Exceptional *in vivo* wound healing following destruction of cutaneous and subcutaneous tumours in companion animals treated with the novel epoxy-tigliane drug EBC-46

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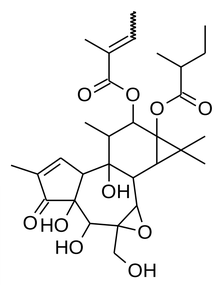
EBC-46 is a novel small molecule discovered from an Australian rainforest plant

Red fruit of *F. picrosperma* in tropical montane rainforest, Queensland

EBC-46 is one of a novel class of epoxy-tiglianes discovered in the seed of *Fontainea picrosperma* (Euphorbiaceae), a small tree endemic to Australia's tropical rainforest. The likely biological role of EBC-46 in the seed of this species is as a feeding deterrent against mammalian herbivores.

Fruit of plantation-grown *F. picrosperma* used for production of EBC-46



Chemical structure of EBC-46

EBC-46 is under development to treat cancers in humans and companion animals

EBC-46 is currently being developed, by Australian biotechnology company QBiotics Ltd, for intratumoural treatment of cutaneous and subcutaneous tumours in humans and in companion animals.

To date, more than 300 client-owned companion animals (dogs, cats and horses) with spontaneous tumours have been treated with EBC-46 in veterinary case studies.

In these studies EBC-46 has:

- caused significant local ablation of tumours in more than 80% of cases;
- shown efficacy against a wide range of tumour types including sarcomas, carcinomas, mastocytomas and melanomas; and,
- been well tolerated with a lack of any significant side effects at efficacious doses.

EBC-46 initiates rapid haemorrhagic necrosis of tumours followed by exceptional wound healing

EBC-46 is a signalling molecule, rather than a cytotoxic, and causes rapid haemorrhagic necrosis of treated tumours through a combination of:

i. local recruitment and activation of leukocytes, especially neutrophils and macrophages;

ii. initiation of an acute, but highly localised, pro-inflammatory response immediately surrounding the tumour; and,

iii. direct disruption of the integrity of tumour vasculature.

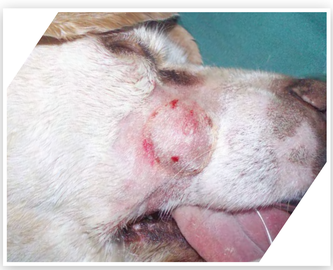
Short-term activation of specific isoforms of protein kinase C is fundamental to the mode of action of the drug in destroying tumours.

Dependent on tumour type and position on the body, necrotic remnants of cutaneous tumours treated with EBC-46 generally slough within 4 to 14 days of injection leaving open wounds. Without further intervention, these wounds then consistently show exceptional resolution characterised by:

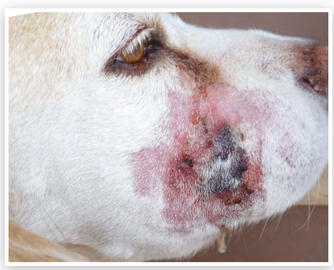
- rapid granulation tissue development;
- enhanced re-epithelialisation;
- minimal tissue deficit; and,
- minimal scarring.

Case study 1 illustrates the course of tumour destruction and subsequent wound healing following a single intratumoural treatment with 1mg of EBC-46.

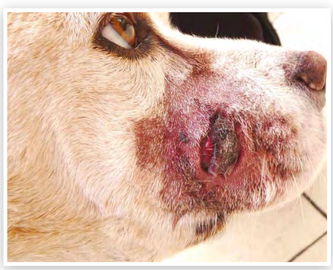
Case study 1: 11 year-old Beagle, spindle cell tumour



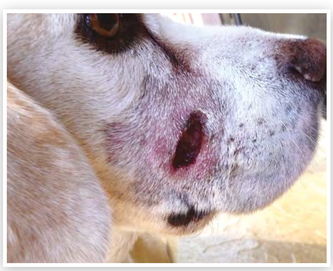
Immediately prior to intratumoural injection with EBC-46



Tumour necrosis evident 24 hr post treatment

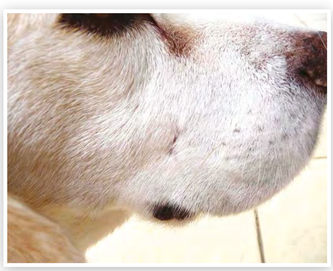


Day 3 post injection, just prior to tumour slough



Day 7 post injection

Day 15 post injection



Day 43 post injection

Rapid formation of granulation tissue and enhanced re-epithelialisation characterise wound resolution following tumour destruction by EBC-46

Time to closure and basic macroscopic wound characteristics were assessed following destruction of cutaneous tumours by EBC-46 in case studies of 28 dogs and 10 horses. In all instances there were no active wound management interventions (e.g. dressings, lotions, antibiotics, or other concomitant medications) following the initial treatment of the tumour with EBC-46.

Time for full wound closure was strongly related to wound size (**Figure 1**). Closure rates were favourable compared to data available in the veterinary literature (e.g. Bohling *et al*. 2004, *Vet Surg.* 33: 579; Wilwink & Weeren 2005, *Vet Clin Equine* 21: 15).

Healing of canine and equine trunk wounds was primarily by contraction, whereas re-epithelialisation was more important for wounds on extremities (and consequently these wounds are more representative of healing processes in humans).

Case studies 2 to 4 below provide typical examples of the time course and features of wound resolution that followed tumour sloughing.

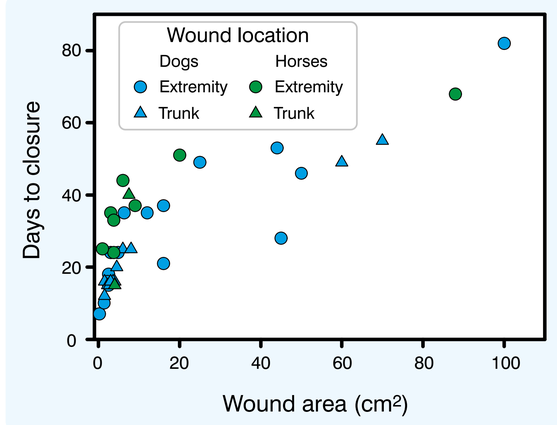
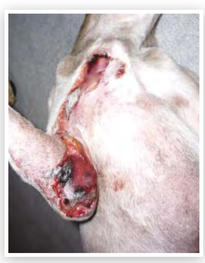
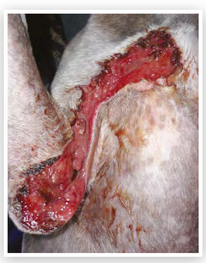


Figure 1: Wound size versus days to complete closure of open wounds that developed following destruction of spontaneous tumour by EBC-46 in 28 dogs (blue symbols) and 10 horses (green symbols).

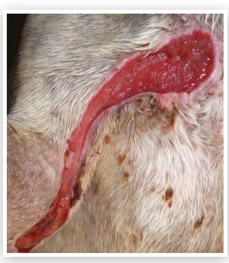
Case study 2: 11 year-old Whippet, soft tissue sarcoma



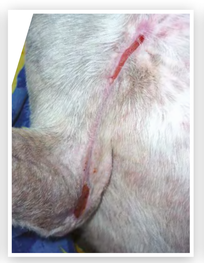
Tumour slough 6 days after injection



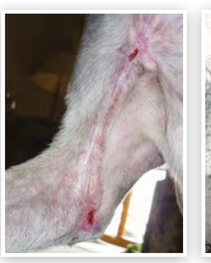
Day 3 post tumour slough



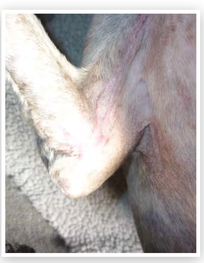
Day 8 post tumour slough



Day 17 post tumour slough



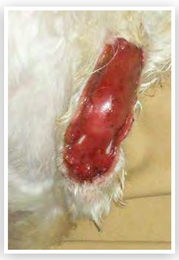
Day 22 post tumour slough



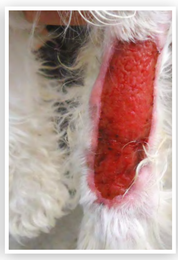
Day 30 post tumour slough

Case study 3: 6 year-old Shih Tzu, mast cell tumour

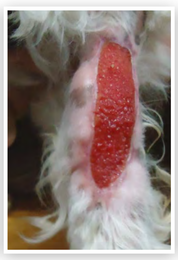
Tumour slough 6 days after injection



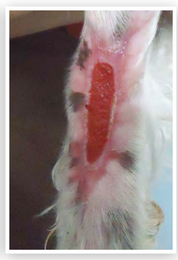
Day 2 post tumour slough



Day 9 post tumour slough



Day 16 post tumour slough



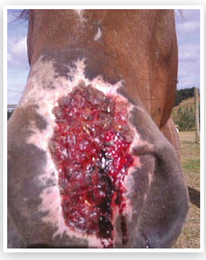
Day 28 post tumour slough

Day 35 post tumour slough

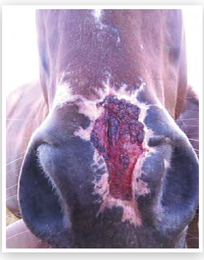
Day 44 post tumour slough

Case study 4: 8 year-old Stock horse, equine sarcoid

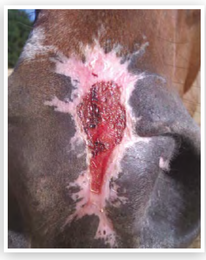
Tumour slough 10 days after injection



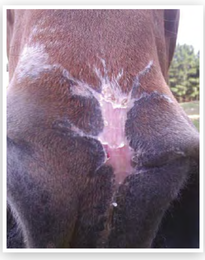
Day 18 post tumour slough



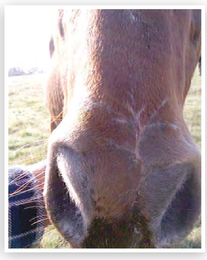
Day 32 post tumour slough



Day 50 post tumour slough



Day 90 post tumour slough



Day 138 post tumour slough

Minimal tissue deficit and minimal scarring were consistent features of the resolved wounds

'Cosmetic' features of the resolved wounds in the 38 case studies are summarised in **Table 1**.

Close to complete tissue infill was evident for all but one large canine wound.

Scarring was minimal in the majority of cases and was generally restricted to larger wounds on limbs subject to frequent weight-bearing during healing phases.

Wound site feature and outcome		No. of dogs	No. of horses
Tissue deficit on resolved wound (% of the original wound area where deficit is present)	Nil or minimal (<5%)	27	10
	Minor (5 to 10%)	1	0
	Substantial (>10%)	0	0
Scarring (% of the original wound area affected)	Nil or minimal (<5%)	21	6
	Minor (5 to 10%)	6	3
	Substantial (>10%)	1	1
Hair coverage on resolved wound (% of original wound area)	Full (>95%)	21	7
	Partial (>50%)	4	2
	Sparse (<50%)	3	1

Table 1: Tissue, skin and hair features of healed wound sites in 38 companion animals following sloughing of spontaneous tumours treated with EBC-46.

EBC-46, and related epoxy-tiglianes, have significant potential as novel pharmaceuticals to aid in wound healing

The exceptional dermal wound healing observed in companion animals following tumour destruction by EBC-46 provides strong evidence that epoxy-tiglianes can directly facilitate wound repair. We hypothesise that the initial, acute pro-inflammatory response is one of the key mechanisms underlying the enhanced healing initiated by EBC-46.

Studies currently in progress are:

(a) examining the direct effects of topically formulated epoxy-tiglianes on chronic wounds in companion animals; and,

(b) investigating the apparent multi-factorial mode of action of epoxy-tiglianes in initiating favourable wound outcomes.

Epoxy-tigliane drugs may have significant future application in addressing dysfunctional healing such as chronic wounds and excessive scarring.

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