



AMERICAN KENNEL CLUB  
**CANINE HEALTH FOUNDATION**

November 20, 2007

Liz Wertz  
Rottweiler Health Foundation  
PO Box 391  
Chesterland, OH 44026-0391

Re: Grant Number 778: Role of Regulatory T Cells in Dogs with  
Osteosarcoma  
Principal Investigator: Barbara Biller

Dear Ms. Wertz:

We are pleased to forward to you the next progress report for the above referenced grant, which your organization has co-sponsored.

This progress report has been reviewed and approved. If you have any questions regarding the progress of this research please feel free to contact me by phoning toll free at 888-682-9696 or emailing [eaw@akcchf.org](mailto:eaw@akcchf.org).

We extend our thanks and appreciation to you and your club members for your support of canine health.

Sincerely,

Erika Werne  
Director of Canine Research & Education

Enclosures

# AKC Canine Health Foundation

## REVIEW OF SCIENTIFIC PROGRESS

**Grant No.:** 778

**Date Report Received:** September 28, 2007

**Title of Grant:** Role of Regulatory T Cells in Dogs with Osteosarcoma

**Amount Granted:** \$67,635.00      **Amount Paid:** \$13,527.00      **Amount Owed:** \$54,108.00

**Principal Investigator:** Barbara Biller

**Research Institution:** Colorado State University

**Start Date:** 4/1/2007

**Length of Grant:** 2

**Progress Report:** Mid Year 1

**Date of Review:**

**Recommended for Approval:**

**Performance Summary:**

**Objectives completed:**

**Publications:**

**Significant Accomplishments:**

*See next page*

# AKC Canine Health Foundation

## REVIEW OF SCIENTIFIC PROGRESS

**Grant No.:** ~~987~~ <sup>776</sup>      **Date Report Received:** 9/28/07  
**Title of Grant:** In vivo Effects of Tetracycline on Canine Refractory Ulcers  
**Amount Granted:**      **Amount Paid:**      **Amount Owed:**  
**Principal Investigator:** Barbara Biller, DVM  
**Research Institution:** Animal Cancer Center/VTH  
**Start Date:**  
**Length of Grant:**      **Progress Report:**  
**Date of Review:** 10.30/07      **Recommended for Approval:** yes

**Performance Summary:** problematic in that a complete set of samples for study dogs are not on hand due to difficulty in getting aspirates from lymph nodes; hopefully, this roadblock will be removed; they admit they need more samples to make data statistically sound so we should await and see if that can be achieved

**Objectives completed:** 0; obj 1 may be hard to complete but 2 will hopefully yield seminal clinical information

**Publications:** 0

**Significant Accomplishments:** 0

**Aim 1.** Determine whether dogs with OSA have increased Treg in blood or lymph nodes compared to normal dogs.

**Hypothesis 1:** Dogs with OSA will have increased Treg compared to normal dogs, particularly in their tumor draining lymph nodes.

We currently have 9 normal control dogs that are an average of 8.4 years in age. Peripheral blood mononuclear cells (PBMCs) have been successfully collected from all 9 animals and have been analyzed for CD4, CD8, and Foxp3 expression. Lymph node aspirates (LNA) have been successfully collected from 2 of these dogs. The aspirates have proven to be difficult to obtain due to their small size and the fact that the dogs are not under anesthesia for all of the lymph node aspirate samples needed. We plan on obtaining more normal controls and concentrating on getting as many lymph node aspirates as possible to allow for statistical analysis of our data. We also have 4 OSA dogs (average age=8.7 years) that have finished the study. Unfortunately, we do not have a complete set of samples on any of these dogs due to the inability to get lymph node aspirates at all of the proposed time points. Reasons for this include: small lymph nodes, uncooperative dogs and dogs being seen by their referring veterinarian. However, we do have complete blood work and PBMC on all of these four patients. Based on our current sample size the percentage of CD4+ cells that are foxp3+ (Tregs) are increased in dogs with OSA (6.73%) versus normal dogs (4.29%) but these results are also not statistically significant most likely due to the small sample size collected thus far.

Lymph node aspirates obtained thus far show no differences in the percentage of Treg cells in the non-draining lymph nodes of dogs with OSA vs normal dogs (average of about 16% of CD4+ cells expressing Foxp3 in all samples obtained). Interestingly, it appears that draining lymph nodes contain a smaller percentage of Treg cells (~12% of CD4+ cells are Foxp3+) than non-draining lymph nodes. However, these results are also only based on a small samples size. Tumors appear to contain, on average about 12% Tregs.

**Aim 2.** Determine whether treatment with doxorubicin or carboplatin has the greatest effect on Treg levels in dogs with OSA.

**Hypothesis 2:** Treatment with doxorubicin will increase Treg levels compared to treatment with carboplatin.

Using a randomization table, 3 of the 4 dogs in the study have received doxorubicin thus far; the last one receiving carboplatin. Current data shows that dogs pre-Sx have on average 6.73% Tregs. This percentage is decreased slightly to 6.03% following surgery, but then increase to 6.45% about one week post doxorubicin-treatment. The one dog that has received carboplatin has a post-chemotherapy Treg percentage of 5.81. Of interest, this dog's starting Treg percentage was 6.09%, indicating a possible negative effect of carboplatin on Treg percentages.