AMERICAN KENNEL CLUB
CANINE HEALTH FOUNDATION

GRANT PROGRESS REPORT

Grant: 01064-A: *Immunocytochemical Detection and siRNA-Mediated Knockdown of Survivin in Canine Lymphoma*

Principal Investigator: Dr. Douglas Thamm, VMD

Research Institution: Colorado State University

Grant Amount: $12,749.00

Start Date: 2/1/2008 **End Date:** 7/31/2009

Progress Report: 18 month

Report Due: 7/31/2009 **Report Received:** 7/31/2009

Recommended for Approval: Approved

Original Project Description:
Background: Survivin, a member of the XIAP family of anti-apoptotic proteins, has been shown to be a key mediator of cell survival and chemotherapy resistance in human lymphoma and many other cancers. The researchers have previously confirmed the appearance of survivin in canine lymphoma cell lines and tissues, and have furthermore identified survivin appearance as a novel, independent prognostic factor in dogs with stage III-IVa B-cell lymphoma treated with a multi-agent, injectable chemotherapy protocol.

Objective: The goals of this grant are (1) To determine if survivin appearance can be assessed accurately using immunocytochemistry, performed on canine lymphoma samples obtained through needle aspiration cytology; and (2) To determine if surviving breakdown using small interfering RNA is capable of inhibiting creation, inducing apoptosis, and enhancing chemosensitivity in canine lymphoma cells in vitro. Successful appearance that survivin reactivity can be detected using immunocytochemistry will increase the applicability of this novel prognostic marker for dogs with lymphoma, and successful demonstration of antitumor effects associated with survivin inhibition will justify future studies targeting survivin as therapy for canine lymphoma.

Original Grant Objectives:
Objective 1: Correlate survivin expression in canine NHL from fine-needle aspirates assessed by immunocytochemistry with immunohistochemical expression from contemporaneous histologic samples.

Objective 2: Examine the effects of survivin gene knockdown by siRNA on canine NHL cell proliferation, apoptosis and chemosensitivity.

Publications:

- Comparative Analysis of Survivin Expression in Untreated and Relapsed Canine Lymphoma. R.B. Rebhun, S.E. Lana, E.J. Ehrhart, J.B. Charles, and D.H. Thamm. JVIM, Vol.22, No.4. July/August 2008, pp 989-95.

Report to Grant Sponsor from Investigator: (Lay Update allowed to be reproduced)

Survivin is a protein found within many cancer cells that can function to prevent cancer cells from being killed by chemotherapy. We recently published data describing methods for measuring survivin in canine lymphoma and found that dogs with low levels of survivin lived longer than those dogs that expressed high levels of survivin. The problem with our previous study is that in order to analyze survivin expression, all dogs needed to undergo a lymph node excision that required anesthesia and surgical removal of a lymph node.

One purpose of this study was to determine whether we could measure survivin expression using less invasive techniques such as a fine needle aspirate, which can be done without surgery. We collected and stained 20 planned samples, and evaluated corresponding tissue blocks, and determined that the smearing technique routinely used for evaluation of fine-needle aspirates was not suitable for the evaluation of survivin levels. We will be evaluating an alternate cell preparation technique in the coming months, at no additional cost to AKCCHF.

The second purpose of this study was to molecularly interfere with survivin levels in canine lymphoma cells that can be grown in the laboratory, in order to test whether these lymphoma cells are more easily killed. Despite numerous attempts to decrease survivin production from the canine lymphoma cells, we were only able to modestly decrease it in either cell line (<50%). This degree of survivin reduction was not able to increase cell death. We are planning to evaluate 2 alternative strategies for survivin inhibition, including a more potent method of gene knockdown, as well as a drug that has been recently shown to inhibit survivin production. These investigations will be completed in the coming months, also at no additional cost to AKCCHF.