



Morris Animal Foundation Projects

Funded in Part by the Golden Retriever Foundation

**Morris Animal Foundation Project D10CA-501:
MADGiC: Making Advanced Discoveries in Golden Cancers
Grant Status: Open**

Drs. Jaime F. Modiano, Matthew Breen and Kerstin Lindblad-Toh, Institutions: North Carolina State University, University of Minnesota and Uppsala University, Sweden
January 1, 2011 – January 31, 2014

Golden retrievers have been one of the most popular breeds in America for decades, but unfortunately these dogs also have one of the highest incidences of cancer. Hemangiosarcoma and lymphoma account for more than 30 percent of the deaths in this breed. Although breed susceptibility to cancer was first reported 30 years ago, the relationship between inherited traits and susceptibility for these cancers is still not known. The Golden Retriever Foundation and Morris Animal Foundation are funding MADGiC (Making Advanced Discoveries in Golden Cancers), a study that aims to discover and characterize heritable and somatic cancer mutations in golden retrievers. The three-year, \$1 million project will examine heritable (genetic) traits that contribute to risk and progression of hemangiosarcoma and lymphoma in golden retrievers. The long-term goal is to understand what causes these diseases. Because both cancers occur with such high frequency, reducing their incidence (while retaining the positive phenotypes of the breed) will be a complex task, but the development of reliable genetic tests would allow breeders to build programs whereby high-risk combinations of factors could be avoided. In addition, effective strategies could be developed to control and treat hemangiosarcoma and lymphoma in golden retrievers and other dogs, and as importantly, what is learned from this research also may be applicable to develop effective prevention and treatment strategies for these diseases in people.

**Morris Animal Foundation Project D09CA-605:
Veterinary Student Scholars Program
MicroRNA Expression of Biologically High Grade Canine Mast Cell Tumors
Grant Status: Closed**

Bonnie K Harrington, The Ohio State University
June 1, 2010 – September 30, 2010

Mast cell tumors are the most common skin tumor in dogs, but there is wide variation in their biological makeup. Complete surgical removal often cures low-grade tumors, but high-grade tumors often spread and result in death. Previously, the student used the university's pathology archive to identify canine mast cell tumor cases that had sufficient tumor tissue and clinical outcome data available to classify them as biologically low or high grade. By comparing and

studying genes in low-grade and high-grade tumors, she sought to gain insight into ways to regulate the biological behavior of the tumors and determine how low-grade tumors transform to high-grade tumors in dogs. The primary objective of this study was to determine whether a certain intracellular molecule, known as microRNA-9, can be used to distinguish between high- and low-grade canine mast cell tumors. MicroRNA-9 is found in healthy canine mast cells, and the student hypothesized that the levels of microRNA would be higher in the cells of high-grade tumors than in cells of their low-grade counterparts. Although results from the study were not conclusive, the data display a clear trend for overexpression of microRNA-9 in the high-grade tumors; expression levels are, on average, about six times higher in the high-grade tumors than in the low-grade tumors. This difference in expression suggests that microRNA-9 may be a good marker to distinguish between high-grade and low-grade mast cell tumors. However, more research is required to confirm that a difference does, indeed, exist between the two tumor categories.

**Morris Animal Foundation Project D09CA-909:
Pfizer Animal Health-MAF Fellowship
Identification of a Genetic Marker for Familial Subvalvular Aortic Stenosis in the Golden Retriever**

Grant Status: Open

Dr. Joshua A. Stern, Washington State University
January 1, 2010 – December 31, 2013

With his Pfizer Animal Health-MAF Fellowship, Joshua A. Stern, DVM, joins a team at Washington State University that may discover the genetic mutation that causes a fatal congenital heart defect, subvalvular aortic stenosis, in golden retrievers, one of America's most beloved breeds. Stern's fellowship creates a new position in the university's Veterinary Cardiac Genetics Laboratory, where he will be mentored by Kathryn Meurs, DVM, PhD, a leading veterinary cardiologist.

Stern has been involved with golden retriever breed organizations for many years. He has also been involved in a study that evaluated a genetic marker for a heart disease affecting boxers and one that tested for a genetic mutation that causes an often fatal heart disease in cats. He has also studied cardiovascular changes in racehorses and Alaskan sled dogs.

"The Pfizer Animal Health-MAF fellowship will provide an opportunity for me to complete my training goals and obtain my dream of working in veterinary cardiovascular research," Stern says.

**Morris Animal Foundation Project D08CA-620:
Veterinary Student Scholars Program
Effect of Alternative and Complementary Treatments on Dogs with Osteoarthritis of the Hip or Stifle**

Grant Status: Closed

Anna K Keo, University of Tennessee
June 1, 2009 – September 30, 2009

Osteoarthritis is a common, crippling orthopedic problem affecting an estimated 1 in 5 adult dogs. While many pharmacological therapies, including non-steroidal anti-inflammatory drugs, are currently used to alleviate the pain of osteoarthritis, pet owners are increasingly using alternative and complementary treatments. Little is known about the efficacy of these alternative treatments in dogs, however. This study evaluated the efficacy of three alternative treatments commonly used to alleviate chronic pain in humans with osteoarthritis- transcutaneous electrical nerve stimulation, low level laser therapy and acupuncture - to determine their effectiveness in helping dogs with osteoarthritis of the hip or stifle. This veterinary scholar project is part of a larger MAF-funded grant. Results may provide scientific proof as to whether these treatments work in dogs.

**Morris Animal Foundation Project D07CA-627:
Veterinary Student Scholars Program
Development of a Genomic Assay to Profile Archival Canine Hemangiosarcomas
Grant Status: Closed**

Stephanie Montgomery, PhD, North Carolina State University
June 1, 2008 – September 30, 2008

To identify the mutations responsible for hemangiosarcoma, this comparison must be made over thousands of samples. Presently, there are extensive archives of hemangiosarcoma tumors samples, but the process used to preserve these tumors makes their DNA more difficult to use to identify genetic mutations. The student and her mentor have identified a technique to isolate and utilize the DNA from existing archival samples, rather than having to collect fresh samples from thousands of newly diagnosed cases. This approach will allow them to rapidly identify the location of the genetic mutations leading to hemangiosarcoma. Ultimately, this knowledge may lead to new cancer therapies and allow them to identify dogs susceptible to this disease before they get sick.

**Morris Animal Foundation Project D07CA-620:
Veterinary Student Scholars Program
Stereotactic Radiosurgery for Curative-Intent Treatment of Appendicular Osteosarcoma in Dogs
Grant Status: Closed**

Joanne Tuohy, Colorado State University
June 1, 2008 – September 30, 2008

Osteosarcoma is the most common primary bone tumor in dogs, and it commonly affects large breed dogs such as Rottweilers. The current standard of care therapy is removal of the primary tumor via amputation, or surgical limb salvage of the distal radius, followed by chemotherapy. Complications associated with limb spare surgery include local tumor recurrence and infection, which can occur in as many as 30-50% of patients. Stereotactic radiosurgery (SRS) is a new type of radiation available in veterinary medicine that can deliver high doses of radiation to the tumor while minimizing the radiation to the surrounding normal tissues. The investigators evaluated the possibility of using SRS as a novel non surgical limb salvage option for a variety of anatomic sites to treat the primary tumor in canine osteosarcoma. Results have shown improvement in patient lameness, significant pain palliation and no incidence of tumor recurrence. Fracture of the affected limb is the most common complication of SRS, and occurred in 5 of the 17 dogs in this study. All of these dogs have been managed appropriately for their fractures. SRS has displayed great potential to be a new non-surgical limb salvage option for dogs with osteosarcoma, and not being limited to just the distal radius, it can be used for tumors found in other anatomic sites such as the humerus. SRS promises to be a viable treatment option that can be offered to owners with dogs suffering from osteosarcoma, and expands the arsenal that veterinarians have to fight this devastating disease.

**Morris Animal Foundation Project D06CA-624:
Veterinary Student Scholars Program
Activity of Decitabine, a Demethylating Agent, on Tumor Suppressor Genes in Canine Lymphoma
Grant Status: Closed**

Hillary Voris, The Ohio State University
June 1, 2007 – September 30, 2007

In this study, the student used a canine lymphoma cell line to examine the effect of a new drug, decitabine, which is currently being tested in humans with cancer. Treating the lymphoma cells with decitabine resulted in cell death and decreased growth of cancer cells. Additionally,

treatment with decitabine restored the expression of one of the tumor suppressor genes examined. Though the drug will need to be tested further, the results from this study suggest that decitabine could one day be another tool that veterinarians can use to treat canine cancer.

**Morris Animal Foundation Project D06CA-065:
Reciprocal Relationship of PTEN and p21 in Canine Cancer
Grant Status: Complete**

Dr. Jaime F. Modiano, University of Minnesota
October 1, 2006 – June 30, 2009

Insight Into Chemotherapy Resistance

Cancer is the leading cause of disease-related death in dogs, which is why Morris Animal Foundation has invested considerable resources into understanding this group of diseases and developing more effective treatments. Cancer develops when genes that control the balance of cell division and survival cease to function normally and cause a cell to become malignant. One of these genes is called PTEN. The protein product of this gene generally restrains cell division, in part by controlling p21, another protein. Scientists at the University of Minnesota and the University of California-Davis found that using compounds to lower the levels of p21 in some tumors decreased resistance to conventional chemotherapy drugs. They also concluded that chemotherapy resistance is sometimes unrelated to abnormalities of PTEN, though it is often associated with elevated p21 levels. The results from this MAF-funded project allowed the investigators to justify efforts to move these compounds to the next step of clinical development.

**Morris Animal Foundation Project D06CA-026:
Investigating the Biology of Canine Met Mutations
Grant Status: Nearly complete**

Dr. Cheryl A. London, The Ohio State University
September 1, 2006 – January 31, 2011

Research has shown that mutations in a gene called Met contribute to the development of numerous types of cancer in people and mice. Met inhibitors that may stop these mutations are entering human clinical cancer trials, and with further scientific work, the same trials could take place for dogs. Researchers in this study already have identified two similar mutations in dogs, one of which has been found in about 70 percent of Rottweilers, which have a high risk of developing cancer. This study will continue investigating the biology of Met mutations in dogs and lay the foundation for using Met inhibitors in dogs with cancer.

**Morris Animal Foundation Project D06CA-027:
Transcriptional Profiling of Canine Mast Cell Tumors
Grant Status: Complete**

Dr. Cheryl A. London, The Ohio State University
September 1, 2006 – November 30, 2008

Mast cell tumors (MCTs) are one of the most common tumors in dogs. Although many MCTs are benign, others can behave in a highly aggressive manner (malignant), spreading to distant skin sites, lymph nodes and the spleen and ultimately resulting in death. Scientists at the Ohio State University were interested in establishing a more accurate method to distinguish MCTs more likely to behave in a malignant manner and, therefore, identify dogs in need of aggressive treatments. They theorized that certain genetic factors, specifically a subset of genes called microRNAs, would be differentially altered in the malignant tumors. Scientists successfully profiled microRNA changes in benign and malignant MCTs and have identified a set of microRNAs preferentially expressed at high levels in the malignant tumors. Further research is

now needed to use this new information to develop earlier diagnostic tools and better treatments for this deadly cancer in dogs.

**Morris Animal Foundation Project D05CA-019:
Canine Lymphoma - the Prognostic Significance of Cytogenetic Changes**

Grant Status: Complete

Dr. Matthew Breen, North Carolina State University
January 1, 2006 – February 20, 2009

The aim of this project was to assess the prognostic value of a series of five chromosome aberrations that Dr. Breen's lab had identified previously. The study population used for this project was a set of archival lymph node specimens from dogs with lymphoma that were part of a multi-center clinical trial and which had been treated with the same chemotherapy protocol. Each dog had been followed from the time of their diagnosis of lymphoma to the time of death and so provided a key resource to allow us to determine if genetic changes were of any prognostic value. Cells from each patient were isolated from the archival specimens and interrogated subsequently using multi color molecular cytogenetics. In these experiments, regions of each of the five chromosomes were labeled with a fluorescent dye and then used to probe cells isolated from each specimen. Using fluorescence microscopy, the number of copies of each of the five chromosomal regions was assessed in a population of cells from each patient. The mean copy number of each locus in the lymph node cell population was then evaluated using appropriate statistical methods. The data revealed that one of the five aberrations tested correlates significantly with the time that the lymphoma patients went into remission following first chemotherapy treatment. Further analysis of this chromosome aberration is underway and early data indicate that assessment of this chromosomal region may provide a means to predict reliably and accurately for how long canine lymphoma patients will respond to their first series of chemotherapy treatments.

**Morris Animal Foundation Project D05CA-049:
Candidate Gene Analysis in Dogs Affected with Hereditary Cataracts**

Grant Status: Complete

Dr. D.J. Sidjanin, The Medical College of Wisconsin
December 1, 2005 – December 31, 2008

Hereditary canine cataracts are one of the most common disorders affecting various dog breeds; however, very little is known about cataracts. To date only mutations in one specific gene (the HSF4 gene) have been identified as associated with canine cataracts in some breeds. Yet numerous breeds, including Labrador and golden retrievers, Portuguese water dogs and huskies, analyzed by scientists at the Medical College of Wisconsin do not carry mutations in the HSF4 gene. In this Morris Animal Foundation-funded study the researchers evaluated 26 genes associated with hereditary cataracts in humans and mice to see if mutations in these genes may be responsible for hereditary cataracts in Labrador and golden retrievers, Portuguese water dogs and huskies. In spite of their enormous efforts, the researchers did not identify cataract-associated mutations in these particular breeds. Still, these results suggest that certain breeds of dogs may harbor mutations in novel, not yet identified canine genes associated with cataracts.

**Morris Animal Foundation Project D04CA-026;
Genome Scanning for Aberrant DNA Methylation in Canine Lymphoma,
Grant Status: Discontinued**

Laura Rush, DVM, PhD, The Ohio State
January 1, 2004 – December 31, 2007

This study was discontinued due to the unexpected departure of the PI from the institution. Below is a summary of progress from the most recent progress report.

DNA promoter methylation occurs frequently in human cancers and enhances tumor survival by inactivating genes necessary for normal growth control. We hypothesized that methylation plays a key role in canine lymphoma. Using a technique called Restriction Landmark Genomic Scanning we have shown that canine lymphoma samples exhibit widespread abnormalities in their genomic DNA methylation patterns which is very similar to human tumors. We have also investigated individual genes for a more comprehensive assessment of their promoter methylation and found that the promoter regions of some genes could be demethylated and reactivated after exposure to a demethylating drug. Our approach is to identify these genes based on a computational 'in silico' computer program, an approach that has been successful for the human, mouse and rat genomes.

Thus far the project has shown that dogs with lymphoma have abnormal DNA methylation in their cancer cells, just as humans do. Some of the same genes methylated in humans are methylated in dogs, and our studies in cell culture show that the methylation can be partially reversed by treatment with a demethylating drug. This treatment has been used with success in human cancer and we hope that our work leads to clinical trials in pet dogs.