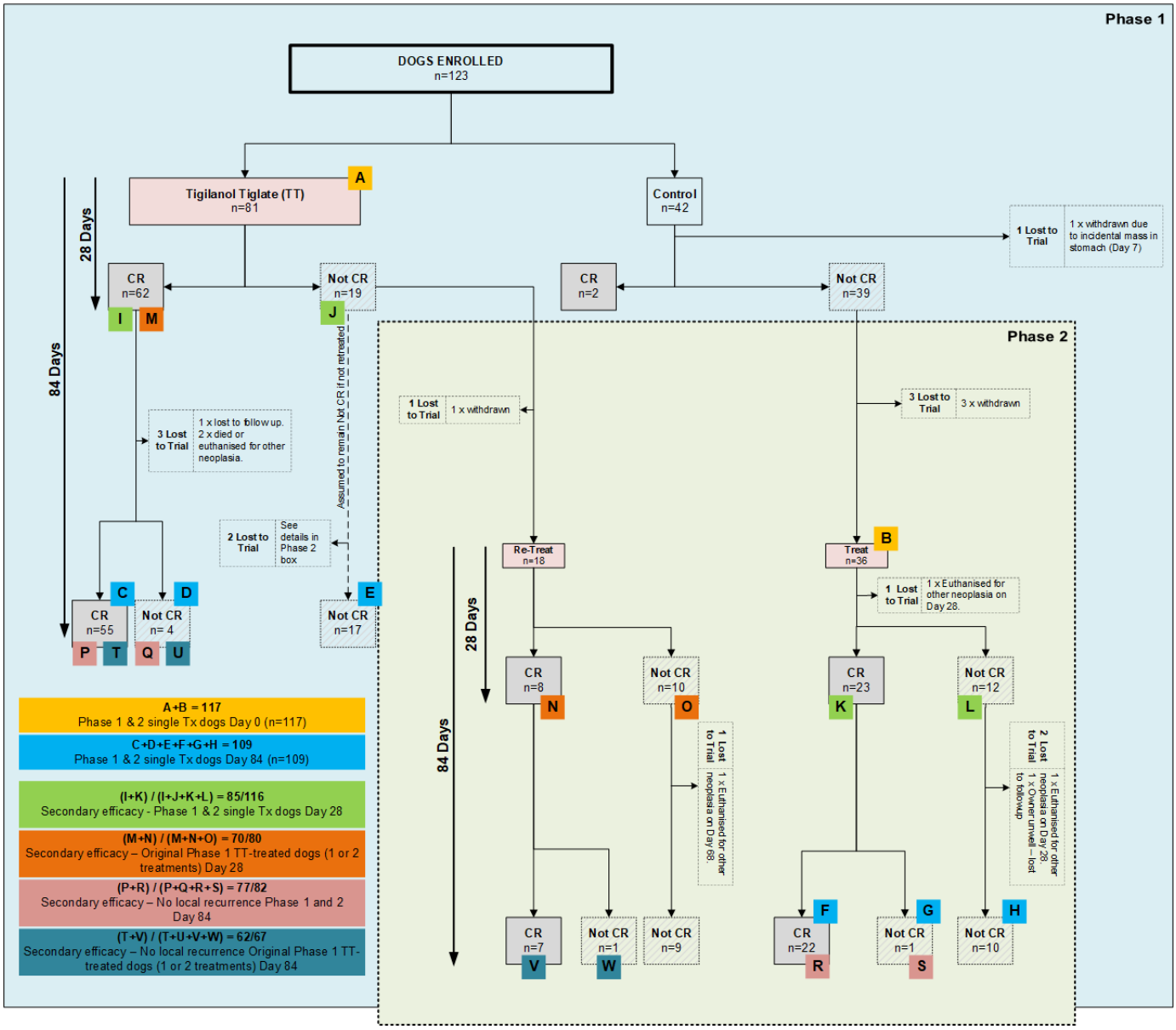
Supporting Information Figure 1

Figure 1: Summary of the number of dogs in each treatment group at each assessment time (Day 28 or Day 84) in each phase of the study, their response (CR or Not CR), and the number and reason for those lost to follow up. For the combined data set for a single treatment (i.e. dogs treated with in Phase 1 and control group that crossed over to TT treatment in Phase 2), there were 116 assessable dogs at day 28 and 109 dogs at day 84.



Supporting Information Table 1

Table 1. Odds ratios for complete resolution of mast cell tumors at day 28 after first treatment with tigilanol tiglate (TT; n=80) or at 28 days after day 0 in control dogs (n=38)

Analysis and group	No. dogs	% (no.) where CR was achieved by day 28 after treatment	Odds ratio	95% CI	P-value
Crude odds ratio					
TT treated dogs	80	75% (60)	51.8	13.2 to 345.2	<.001
Control dogs	38	5% (2)	Reference category		
Odds ratio adjusted for clustering by trial site					
TT treated dogs			57.0	11.7 to 277.6	<.001
Control dogs			Reference category		
Odds ratio adjusted for tumor location					
TT treated dogs			47.8	12.1 to 318.8	<.001
Control dogs			Reference category		
Odds ratio adjusted for tumor volume					
TT treated dogs			51.6	13.0 to 348.6	<.001
Control dogs			Reference category		
Odds ratio adjusted for cytological grade					
TT treated dogs			90.6	15.7 to 1980.7	<.001
Control dogs			Reference category		
Odds ratio adjusted for regional lymph node(s) status (enlarged/not enlarged)					
TT treated dogs			48.6	12.4 to 322.5	<.001
Control dogs			Reference category		

Note: Odds ratio remained large after accounting for clustering by study site and adjustment for various baseline covariates, demonstrating that the strong association was not due to confounding by these factors.

Supporting Information Table 2

Table 2: Owner assessed, health-related Quality of Life (QoL) of dogs treated with tigilanol tiglate compared to control (untreated) dogs using a questionnaire developed by Lynch S *et al. Vet Comp Oncol*. (2011) 9: 172–82. Assessments were made at screening, day of treatment, and days 7, 14 and 28 after treatment.

QoL Categories headings (parameters)	**Mean difference**(TT treated vs control) [1]	**P-value when statistical difference** [2]
Happiness		
Pet enjoys life [3]	Yes Day 7 (4.3 vs 4.7), Day 14 (4.6 vs 4.8)	Day 7 (.004), Day 14 (.049)
Pet wants to play	No statistical difference	
Pet response to owner's presence	No statistical difference	
Mobility		
Pet moves normally [3]	Yes Day 7 (4.1 vs 4.6), Day 14 (4.3 vs 4.7)	Day 7 (.011), Day 14 (.024)
Pet is active [3]	Yes (3.6 vs 4.2, 4.2 vs 4.7)	Day 7 (.002), Day14 (.003)
Pet lays in once place all day	No statistical difference	
Pain		
Pet is in pain [3]	Yes Day 7 (2.3 vs 1.6)	Day 7 (.002)
Presence of panting even at rest	No statistical difference	
Shaking or trembling	No statistical difference	
General health		
Compared to last visit [4]	Yes Day 0 (3.5 vs 3.0), Day 7 (4.1 vs 3.4), Day 14 (4.0 vs 3.3)	Day 0 (.002), Day 7 (.0001), Day 14 (.0001)
Compared to initial diagnosis of cancer [4]	Yes Day 14 (3.6 vs 3.2), Day 28 (3.8 vs 3.3)	Day 14 (.013), Day 28 (.002)
Mental Status		
Good days vs bad days	No statistical difference	
Sleepiness	No statistical difference	
Alertness	No statistical difference	
Appetite		
Normal eating	No statistical difference	
Vomiting or nausea	No statistical difference	
Eats snacks and treats	No statistical difference	
Hygiene		
Keeps self-clean	No statistical difference	
Smells like urine or has skin irritation	No statistical difference	
Abnormal and unkempt hair	No statistical difference	
Water intake		
Drinking adequate	No statistical difference	
Has diarrhea	No statistical difference	
Urinating normally	No statistical difference	

[1] For categories of Happiness, Mental Status, Pain, Appetite, Hygiene, Water intake and Mobility, responses were graded on a scale of 1 to 5, 1 = Disagree, 2, 3 = Neutral, 4, or 5 = Agree. For the 2 questions in the General Health category, responses were graded on a scale of 1 to 5, 1 = Worse, 2, 3= Same,4, or 5 = Better.

[2] Kruskal-Wallis non-parametric analysis of medians, P-value adjusted for ties

[3] Blue shading shows questions where there was a statistical difference between TT treated and control dogs at specific assessment times - in this case where control dogs were considered better than TT treated dogs.

[4] Green shading where shows questions where there was a statistical difference between TT treated and control dogs at specific assessment times – in this case where TT treated dogs were considered better.

Supporting Information Table 3

Table 3: Percent complete resolution of mast cell tumors treated with tigilanol tiglate in preliminary Australian clinical studies.

Study[1]	Compliance[2]	Dose (%v/v)	No. of patients	CR at Day 28	% Complete Response
C01	GCP	50%	10	9	90%
C02	GCP	50%	10	9	90%
C08	Non-GCP	50%	31	26	84%
C15	Non-GCP	50%	10	7	70%
C20	Non-GCP	50%	11	8	73%

[1] Five preliminary studies (C01, C02, C08, C15 and C20) conducted in Australia to investigate aspects of the efficacy of 1mg/mL tigilanol tiglate for intratumoral treatment of single mast cell tumors (all dosed at 50% TT v/v) are summarized below.

C01 - Characterize safe and effective dose of tigilanol tiglate (TT) for treatment of canine cutaneous MCT.
- Investigate systemic concentrations of TT following intratumoral injection in dogs.
- Select an appropriate dose of TT for investigating the drug's efficacy in proposed pivotal trial.

C02 - Characterize safe and effective dose of tigilanol tiglate (TT) for treatment of canine cutaneous and subcutaneous MCTs anywhere on the body.
- 50% v/vol dose.
- Concomitant medications used - NSAIDS, H1 antagonist (Cetirizine) and Proton pump inhibitor (Omeprazole).

C08 - Investigate efficacy of EBC46 in treating MCT and at 50% tumor volume with corticosteroids.
- 50% v/vol dose with a minimum dose of .1mL.
- Concomitant medications used - mandated prednisolone (Day -2), and H1 & H2 antagonists.

C15 - Investigate efficacy of EBC46 in treating MCT following triamcinolone injection 7 days prior to treatment.
- Cutaneous MCT at any site on the body.
- 50% v/vol dose with a minimum dose of .1mL.
- Concomitant medications used - mandated prednisolone (Day -2), and H1 & H2 antagonists.

C20 - Investigate concentrations of tigilanol tiglate in patients treated with MCT- urine, faeces and saliva.
- Cutaneous at any site on the body and subcutaneous MCT below the elbow and hock.
- 50% v/vol dose with a minimum dose of .1mL.
- Concomitant medications used - mandated prednisolone (Day -2), and H1 & H2 antagonists.

[2] GCP - Good clinical practice