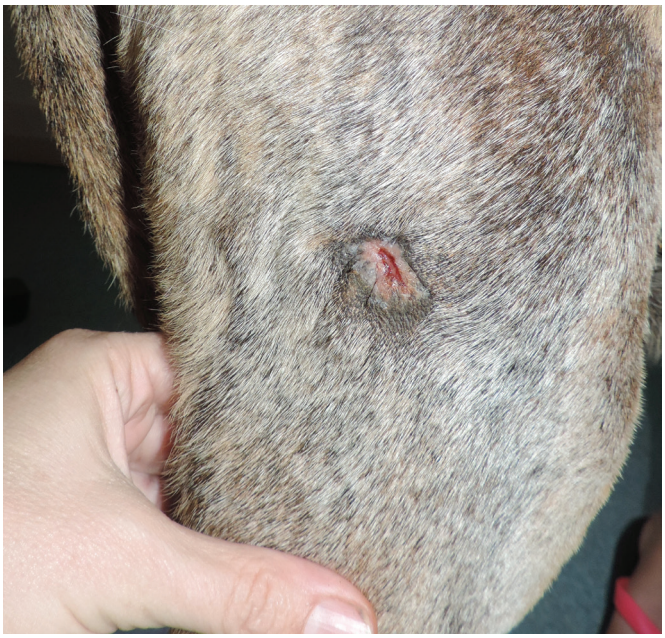

Figure 9. Day 42 post EBC-46 treatment. Wound almost completely resolved.

Intratumoural treatment with EBC-46 Injection does not usually require wound intervention. In treatment of 62 MCT cases by the lead author, only 11 wounds have been prescribed prophylactic or supportive antibiotics. All wounds have healed uneventfully in ambient air. It was disappointing that the wound in this case became infected due to unrestricted farmyard access.

EBC-46 Injection is currently being evaluated under an INAD with the FDA-CVM in the US where a Pivotal Field Efficacy Study treating MCT in dogs is currently underway. EBC-46 Injection is also being evaluated under an ATC with the VMD in the UK where a Pivotal Field Efficacy Study treating STS in dogs is currently underway. Both of these applications protect and promote animal health and welfare by assuring the safety, quality and efficacy of veterinary medicines before they are approved and go to market. The objective is to advance the product through this formal application and registration process as quickly as is feasible in order to make it widely available to the veterinary community.

References

Blackwood, L., Murphy, S., Buracco, P., De Vos, J.P., De Fornel-Thibaud, P., Hirschberger, J., Kessler, M., Pastor, J., Ponce, F., Savary-Bataille, K. and Argyle, D.J. (2012) European consensus document on mast cell tumours in dogs and cats. *Vet Comp Oncol:* doi:10.1111/j.1476-5829.2012.00341.x

Bostock, D.E (1986) Neoplasms of the skin and subcutaneous tissues in dogs and cats, *Br Vet J* 142: 1-19.

Boyle, G.M., D'Souza, M.M.A, Pierce, C.J., Adams, R.A., Cantor, A.S., Johns, J.P., Maslovskaya, L., Gordon, V.A., Reddell, P.W. and Parsons, P.G. (2014) Intralesional injection of the novel PKC activator EBC-46 rapidly ablates tumors in mouse models, *PLoS ONE* 9(10): e108887. doi:10.1371/journal.pone.0108887, 2014.

Campbell, J., Miller, J., Blum, A., Toole, S., Ayerbe, J., Verning, M., Poulos, C., Boyle, G., Parsons, P., Moses, R., Steadman, R., Moseley, R., Schmidt, P., Gordon, V. and Reddell, P. (2014) Excetional *in vivo* wound healing following destruction of cutaneous and subcutaneous tumours in domesticated animals treated with the novel epoxy-tigliane drug EBC-46. *Wound Rep Regen* 22: A76.

Celikoglu, F., Celikoglu, S.I., and Goldberg, E.P. (2008) Techniques for intratumoural chemotherapy of lung cancer by bronchoscopic drug delivery. *Cancer Therapy* 6:545-552.

Dennis, M.M., McSporran, K.D., Bacon, N.J., Schulman, F.Y., Foster, R.A., and Powers, B.E. (2011) Prognostic factors for cutaneous and subcutaneous soft tissue sarcomas in dogs. *Vet Pathol* 48: 73-84.

Eisenhauer, E.A., Therase, P., Bogaerts, J., Schwartz, L.H., Sargent, D., Ford, R., Dancey, J., Arbuck, S., Gwyther, S., Mooney, M., Rubinstein, L., Shankar, L., Dodd, L., Kaplan, R., Lacombe, D. and Verweij, J. (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1), *Eur J Cancer* 45(2): 228-247.

Fox, F.E., Rosentahl, R.C., Twedt, D.C., Dubielzig, R.R., MacEwen, E.G. and Grauer, G.F. (1990) Plasma histamine and gastrin concentrations in 17 dogs with mast cell tumors. *J Vet Intern Med* 4(5):242-246.

Garrett L.D. (2014) Canine mast cell tumours: diagnosis, treatment and prognosis. *Veterinary Medicine Research and Reports* 4(5): 49-58.

Harðardottir, H., Yool, D., Lawrence, J., and Duncan, J. (2015) Management of an anaphylactoid crisis due to mast cell degranulation in a dog during general anaesthesia. *Vet Rec Case Reports* 3(1): doi: 10.1136/vetreccr-2015-000177

Hilton, R. (2005) Canine and feline cutaneous mast cell tumours: a clinical update. *The Veterinarian*. Oct 2005: 39- 44.

Ishiguro, T., Kadosawa, T., Takagi, S., Kim, G., Ohsaki, T. and Bosnakovski, D. (2003) Relationship of disease progression and plasma histamine concentrations in 11 dogs with mast cell tumors. *J Vet Intern Med* 17(2):194 - 198. doi: 10.1111/j.1939-1676.2003.tb02433.x

London, C. (2008) Management of canine mast cell tumours. *Proceedings of the Southern European Veterinary Conference & Congreso Nacional AVEPA,* Barcelona, Spain

McCaw, D.L. Miller M.A., Ogilvie G.K., Withrow, S.J., Brewer Jr., W.G., Klein, M.K., Bell, F.W. and Anderson, S.K. (1994) Response of canine mast cell tumours to treatment with oral prednisone. *J Vet Intern Med.* 8: 406-408.

Monga, S.P., Wadleigh, R., Sharma, A., Adib, H., Strader, D., Singh, G., Harmon, J.W., Berlin, M., Monga, D.K. and Mishra, L. (2000) Intratumoural therapy of cisplatin/epinephrine injectable gel for palliation in patients with obstructive esophageal cancer. *Am J Clin Oncol* (CCT) 23: 386-392.

Pimental, T.A., Sampaio, A.L.F., D'Acquisto, F., Perretti, M. and Oliani, S.M. (2011) Blood, lymphatics, immune system, stem cells. An essential role for mast cell as modulators of neutrophils influx in collagen-induced arthritis in the mouse. *Lab Invest.* 91: 33-42, 2011.

Pratschke K.M. (2015) Mast cell tumours in dogs. *Veterinary Ireland Journal* 5(4): 179-184. Available online: < http://www.veterinaryirelandjournal.com/images/sa_apr_2015.pdf > [Access date: 20 Apr 2016]

Rothwell, T.L.W., Howlett, C.R., Middleton D.J. *et al.* (1987) Skins neoplasm of dogs in Sydney, *Aust Vet J* 64: 161-164, 1987.

Sano, H., Ichioka, S. and Sekiya, N. (2012) Influence of oxygen on wound healing dynamics: assessment in a novel wound mouse model under a variable oxygen environment. *PLoS ONE* 7(11): e50212. doi:10.1371/journal.pone.0050212, 2012.

Sano, H. and Ichioka, S. (2014) Influence of oxygen on wound healing dynamics in healing-impaired diabetic mice. *J Plast Surg Hand Surg.* Early online: 1-6. doi:10.3109/2000656X.2014.964723.

Stanclift R.M., Gilson S.D. (2008) Evaluation of neoadjuvant prednisone administration and surgical excision in treatment of cutaneous mast cell tumours in dogs. *J Am Vet Med Assoc.* 232:53-62.

Thompson, J.J., Pearl, D.L., Yager, J.A., Best, S.J., Coomber, B.L. and Foster, R.A. (2011) Canine subcutaneous mast cell tumor: characterization and prognostic indices. *Vet Pathol* 48(1): 156-168

Veterinary Cooperative Oncology Group – common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1, *Vet Comp Oncol.* July 2011. Available online from: < http://www.vetcancersociety.org/members/files/2012/01/CTCAE-v1-1.pdf> [18 Mar 2015]

Warland J., Amores-Fuster I., Newbury W., Brearley M. and Dobson J. (2012) The utility of staging in canine mast cell tumours. *Veterinary and Comparative Oncology* 12(4) 287-298

Withrow, S.J. and Vail, D.M. (2007) *Small Dog Clinical Oncology,* Elsevier Inc., Canada 402-421.