Canine osteosarcoma (OSA) is a good model for human OSA due to similarities in their clinical presentation, molecular features, and the fact that dogs develop the disease naturally, sharing environmental and genetic risk factors with humans.[1]

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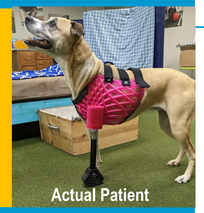
Chemotherapy & Immunotherapy Combination Results In ***Extended Survival*** in Dogs with Newly-Diagnosed **Osteosarcoma**



While combined therapy improved overall survival, the subset of dogs receiving a single carboplatin administration followed by ECI® immunotherapy had the greatest improvement.

TIMELINE	DVM	ELIAS
Week 0	Remove and submit cancer tissue	Vaccine preparation
Weeks 1 - 3	Administer vaccine series	
Week 5	Apheresis to collect MNCs	Ex vivo T cell expansion
Week 6	Infusion of activated 'killer' T cells	
Weeks 7 - 8	Administer IL-2 injection series	

INTRODUCTION

Treatment Overview

ECI® is an adoptive cell therapy that uses a series of vaccines derived from the dog's tumor to condition immune cells to the cancer antigens. Immune cells are then collected by leukapheresis but need to be activated to attack cancer cells. *Ex vivo* expanded and activated T cell infusate is reinfused and followed by a series of low-dose IL-2 injections.

Actual Patient

DESCRIPTION AND BACKGROUND

T cell Characterization and Cytokine Production[2]

TABLE 2 Mean values of leukapheresis and activated T cell products of normal, health laboratory dogs. All percentages are based on % of totals cells in population. Note the increase in proportion of CD4+ and CD8+ cells with the majority carrying the CD25+ activation marker

	Total cells (billions)[a]	Viability (%)[a]	Lymphocyte (%)[a]	Lymphoblast (%)[a]	Monocytes (%)[a]	Total T-cells (%)[b]	CD4 (%)[b]	CD8(%)[b]	CD25(%)[b]
Leukapheresis	5.4	89	52	< 1	31	54	43	11	< 5
Activated T-cell	2.3	80	39	56	5	87	57	31	> 95

[a]Cell analyses before freezing sample: based on morphologic characterization (cytospin and Wright stain).
[b]Cell analyses after thawing frozen samples: gated on total live/nondoublet cells.

T cell infusates used in ECI therapy were almost exclusively comprised of T cells. CD8+ T cells and CD25 expression were increased compared to apheresis product levels. CD25 was up-regulated dramatically, marking these T cells as being highly activated and potentially highly responsive to subsequent injections of IL-2.

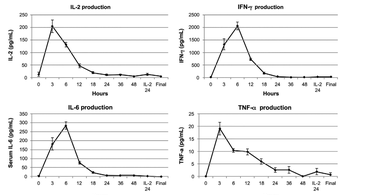
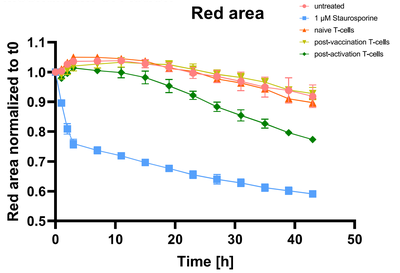


FIGURE 5 Cytokine levels in the serum of 4 laboratory dogs after activated cellular therapy (ACT). IL-2, IL-6, TNF-α, and INF-γ were elevated above baseline for 12 hours after the infusion of the activated T-cells. Presented are cytokine levels by time point ± SEM. Minimum limits of detection are less than approximately 10 pg/mL. TNF-α lower limit of detection is approximately 6 pg/mL

Cytokine production after T cell infusion in dogs was upregulated for IL-2, IL-6, IFNγ, TNFα.

Mechanism of Action[3]



Cancer cells from dogs undergoing ECI therapy were challenged with their respective T cells. Naïve (orange), post-vaccination (yellow), and post-activation (green) T cells were used. Imaging used red staining of cancer cells as surrogate for viability (Y-axis) over time (x-axis). Untreated cancer cells (pink) were used as a negative control. Staurosporine (blue) was used as a positive control causing cancer cell apoptosis. Only the post-activated T cells (green) caused a reduction in the red staining indicating apoptosis of the cancer cells by autologous activated T cells.

BASE (ECI-OSA-01) – Single Arm Pilot Study[2]

In dogs with appendicular OSA and no evidence of metastatic disease at diagnosis, historically reported median survival time is 77-134 days post-amputation.[4]

In a prospectively enrolled single-arm clinical trial in pet dogs (n=14) with newly-diagnosed OSA and no evidence of gross metastatic disease, ECI was evaluated as monotherapy with treatment initiated 2 weeks following therapeutic amputation.

Results demonstrated a favorable safety profile and a preliminary indication of efficacy.

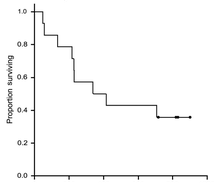


FIGURE 3 Overall survival time (OST) for all dogs on an intent-to-treat basis. The median OST for all dogs (n = 14) was 415 days. Dogs that were alive at the end of the study (n = 5) were censored (dots). The median survival of these 5 dogs at the time of analysis was 822 days

TABLE 1 Toxicoses summarized by organ system. Attributions were assigned by temporal correlation to each phase of the trial. Reactions that occurred before the second dose of IL-2 were attributed to ACT. Reactions that continued after the second dose of IL-2 were attributed to IL-2

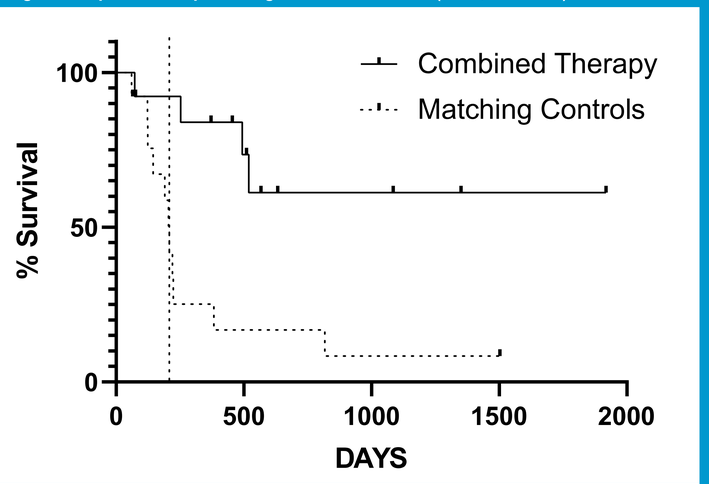
	Toxicosis						
Procedure	Admin site	Gastro-intestinal	Bone marrow	Cardiac	Respiratory	Constitutional	Fever
Vaccine	ISR(14) - I	V(1[b]) - I					
Leukapheresis			M(1) - II	AV(1) - II			
ACT		V(1[a]) - I D(2[a]) - I/II			C(1a) - I	L(1[a]) – II W(1[a]) - II	F(1[a]) - III
IL-2		V(2) - I, N (2) - I D(3) - I/II (2)					

Notes: Letter indicate toxicosis, (n) is the number of patients, and roman numerals indicate grade of toxicosis. Example: D(3) - I/II/III means 3 patients had grade I, II, or III diarrhea.
Abbreviations: AV, AV block; C, cough; D, diarrhea; F, fever; ISR, injection site reaction; L, lethargy; M, myelosuppression; N, nausea; V, vomiting; W, weight loss.
[a]Patient treated before premedicants. [b]Toxicosis unlikely attributable to intervention.

[1]Front. Vet. Sci., 07 December 2022, Sec. Comp. and Clin. I Med, Vol. 9 - 2022.
[2]Flesner BK, Wood GW, Gayheart-Walsten P, et al. J Vet Intern Med. 2020;1–12
[3]Data on file in company records.
[4]MacEwan EG, et al, 1989; Spodnick G, et al, 1992.
[5]Santamaria, AC, 2019; Bergman P, et al, 1996.
[6]Bryan JN, et al, 2024. Poster presented at VCS Annual Conference.
[7]ECI® Product Insert.

ASCENT – Single Arm, Multi-center Study

Single carboplatin dose preceding ECI administration (n=13, Cohort A)



CONCLUSION

Osteosarcoma is highly metastatic in dogs and historically reported post-amputation MST is 77-134 days[4] and 4-dose carboplatin is 196-321 days.[5] Interim analysis of dogs treated with this chemotherapy and immunotherapy combination showed improved outcomes compared to carboplatin alone and the treatment protocol was well-tolerated.

Clinical relevance was shown through improved MST and 1-year survival rates in dogs receiving up to 4 doses of carboplatin before initiation of ECI.

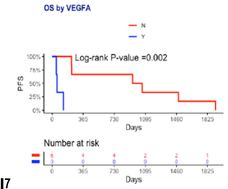
Dogs (n=13, Cohort A) receiving only one dose of carboplatin before initiation of the ECI vaccine-enhanced adoptive cell therapy showed the best outcomes (83% 1-year survival rate compared to 25% in matched controls receiving 4-dose carboplatin). These findings support the hypothesis that this combination therapy may improve canine patient outcomes. Further evaluation of this and other therapeutic combinations such as checkpoint inhibitors and oncolytic virotherapy is warranted and ongoing.

ECI is approved by USDA-CVB for use as treatment for canine osteosarcoma and is manufactured and distributed by ELIAS ANIMAL HEALTH

The translational impact of this research in dogs may have important implications for treatment of osteosarcoma and other cancers in human medicine.

Gene Sequencing Identifies Potential Biomarkers of Interest[6]

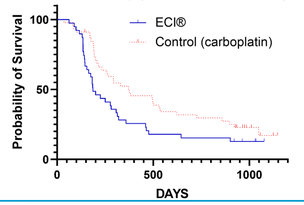
Actionable biomarkers of early metastatic failure in canine osteosarcoma are lacking. Targeted gene sequencing assessing copy number variation, single nucleotide variations, and internal tandem duplicates in 120 genes was performed on 9 dogs with suitable FFPE undecalcified samples collected in the BASE (ECI-OSA-01) study. Shown at right is an example of the correlation between overall survival of dogs with VEGFA overexpression (blue line) compared to dogs without (red line). The study results support further prospective study in dogs with the goal of improving therapeutic options.



BASE[R] (ECI-OSA-04) – Randomized Pivotal Study for Regulatory Approval[7]

A reasonable expectation of efficacy was demonstrated in a multi-site, prospective, randomized, positive-controlled, open label clinical trial. Dogs were randomized into one of two treatment cohorts of either ECI or the positive control (carboplatin). All dogs underwent amputation surgery to remove the primary tumor and then started their designated treatment. Treatment was well-tolerated, low-grade adverse reactions were transient, and no serious adverse reactions were observed in the ECI arm. A total of 86 dogs were evaluable for efficacy (ECI® = 40; Control = 46).

	Total Dogs Evaluated	Adverse Reaction	Any Grade AR	Serious Adverse Reactions VCOG Grades 3-5
Autologous Vaccine	49	Lethargy/fatigue	8 (16.3%)	0
		Anorexia	6 (12.2%)	0
		Diarrhea	5 (10.2%)	0
		Personality/behavior	5 (10.2%)	0
		Nausea/ptyalism	3 (6.1%)	0
		Vomiting	3 (6.1%)	0
T cell Infusate	45	Lethargy/fatigue	8 (17.8%)	0
		Anorexia	7 (15.6%)	0
		Nausea/ptyalism	6 (13.3%)	0
		Fever	5 (11.1%)	0
		Diarrhea	3 (6.7%)	0



METHODS

Twenty-one pet dogs with newly diagnosed appendicular osteosarcoma (OSA) were therapeutically amputated, received up to 4 doses of carboplatin, then received ECI® ~21 days after the final carboplatin dose. Survival outcomes were compared to historical matched controls selected from the COTC-022 dataset published on NCI's Canine Data Commons based on randomization to a 4-dose carboplatin arm (standard of care), survival to planned ECI initiation date (to reduce bias), tumor location, breed, weight and age, in order of priority. Kaplan-Meier analysis was performed to evaluate survival outcomes. Survival distributions were compared using both the log-rank and Gehan-Breslow-Wilcoxon tests. Dogs were followed until death or lost to follow up. One dog was lost to follow-up at 225 days and was censored in the analysis. Dogs were divided into subsets for further analysis based on the number of chemotherapy doses administered. Shown here is the subset of dogs (n=13, Cohort A) that received one dose of carboplatin (250-300 mg/m^2) preceding ECI administration.

ASCENT Combination Protocol

TIMELINE	DVM	ELIAS
Week 0	Remove and submit cancer tissue	Vaccine preparation
Week 1	Administer chemotherapy	
Weeks 4-6	Administer vaccine series	
Week 8	Apheresis to collect MNCs	*Ex vivo* T cell expansion
Week 9	Infusion of activated "killer" T cells	
Weeks 10-11	Administer IL-2 injection series	

RESULTS

Cohort A: Dogs receiving one carboplatin dose preceding ECI (n=13) showed median survival time (MST) was not reached (range, 73-1918 days) compared to 208 days (range, 61-1503 days) for matched controls. Hazard ratios were $p=0.002$** and $p=0.004$** using log-rank and Gehan-Breslow-Wilcoxon tests, respectively. One-year survival rates were 83% and 25%, respectively. Kaplan-Meier survival analysis for this subgroup is shown at left.

All Dogs: Dogs receiving 1-4 chemotherapy doses preceding ECI (n=21), including 2 dogs that instead received 2 doxorubicin doses during a carboplatin shortage, showed MST of 519 days (range, 73-1918 days) compared to 220 days (range, 61-1503 days) for matched controls. Hazard ratios were $p=0.012$* and $p=0.009$** using log-rank and Gehan-Breslow-Wilcoxon tests, respectively. One-year survival rates were 70% and 26%, respectively.

No serious adverse events were reported.

Note: Survival time for 2 dogs receiving doxorubicin instead of carboplatin was 225 and 486 days.
Disclaimer: Carboplatin and doxorubicin are currently not approved for use in dogs but are used historically as standard of care due to a lack of other approved therapeutics.

Actual Patients

Poster presented at **18th AACR Conference on The Science of Cancer Health Disparities, September 18-21, 2025**, Baltimore, Maryland
Results reported at **2025 Veterinary Cancer Society Annual Conference, September 25-27, 2025**, Salt Lake City, Utah