Canine Health Foundation Projects

Funded in Part by the Golden Retriever Foundation

Canine Health Foundation Project 01521-A:
Assessment of grass awn disease in dogs & CRP plantings of grasses with barbed awns
Grant Status: Open
Dr. William K Lauenroth, , University of Wyoming
October 1, 2010 - January 31, 2011

In the sporting dog world, there appears to have been a dramatic escalation in in the incidence of grass awn migration disease. This is a well-established disease in the veterinary medical profession with many published case studies. It is suspected that the incidence of grass awn migration disease has increased over the past 2 decades, due to inclusion of problem grasses in the approved lists for CRP lands. This idea is at the core of the proposed study. If it can be determined that there has been a dramatic increase in both the incidence of the disease and the quantity of barbed seeds planted in CRP lands, such determination would surely factor significantly into improvements in veterinary diagnostic and treatment protocols, overall education for dog owners, and preventative or remedial measures for the U.S. Department of Agriculture's approach to CRP plantings. The proposed study will conduct an analysis of the frequency of treatment of grass awn disease at 8 veterinary teaching hospitals over the past 2 decades and create a list of the problem seeds and survey USDA office over a 10 state area to determine the frequency of planting of the problem grasses in CRP lands. The work will be carried out by students under the direction of the principal investigator. At a future date, one or more students might possibly visit regional governmental offices to sample documents relative to particular CRP contracts, but such site visits are beyond the scope of the initial phase of the proposed study.

Canine Health Foundation Project 01488-A:
Health Implications of Spay and Neuter: Golden Retriever and Labrador Retriever
Grant Status: Open
Dr. Benjamin Hart, DVM, PhD, University of California, Davis
July 1, 2010 - June 30, 2011

Recent published studies have profiled several examples of adverse effects of the traditional spaying and neutering procedures on different types of cancer and bone articular disorders in dogs. A markedly increased risk of bone cancer (osteosarcoma), a blood system cancer (hemangiosarcoma) and the bone articular disorder of cranial cruciate ligament deficiency, are among the most disturbing. While these published studies focus on just one disease and one breed, or lump several breeds together for analyses, our long-term goal is an epidemiological study examining all relevant disease syndromes with sufficient prevalence, on a breed-by-breed basis, that may be increased or decreased by spay/neuter.

This project focuses primarily on the Golden Retriever and secondarily on the Labrador Retriever, using our extensive hospital computerized database, for an analysis of several diseases. Our preliminary data

indicate that hip dysplasia in both breeds is increased by about 2-fold by neutering males and spaying females. This project will complete our analysis of hip dysplasia, examining early versus later spay/neuter. Next, we will examine the effects of spay/neuter on relevant disease syndromes of the Golden. For disease syndromes that show trends, we will examine the same syndromes in Labs and pool data for significance testing. The goal is to provide caregivers with the information they need to make the best choice for the long-term health of their dog.

Canine Health Foundation Project 01248:
Whole Genome Association Analyses for Cryptorchidism in Dogs
Grant Status: Open
Dr. Max F. Rothschild, PhD, Iowa State University
January 1, 2010 - December 31, 2010

Background: Cryptorchidism, or retained testicles, is one of the common congenital problems in dogs. The testes of cryptorchids are more prone to testicular cancer and infertility. Therefore, cryptorchids and animals carrying genes for cryptorchidism should be eliminated from the breeding population. Some evidence exists to suggest that it appears to be a multigenic trait but single genes with large effects may exist. In earlier studies, the researchers utilized a candidate gene approach using 50 polymorphisms (called SNPs) in 22 candidate genes and found that collagen 2A1 (COL2A1) was significantly associated with cryptorchidism in Siberian Huskies. Now, they need to use the whole genome association analysis which typically provides more comprehensive analyses of chromosomal regions associated with a particular trait. This will then allow them to confirm previous findings or suggest other contributing regions or genes.

Objective: The researchers will utilize a new tool called the canine SNP chip which allows them to genotype for over 200,000 genetic differences between affected and unaffected animals. All results will be published and available freely to all dog breeders and they also aim to develop a test to remove the defect from the population.

Canine Health Foundation Project 01317:
Mutation Detection and Functional Analysis of Multiple Loci for Osteosarcoma
Grant Status: Open
Dr. Kerstin Lindblad-Toh, PhD, Broad Institute
January 1, 2010 - December 31, 2011

Background: Osteosarcoma (OSA) is a considerable canine health concern, affecting 8,000-10,000 dogs in the United States annually. In the completed CHF study "Mapping Genes Associated with OSA in Large Breed Dogs", the researchers identified six genomic regions associated with osteosarcoma (OSA) in Rottweilers and Greyhounds. In study 758, they conducted fine-mapping of these candidate regions using additional Rottweiler and Greyhound samples as well as samples from eight other breeds (Golden Retrievers, Labrador retrievers, Leonbergers, Great Pyrenees, Mastiffs, Great Danes, Irish Wolfhounds and boxers). All six loci are supported and have been narrowed to a discreet size. Some loci are present in some breeds, some in others. The majority of candidate genes in associated regions have been re-sequenced, but no associated protein changes identified.

Objective: The researchers will re-sequence the whole regions of association in cases and controls to identify candidate mutations. Once mutations have been found they aim to survey multiple breeds to see if they share the same mutations. This will make it possible to develop and apply genetic tests for OSA. The researchers will also study the functional consequences of the mutations, which will lead to a better understanding of the disease, enabling development of more targeted treatment options.

Canine Health Foundation Project 01262:
Sequencing and Functional Analysis of the Canine Y Chromosome
Grant Status: Open
Dr. William J. Murphy, PhD, Texas A&M University
January 1, 2010 - December 31, 2011

Background: Studies of the human and mouse Y chromosomes have shown they contain many testis-specific genes that, when defective, cause infertility and sperm abnormalities. The causes of male infertility in dogs are not well known. Though a high quality draft genome sequence exists for the canine autosomes and X chromosome, virtually nothing is known about the canine Y chromosome and the genes it harbors.

Objective: The researchers will use an existing map of the canine Y chromosome to generate a comprehensive sequence of the chromosome, and explain the sequence for coding and non-coding potential using cDNAs selected from a large number of canine tissues. The identification of a comprehensive set of canine Y chromosome genes, their regulatory regions and noncoding RNAs will provide targets for development of molecular diagnostic tests that examine the influence of these genetic elements on canine male infertility as well as many other sexually dimorphic traits.

Canine Health Foundation Project 01131:
Genetic Background and the Angiogenic Phenotype in Cancer
Grant Status: Open
Dr. Jaime F Modiano, VMD PhD, University of Minnesota
January 1, 2010 - December 31, 2012

Background: Certain dog breeds are prone to develop certain types of cancer; yet, there has been little progress to define genes or other factors that account for this risk. The researchers' recent work on hemangiosarcoma is the first to clearly demonstrate that a dog's genetic background, defined by "breed," can influence the type of genes that show up as tumors. This means that certain breeds are diagnosed with specific cancers more frequently than others because of the behavior of tumors after they show up, and not simply because they show up more frequently. Specifically, this may apply to the observed tendency for hemangiosarcoma seen in Golden Retrievers, German Shepherd Dogs, and Portuguese Water Dogs. In addition, one-size-fits-all therapies may be not enough to effectively treat this disease.

Objective: This project will continue the researchers' observations on gene appearance profiles in hemangiosarcoma from Golden Retrievers to German Shepherd Dogs and Portuguese Water Dogs, and it also will define how new targeted therapies may effectively control the disease in these and other dog breeds.

Canine Health Foundation Project 01272:
Isolation and Characterization of Canine Induced Pluripotential Stem Cells (iPS)
Grant Status: Open
Dr. Jorge Piedrahita, PhD, North Carolina State University
January 1, 2010 - December 31, 2011

Background: Stem cells have tremendous promise to alleviate clinical conditions in dogs such as spinal cord damage, hematopoietic malignancies, and cardiac and hepatic disease. While a range of adult stem cells have been isolated and studied, most of these have a limited capacity to differentiate outside a living organism and inside a living organism. Recently, approaches have been developed to convert differentiated cells into cells resembling embryonic stem (ES) cells by the use of "reprogramming" factors. These cells referred to as induced pluripotential stem cells (iPS) have the ability, like ES cell, to

differentiate into multiple tissue types. As virtually any cell can be converted to an iPS cell this means that it is now possible to isolate patient-derived stem cells.

Objective: The researchers will utilize this technology for the development of canine iPS. Briefly, adipocyte-derived mesenchymal cells and keratinocytes will be transformed with the required reprogramming factors and plated under a condition that allows development of iPS cells. Colonies will be selected, expanded, and studied for their ability to differentiate outside a living organism into multiple tissue types. The development of patient-specific pluripotential stem cells is a critical step toward the successful scientific application of this promising technology.

Canine Health Foundation Project 01231-A:
Prevalence and Localization of Bartonella spp. in Vascular Tumors from Dogs
Grant Status: Closed
Dr. Edward B Breitschwerdt, DVM, North Carolina State University
April 1, 2009 - March 31, 2010

This study showed a statistically higher prevalence of Bartonella spp. DNA in surgical biopsy samples obtained from dogs with hemangiosarcoma (the most commonly encountered vascular neoplasm of spleen in dogs) compared to dogs with lymphoid nodular hyperplasia (the most commonly encountered non neoplastic splenic disease). These results provide preliminary evidence that bacteria of the genus Bartonella may contribute to the development of vascular tumors in dogs. One future direction the researchers hope to explore will be to test the prevalence of Bartonella spp. in other vascular tumors, particularly hemangiopericytomas. Also, in an effort to obtain additional environmental control data they are planning to test the prevalence of Bartonella spp. in the spleens of dogs euthanized at a local animal shelter. Studying additional control populations will be required to determine the medical relevance of the data generated to date and will provide information regarding the prevalence of Bartonella spp in the spleens of dogs within the general dog population.

Publication(s)
None at this time- ACORN provided preliminary data - 3/31/10

Canine Health Foundation Project 01232-A:
Investigation of NF-kB as a Therapeutic Target in Canine Lymphoma
Grant Status: Open
Dr. Nicola J Mason, BVetMed, PhD, University of Pennsylvania
March 1, 2009 - August 31, 2010

Background: Lymphoma is the most common hematopoietic cancer in dogs and is currently treated using a combination of chemotherapeutic agents which inhibit cell division and induce cell death. Over the past 30 years, many chemotherapeutic protocols have been used and most induce remission in 65-96% of patients. However, regardless of induction protocol, 85-90% of patients relapse with lethal, drug-resistant lymphoma within 6 to 9 months of diagnosis.

Objective: The researchers hypothesize that constant activation of a major intracellular signaling pathway (the NF-kB pathway) in the cancerous lymphoma cells contributes to their enhanced increase, survival, and resistance to chemotherapeutic drugs. The researchers aim to investigate NF-kB signaling pathways as potential targets for inhibition. This preliminary work aims to build the groundwork for the use of selective NF-kB inhibitors as extra agents to increase the sensitivity of cancer cells to chemotherapy. It is anticipated that this work will provide the preliminary data necessary to initiate a pilot clinical trial to evaluate NF-kB inhibition as an adjunct to chemotherapy to enhance clinical response at the time of diagnosis and/or during rescue therapy.

Publication(s)

Anita Gaurnier-Hausser, Reema Patel, Karen Jackson, Al Baldwin, Michael May, Nicola J. Mason. "Nemo Binding Domain Peptide inhibits constitutive NF-!B activity in dogs with ABC-DLBCL" Manuscript in Preparation

Canine Health Foundation Project 01142:
Cutting Balloon Valvuloplasty for Dogs with Subaortic Stenosis
Grant Status: Open
Dr. Amara H. Estrada, DVM, University of Florida
January 1, 2009 - December 31, 2010

Background: Treatment for subaortic stenosis (SAS) in canine clinical patients is frustrating and there exists a great need from both the veterinary community and the dog owner/breeder population for better treatment options. Balloon dilation of the stenosis is a type of interventional procedure used in veterinary medicine for dogs with stenosis of the pulmonary valve. Balloon dilation in dogs with SAS however, has not proven effective or beneficial. A new balloon dilation catheter has been developed for use in the management of resistant coronary artery and peripheral pulmonary artery lesions in humans. The balloon has been modified to also have very small blades, approximately 2mm, which are used to score or cut the stenotic, or narrowed, tissue when the balloon is maximally inflated. This technique has proven successful in children and young adults with lesions previously resistant to balloon dilation.

Objective: The study is using these new techniques and procedures in dogs with severe SAS, in hopes of providing a new treatment option for this currently untreated disease in dogs.

Canine Health Foundation Project 01152:
Liposomal BisphosphonateTherapy for Malignant Histiocytosis
Grant Status: Open
Dr. Steven W. Dow, DVM PhD, Colorado State University
January 1, 2009 - December 31, 2010

Background: Malignant histiocytosis (MH) is a common and aggressive and often fatal tumor of Bernese Mountain dogs. The tumor also affects flat-coated retrievers, Golden Retrievers, and Rottweilers. There are currently no effective medical treatments for the disease, which often spreads widely. Preliminary studies in the laboratory suggest that a compound known as liposomal clodronate (LC) can kill dog MH cell lines in vitro and elicit significant tumor shrinkage in at least one dog with MH.

Objective: The researchers are conducting additional studies to understand better how LC kills MH cells, whether LC can be combined with other types of chemotherapy to increase tumor control, and to assess the potential safety and efficacy of this novel treatment approach in a pilot study of dogs with MH. If shown to be effective, LC would offer a non-toxic and relatively inexpensive new approach to the treatment of MH In dogs.

Publication(s)
- Hafeman S, London C, Elmslie R, Dow S.; 2010 "Evaluation of liposomal clodronate for treatment of malignant histiocytosis in dogs." Cancer Immunol Immunother. 59:441-452
  - 6/30/10 Manuscript submitted to Veterinary and Comparative Oncology
  - 6/30/10 Manuscript submitted to Veterinary Immunology and Immunopathology

Canine Health Foundation Project 01139:
Immune Targeting of Canine Hemangiosarcoma Using a Canine Derived Single Chain Antibody Approach
Grant Status: Open

Dr. Nicola J Mason, BVetMed, PhD, University of Pennsylvania
January 1, 2009 - December 31, 2010

Background: Canine hemangiosarcoma is a common and highly aggressive tumor of blood vessels that is often fatal. At diagnosis most dogs have evidence of metastatic disease and despite chemotherapy, survival times rarely exceed 6 months. New approaches to the treatment of this disease are needed. The use of monoclonal antibodies and antibody fragments to directly target different tumors has shown promise in clinical trials in man.

Objective: This project aims to use a new canine synthetic antibody system to target the tumor and deliver cytotoxic agents directly to both primary and metastatic lesions. Using advanced molecular techniques, the researchers intend to review antibody responses that dogs with hemangiosarcoma may make against their own tumors and use these as a template to generate canine antibody fragments that specifically recognize tumor particles. Tumor-specific antibody fragments will be linked to an exotoxin and evaluated for their ability to kill canine hemangiosarcoma cells in vitro. This allows for the direct delivery of cytotoxic agents to the tumor, which decreases side effects and increases therapeutic value. This work aims to develop the first canine-derived, tumor-specific targeting approach for the treatment of HSA and to provide proof-of-principal for this approach that can then be used to therapeutically target many other tumor types in this species in vivo.

Publication(s)

- Braganza, A., Wallace, K., Pell, L., Parrish, C.R., Siegel, D.L., Mason, N.J., 2010, Generation and validation of canine single chain variable fragment phage display libraries. Veterinary Immunology and Immunopathology In Press, Accepted Manuscript.(Epub) DOI: 10.1016/j.vetimm.2010.07.026

Canine Health Foundation Project 01113:
Canine Non-Hodgkin Lymphoma: Characterization and Prognostic Value of Cancer Stem Cells
Grant Status: Open
Dr. Timothy D. O'Brien, DVM PhD, University of Minnesota
January 1, 2009 - December 31, 2010

Background: Stem cells are cells which, in general, have the ability to increase to multiple different cell types while at the same time maintaining their own population of different stem cells. Embryonic stem cells are the quintessential stem cell and have the ability to form any tissue of the embryo, fetus, and adult. However, in the adult animal, most tissues or organs also have a stem cell population (adult stem cells) with a more limited collection, which can increase to any of the mature cell types of that organ or tissue (eg. skin, brain, liver, or blood and lymphocyte stem cells). Recently, cells have also been identified in several human and animal cancers that have the essential features of cancer stem cells and which are thought to be responsible for the growth and spread of the tumor. The researchers have identified cells within dog lymphomas that have features highly suggestive of a cancer stem cell. They have also found evidence that increased numbers of these cells tend to correlate with worsening prognosis.

Objective: In this study they researchers goals are: 1) to evaluate the numbers of these suspected cancer stem cells in various subtypes of lymphoma to firmly establish whether increasing numbers of these cells do correlate with worsening prognosis across all forms of canine lymphoma, and 2) to obtain key information regarding which genes are characteristically shown in the cancer stems cells in contrast to those found in the remainder of the tumor cells. Thus, this study will potentially give us a new tool to diagnose canine lymphoma and assess prognosis, and secondly, give a detailed look into the biology of the cancer stem cells, revealing much about their origin and functions and possibly indicating new methods to eliminate these common and lethal cancers.

Canine Health Foundation Project 01147:

Identifying Mutations in Genes Associated with Canine Hemangiosarcoma
Grant Status: Closed
Dr. Chieko Azuma, DVM PhD, Tufts University
January 1, 2009 - June 30, 2010

Hemangiosarcoma (HSA), a malignant tumor of blood vessels, is a significant health concern in dogs, with a reported incidence of up to 2% of all tumors. HSA can affect all dogs, but a particularly high disease incidence has been reported in certain breeds, such as Golden Retriever (15%), German Shepherd Dog (10%), and Labrador Retriever, suggesting that genetic risk factors exist. We have identified six regions in the canine genome that differ in golden retrievers with and without HSA and verified the findings with more advanced technology in this project. We are currently identifying the actual mutations. So far no mutations in candidate genes have been found, supporting the major role of regulatory mutations.

Once the mutation has been found, it will be possible to rapidly develop genetic tests for risk assessment for susceptibility of HSA. Interpretation of these DNA tests will require the consideration of markers for all genes simultaneously as well as assessing risks for different types of cancer. The presence of multiple interacting genes, some at high frequency in the population, will make it difficult to reduce the disease frequency quickly, but should still allow for informed breeding. In addition, by examining the frequency of these mutations in other breeds we can determine which other breeds need to be screened for these mutations and to what degree they contribute to the risk of HSA in specific breeds. Still, perhaps the most significant outcome of knowing the actual mutations is that it might suggest which dogs should be under surveillance or preventive care.

The identification of actual mutations should also lead to further study of functional effects of the causative mutations thereby increasing the understanding of the disease mechanism. A better molecular understanding will suggest novel treatment options and possible new drug targets. Due to aggressive nature, HSA is uniquely qualified for studying local invasion, angiogenesis and metastasis, and developing therapeutic intervention in dogs and humans.

Canine Health Foundation Project 00935B:
Positional Cloning of Two Genes for Malignant Histiocytosis (MH) in the Bernese Mountain Dog
Grant Status: Open
Dr. Catherine Andre, PhD, CNRS - University of Rennes
September 1, 2008 - July 31, 2010

Background: Malignant histiocytosis (MH) belongs to a group of histiocyitic disorders, which represent a broad array of clinical symptoms. The disease is found in excess in Bernese Mountain Dogs (BMD), Flat Coated Retrievers (FCR), and a small number of other breeds. MH is an aggressive tumor from which affected dogs die quickly. Working together, the Ostrander and Andre labs have each clearly identified two regions of the genome (on chromosomes 8 and 20) with genes causing MH in the BMD.

Objective: The researchers are working to find the exact genes and genetic variants responsible for the disease. It involves finding a common piece of each chromosome that affected dogs likely inherited from a single affected ancestor. Whether the disease is caused by the same mutations in breeds other then the BMD is unknown. Preliminary data suggest they are distinct. As studies are most advanced in the BMD, once genes are identified the researchers will move to the FCR and other breeds to determine if the same, or different mutations are responsible for the disease. Their long- term goal is to produce the information needed for genetic test development.

Publication(s)
- Abadie, J., Hedan B., Cadieu, E., DeBrito, E., Devauchelle, P., Bourgain, C., Parker, H.G., Vaysse, A., Margaritte-Jeannin, P., Galibert, F., Ostrander E.A., Andr?, C. (2009). Epidemiology,

pathology, and genetics of histiocytic sarcoma in the Bernese mountain dog breed, J. Heredity. 100 Supp 1, S19-27.
- Shearin, A.L., Ostrander, E.A., 2010, Leading the way: canine models of genomics and disease. Disease Models & Mechanisms 3, 27-34.
- Shearin, A.L., Ostrander, E.A., 2010, Canine Morphology: Hunting for Genes and Tracking Mutations. PLoS Biol 8, e1000310.

Canine Health Foundation Project 00615B:
Heritable and Sporadic Genetic Lesions in Canine Lymphoma
Grant Status: Open
Dr. Matthew Breen, PhD, North Carolina State University
August 1, 2008 - July 31, 2011

Background: Certain dog breeds are prone to develop certain types of cancer. Between the late 1960's and the early 1980's researchers related the risk of lymphoma for different dog breeds. Yet, there has been little progress since then to define factors that account for this risk. As part of ongoing programs supported by the AKC CHF, the researchers recently showed that the breed-specific risk of lymphoma extends beyond the simple disease condition to a tendency for specific forms of lymphoma. More importantly, the researchers showed there are frequent chromosomal abnormalities that separate with specific forms of lymphoma and that are more common in Golden Retrievers than in other breeds. This suggests breed-specific profiles of genetic abnormalities will be found in canine lymphoma.

Objective: To continue this work, the researchers are using contemporary "array-based" technologies to identify genes that map to these regions and how they contribute to the disease. The researchers anticipate that the results from this work will allow them to predict how genetic factors influence the occurrence of abnormalities in these genes, and will set the groundwork to identify specific genes associated with breed-dependent cancer risk.

Canine Health Foundation Project 00613:
The Prognostic Significance of Chromosome Aneuploidy in Canine Lymphoma
Grant Status: Open
Dr. Matthew Breen, PhD, North Carolina State University
August 1, 2008 - July 31, 2011

Background: Lymphoma is the most common life-threatening cancer in dogs, accounting for up to 24 percent of all canine malignancies. A large proportion of canine lymphomas are responsive to chemotherapy, increasing both the length and quality of an affected dog's life. However, there is considerable difference in the response to therapy working and overall survival time. This shows that there is a need to develop more improved forms of classification. In human lymphoma, the use of cytogenetics has been used to show the presence of frequent chromosome abnormalities that have both diagnostic and predictive importance. In previous studies the researchers have identified frequent chromosome abnormalities in canine lymphoma, including copy number changes (aneuploidy) of dog chromosomes 6, 15, 16, and 18.

Objective: In this project the researchers will use molecular cytogenetics to study a collection of lymphoma specimens, taken from dogs that were all treated with the same chemotherapy procedure as part of a clinical trial. This approach will allow us to determine if these frequent copy number abnormalities are able to predict response. This project hopes to increase the sophistication of diagnosis and life expectancy for canine lymphoma.

Canine Health Foundation Project 00947A:

Heritable and Sporadic Genetic Lesions in Canine Osteosarcoma
Grant Status: Open
Dr. Matthew Breen, PhD, North Carolina State University
August 1, 2008 - July 31, 2011

Background: Certain dog breeds are prone to develop certain types of cancer. Yet, there has been little progress to define the genes that account for this risk.

Objective: For this project, the researchers' goal is to identify genetic abnormalities that are shared by bone tumors and segregate with risk in two dog breeds (Rottweilers and Golden Retrievers) where the disease is prevalent. In collaboration with their colleagues at the University of Michigan and the Broad Institute, they have identified preliminary regions of the genome that may influence risk in Rottweilers. The work described here represents a next step to pinpoint specific genes that are associated with breed-dependent risk, and to predict how heritable factors influence bone cancer in Rottweilers, Golden Retrievers, and other dogs.

Publication(s)
- Thomas, R., Wang, H., Tsai, P.-C., Langford, C., Fosmire, S., Jubala, C., Getzy, D., Cutter, G., Modiano, J., Breen, M., 2009, Influence of genetic background on tumor karyotypes: Evidence for breed-associated cytogenetic aberrations in canine appendicular osteosarcoma. Chromosome Research 17, 365-377.

Canine Health Foundation Project 00947B:
Heritable and Sporadic Genetic Lesions in Canine Osteosarcoma
Grant Status: Open
Dr. Jaime F Modiano, VMD PhD, University of Minnesota
July 1, 2008 - December 31, 2010

Background: Certain dog breeds are prone to develop certain types of cancer. Yet, there has been little progress to define the genes that account for this risk.

Objective: For this project, the researchers' goal is to identify genetic abnormalities that are shared by bone tumors and segregate with risk in two dog breeds (Rottweilers and Golden Retrievers) where the disease is prevalent. In collaboration with their colleagues at the University of Michigan and the Broad Institute, they have identified preliminary regions of the genome that may influence risk in Rottweilers. The work described here represents a next step to pinpoint specific genes that are associated with breed-dependent risk, and to predict how heritable factors influence bone cancer in Rottweilers, Golden Retrievers, and other dogs.

Publication(s)
Thomas, R., Wang, H., Tsai, P.-C., Langford, C., Fosmire, S., Jubala, C., Getzy, D., Cutter, G., Modiano, J., Breen, M., 2009, Influence of genetic background on tumor karyotypes: Evidence for breed-associated cytogenetic aberrations in canine appendicular osteosarcoma. Chromosome Research 17, 365-377.

Canine Health Foundation Project 00975:
Characterization and Modulation of Canine Mast Cell Derived Eicosanoids
Grant Status: Closed
Dr. Cheryl London, DVM PhD, Ohio State University
April 1, 2008 - March 31, 2010

Dr. London's research team has been investigating the production of inflammatory mediators (also known as eicosanoids) by canine mast cells that contribute to a variety of diseases such as atopy and arthritis.

The purpose of their work is to determine if currently available non-steroidal antiinflammatory drugs (NSAIDs) such as Rimadyl and Zubrin (among others) are capable of modifying their production. The use of NSAIDs would be an attractive alternative to the use of corticosteroids to modulate mast cell function. The preliminary data indicate that Zubrin is very effective at blocking the production of mast cell derived eicosanoids when mast cells are exposed to standard doses of drug. Therefore, results from these studies may provide a new strategy to modify the function of mast cells in several inflammatory disorders.

Publication(s)

- Lin, T.-Y., London, C.A., 2010, Characterization and modulation of canine mast cell derived eicosanoids. Veterinary Immunology and Immunopathology 135, 118-127

Canine Health Foundation Project 00982:
Evaluation of Efficacy of Fasaret in Dogs with Osteosarcoma
Grant Status: Closed
Dr. Don Bellgrau, Ph.D., ApopLogic Pharmaceuticals, LLC
April 1, 2008 - March 31, 2010

The goal of this study is to establish efficacy of Fasaret in treating osteosarcoma in canine companion animals. Fasaret is a human adenovirus vector encoding Fas ligand (FasL), a molecule that binds to a "death receptor" called Fas (CD95) that is often highly up-regulated on rapidly dividing cells such as cancers, leukemias, and lymphomas, and on activated white blood cells. Under the appropriate circumstances, engagement of the Fas receptor by FasL induces programmed cell death. Osteosarcoma predominantly affects larger canine breeds. Spontaneous canine osteosarcoma is also very similar to human osteosarcoma, which, when diagnosed, occurs during childhood. This study involves administering Fasaret to dogs at the time of bone biopsy, 10 days prior to limb amputation, should the biopsy confirm a diagnosis of osteosarcoma. Dogs receive standard of care in addition to treatment with Fasaret, which is limb amputation followed by chemotherapy. Dogs are examined for two years under the study, looking at specified time points for increased numbers of dogs that remain cancer free, to determine efficacy. A total of 50 dogs will be examined at the highest dose in this trial, 26 of these dogs being funded by the AKC CHF. All 26 dogs have been enrolled, 20 at CSU and 6 at UMN. The treatment has shown positive results at 90 day survival. Future studies will address improved dosing regiments.

Canine Health Foundation Project 00976:
Investigating the Role of STAT3 Activation in Canine Osteosarcoma
Grant Status: Closed
Dr. Cheryl London, DVM PhD, Ohio State University
April 1, 2008 - March 31, 2010

The purpose of this research was to characterize the role of STAT3 in canine osteosarcoma to assess whether this protein represents a novel target for future therapeutic intervention. Dr. London's research team has made significant progress over the 2 year project, defining STAT3 as important for the growth and survival of osteosarcoma cells and identifying small molecule inhibitors capable of blocking STAT3 function. More recently they have been investigating the potential utility of a derivative of the spice curcumin that blocks STAT3. This derivative, FLLL32, was engineered from curcumin by the Medicinal Chemistry group at OSU to be more potent and more specific for targeting of STAT3 than curcumin. Work with this exciting new product is ongoing. Lastly, they have begun to identify factors that may be responsible for the observed activation of STAT3 in osteosarcoma and this will likely provide a broader range of future targets for therapeutic intervention. The overriding goal of this work was to eventually bring STAT3 inhibitors into clinical trials of dogs with osteosarcoma.

Publication(s)

- Fossey SL, Liao AT, McCleese JK, Bear MD, Lin J, Li P, Kisseberth WK, and London CA, Characterization of STAT3 activation and expression in canine and human osteosarcoma, BMC Cancer, 10:81; 2009.

Canine Health Foundation Project 00615A-T:
Heritable and Sporadic Genetic Lesions in Canine Lymphoma
Grant Status: Closed
Dr. Jaime F Modiano, VMD PhD, University of Minnesota
October 1, 2007 - March 31, 2009

We are aware that these progress reports are made available to representatives from breed clubs or agencies that have contributed financially to support the project. We respectfully remind readers that data included in this progress report may be preliminary and require further confirmation. We request that all contents of this report be maintained in strict confidence until there is public disclosure (publication). The investigators are generally willing to provide updates on the research for newsletters or other outlets that will describe our findings without compromising the integrity of the data.

It has been apparent for some time that certain dog breeds are prone to develop certain types of cancer. To date, there has been little progress to define factors that account for this risk. We showed recently that the breed-specific risk of lymphoma extends beyond the simple disease condition to a predisposition for specific forms of lymphoma. More importantly, we showed there are recurrent chromosomal abnormalities that segregate with specific forms of lymphoma and that are more common in Golden Retrievers (with that form of the disease) than in other breeds, suggesting breed-specific profiles of genetic abnormalities exist in canine lymphoma. To continue this work, we have used contemporary "array-based" technologies to identify genes that map to these regions and how they contribute to the disease. The results from these analysis are in many ways comforting, as they show differences that are proportional to what would be expected based on the known biology of lymphoma. In other words, tumors differ more based on phenotype (B-cell vs. T-cell) than they do based on histologic grade. Also, T-cell malignancies harbor more differences among them than B-cell malignancies. The data show that breedspecific changes extend to age of onset for lymphoma, providing additional support for the central hypothesis, and we have identified preliminary regions in two chromosomes where gene expression signatures follow the same pattern of segregation for the gains and losses of DNA we identified previously. Nevertheless, gene expression differences are subtle, and in the case of breed and gender, they are few in number. This is a remarkable result, since these small differences may define genes and pathways that are heavily involved in risk and progression of the disease in specific breeds. We predict that the results from this work will allow us to define how heritable factors influence the phenotype and biological behavior of these tumors, setting the groundwork to develop better strategies for diagnosis, control, and treatment.

Canine Health Foundation Project 00760:
Cellular Genomics - Molecular Cytogenetic Investigation of Canine Soft Tissue Sarcomas
Grant Status: Closed
Dr. Matthew Breen, PhD, North Carolina State University
October 1, 2007 - September 30, 2009

Histiocytic malignancies, often referred to as histiocytic sarcoma (HS) and/or malignant histiocytosis (MH) are of major health concern to a variety of breeds, including Bernese Mountain Dog and Flat coated Retriever. In this project we aimed to recruit and analyze 75 cases of MH/HS from these two breeds, using genome-wide array based comparative genomic hybridization (aCGH), multicolour fluorescence in situ hybridization (FISH) analysis and loss of heterozygosity (LOH) analysis. Importantly, this study included BMDs from both the USA and from France. As such we were able to determine that there are no significant differences the genome wide aberrations profiles of either population, suggesting that the Berner populations of the USA and Europe may be considered as a single large population for the

purpose of genetic investigations.

Due to additional resources becoming available, we were able to evaluate a total of 133 cases of MH/HS from BMDs and FCRs, and identify numerous recurrent DNA copy number that are shared between these breeds and unique to each breed, The regions of the genome recurrently involved have been interrogated and contain several key genes that are known to be associated with the cancer process. The data in this study revealed several key findings:

1) MH/HS from BMDs resident in the USA and in France have recurrent DNA copy number changes that do not differ significantly, suggesting that the strong association between breed and disease type is not affected by the gene pool of the breed. This indicates that we may be able to consider the BMDs in USA and Europe as a single population. Importantly, as we develop new treatments for this devastating cancer, they likely will apply to all BMDs with MH/HS, regardless of geographic origin.

2) Analysis of histiocytic malignancies from BMDs and FCRs indicates that, in general, there is a gross level of shared genetic changes, with 23 regions across both breeds being recurrent in >30% of the cases. The five most frequently occurring shared regions were highly recurrent in each breed (present in >50% of the cases) and so these data suggest that there is a strong association between genomic change and histiocytic malignancies.

3) At least three of these five highly recurrent shared regions contain genes that are known to be associated with cancers.

4) This study detected 13 aberrant region of the canine genome (on seven different chromosomes) that are significantly associated with the presence of HS affecting either internal organs or just a limb. This association also separated breed (BMD 'vs' FCR) and so it is not possible to determine if there is a correlation between 1) breed and cytogenetic profile, or between 2) anatomical location of the MH tumors internal masses and cytogenetic profiles, since there is such a strong association between BMDs and the presence of internal masses. To resolve this we would need to investigate the cytogenetic profiles of other breeds that present with internal MH masses. Further analysis would allow us to determine if it mass location or breed that drives the cytogenetic profiles.

5) Analysis of 25 cases of hemangiosarcoma in a range of breeds, using 1Mb resolution aCGH analysis, revealed the presence of highly chaotic genome reorganization. Many of the chromosome changes present are shared with other cancers, but we are unable to form any concrete conclusions about breed association or disease association without further case analysis.

The next phase of this investigation will be to evaluate the status of the genes we have identified to be in regions of significance, both in affected and unaffected individuals.

Canine Health Foundation Project 00779:
Characterization of the Canine Y Chromosome: Identifying Genes that Cause Male Infertility
Grant Status: Closed
Dr. William J. Murphy, PhD, Texas A&M University
July 1, 2007 - December 31, 2009

The causes of male infertility in dogs are not well known. Though much is now known about genes on the dog autosomes and X chromosome, owing to the canine genome sequence, virtually nothing is known about the canine Y chromosome and the genes it harbors. The causes of male infertility in dogs are not well known. Though much is now known about genes on the dog autosomes and X chromosome, owing to the canine genome sequence, virtually nothing is known about the canine Y chromosome and the genes it harbors. Studies of the human and mouse Y chromosomes have shown that they contain many testis-specific genes that when defective cause infertility and spermatogenesis defects. This study aimed

to characterize the gene content of the dog Y chromosome by sequencing from a cDNA selection library that is enriched for Y chromosome gene transcripts, and mapping these in the canine genome. Dr. Murphy and his team identified gene sequences from fifteen canine Y chromosome genes, characterized seven new canine-specific Y genes, and 15 novel candidate genes. Determining the copy number and function of these novel genes are of primary importance, as they are primary infertility candidate genes. Gene expression experiments identified that eight of the novel dog genes are expressed only (or predominantly) in testes, implying a role in spermatogenesis. The researchers assembled a first-generation physical map in collaboration with the Washington University Genome Center, as a prerequisite to eventually obtain the sequence of the dog Y chromosome using Next-Generation DNA sequencing technologies, as funded by CHF grant 1262. A DNA sequence will allow the most detailed information for designing genetic tests to determine whether deletions in these genes lead to abnormal spermatogenesis in infertile dogs.

Canine Health Foundation Project 00768:
A Collaborative Study by Veterinary Oncologists, Pathologists and Diagnostic Laboratories to Enhance the Detection, Diagnosis and Treatment of Canine Lymphoma
Grant Status: Open
Dr. Ted Valli, DVM, University of Illinois
July 1, 2007 - December 31, 2010

Background: Lymphoma is the most common canine cancer treated by chemotherapy and a most common neoplasm that afflicts dogs of all breeds and ages. Many of the malignancies that occur in dogs are like those that occur in humans, especially for the tumors of the lymphoid system. The World Health Organization has devised a new system of recognizing and categorizing the many subtypes of lymphoid tumors with very different characteristics that must be considered in providing effective treatments. Currently lymphomas in dogs are treated as if they are all of the same type, but we now find that like those in humans the canine lymphomas are of many types that also benefit from specific identification and treatment.

Objective: The goal of this study is to demonstrate that veterinary diagnosticians can effectively apply the human criteria to the canine tumors and thus permit much more effective treatment by veterinary oncologists. This application will alter costs of treatment according to tumor type and increase survival in animal companions that share our lives and environments.

Publication(s)
- Valli, V.E., Myint, M.S., Barthel, A., Bienzle, D., Caswell, J., Colbatzky, F., Durham, A., Ehrhart, E.J., Johnson, Y., Jones, C., Kiupel, M., Labelle, P., Lester, S., Miller, M., Moore, P., Moroff, S., Roccabianca, P., Ramos-Vara, J., Ross, A., Scase, T., Tvedten, H., Vernau, W., 2010, Classification of Canine Malignant Lymphomas According to the World Health Organization Criteria. Veterinary Pathology Online.

Canine Health Foundation Project 00757A:
Hereditary Mutations in Genes Associated with Osteosarcoma in Large Dog Breeds
Grant Status: Closed
Dr. Kerstin Lindblad-Toh, PhD, Broad Institute
April 1, 2007 - September 30, 2009

In the completed CHF study "Mapping Genes Associated with OSA in Large Breed Dogs", the investigators have identified genomic regions associated with OSA in Rottweilers and Greyhounds using genome-wide association with the newly developed ~27,000 SNP array. Results of genome-wide scans show that three regions are associated with OSA from the genome-wide screen in Rottweilers and three different and non-overlapping regions are associated with OSA in Greyhounds. In this study, they

conducted further fine-mapping of these candidate regions using additional Rottweiler samples paired with Mastiff-type breeds (Golden Retrievers and Leonbergers) and, likewise, additional Greyhound samples paired with Long-limbed Hound type breeds (Irish Wolfhounds and Great Danes). They have now performed fine-mapping in nine breeds (Rottweilers, Golden Retrievers, Labrador retrievers, Leonbergers, Great Pyrenees, Mastiffs, Greyhounds, Great Danes and Irish Wolfhounds.) All six loci are supported, and several candidate genes have been interrogated for mutations. Since no coding candidate mutations have been identified so far, the researchers believe that regional resequencing will be necessary and the methodology to do this has been developed. They have also continued to fine-map in larger sample numbers to identify the most highly associated regions in preparation for regional resequencing to identify mutations. Regional resequencing is now ongoing to identify mutations.

This project is continuing under CHF Grant 1317.

Publication(s)

- Karlsson EK, Baranowska I, Wade CM,, Salmon Hillbertz NHC, Zody MC, Andersson N, Biagi T, Patterson N, Rosengren Pielberg G, Kulbokas EJ III, Comstock KE, Keller ET, von Euler H, K?mpe O, Hedhammar A, Lander ES,, Andersson G, Andersson L, & Lindblad-Toh K Efficient mapping of mendelian traits in dogs through genome-wide association (2007) Nat Genet. 2007 Nov;39(11):1321-8.

Canine Health Foundation Project 00778:
Role of Regulatory T Cells in Dogs with Osteosarcoma
Grant Status: Closed

Dr. Barbara Biller, D.V.M., Colorado State University

April 1, 2007 - September 30, 2009

The primary goal of this study was to investigate a specific type of lymphocyte, known as a regulatory T cell (Treg), in dogs with osteosarcoma (OSA). Treg are known to be present in abnormally high numbers in human cancer patients where they have been shown to interfere with the immune system?s ability to detect and kill cancerous cells. In addition, determination of Treg levels can provide prognostic (predictive) information for many types of human malignancies.

Our objectives for this project were to: 1) determine whether dogs with OSA have high numbers of Treg compared to healthy dogs, 2) determine if surgical removal of the tumor (limb amputation) or the type of chemotherapy given after surgery changed Treg numbers compared to their pre-treatment values, and 3) evaluate whether OSA dogs with high Treg numbers had a poorer prognosis than dogs with low numbers of Treg.

We enrolled a total of 18 dogs with OSA and followed their progress through the study and afterwards. We also compared Treg and other important T lymphocyte subsets (CD4+ and CD8+ T cells) between the dogs with cancer and 22 healthy control dogs. We found that dogs with OSA had significantly more Treg in their blood compared to healthy dogs. Although removal of the tumor did not change Treg numbers within a 24 hour period following surgery, there was a significant increase in Treg between pre-treatment blood samples and those collected 7 - 10 days after the first chemotherapy treatment. This suggests that Treg numbers may continue to rise despite the initiation of treatment for OSA.

One of the most important findings to emerge from this study was that determination of the ratio of the percent of CD8+ T cells (a subset of T lymphocytes) to the percent of Treg was predictive for survival (short versus long) in the dogs with OSA. This ratio has been used to determine outcome in some types of human cancers but this is the first time we have applied this ratio to better understand cancer in dogs. This information could be very useful to veterinary professionals in helping owners of dogs with OSA make informed treatment decisions.

Publication(s)

- Biller, B., Guth, A., Burton, J., Dow, S., 2010, Decreased Ratio of CD8+ T Cells to Regulatory T Cells Associated with Decreased Survival in Dogs with Osteosarcoma. Journal of Veterinary Internal Medicine 24, 1118-1123.

Canine Health Foundation Project 00790:
MicroRNA Profiling and MicroRNA-Based Treatment of Canine Cancers
Grant Status: Open
Dr. William C Kisseberth, DVM PhD, Ohio State University
April 1, 2007 - September 30, 2010

Background: Cancer is a common disease in dogs of all breeds and is a leading cause of disease-related death. Because cancer is basically a genetic disease, both understanding the genetic basis of cancer and treating cancer are important to dog owners and breeders alike. Much of the progress in diagnosis, prognosis, and treatment of cancer in people has been the result of advances in studying genomes. MicroRNAs (miRNA) are small non-protein coding molecules that have been implicated in humans as having an important role in cancer and a variety of other diseases.

Objective: The goals of this study are to identify important canine miRNAs that can be used to improve cancer diagnosis and treatment in the dog. The researchers will identify miRNAs in selected common canine cancers (osteosarcoma, transitional cell carcinoma, melanoma) using miRNA microarrays. They will then identify miRNAs that are potential targets for new cancer therapy drug development. MiRNAs that significantly effect cancer cell growth will be investigated further to determine how they exert their effects.

Canine Health Foundation Project 888-A:
Generation of Canine Single Chain Fragment Variable Antibody Libraries for the Identification and Targeting of Tumor-Associated Antigens in the Dog
Grant Status: Closed
Dr. Nicola J Mason, BVetMed, PhD, University of Pennsylvania
February 1, 2007 - January 31, 2008

Cancer is the leading cause of disease related death in our current canine pet population. The mainstay of cancer therapy is the systemic administration of chemotherapeutic agents that inhibit cell division and induce cell death. These agents however are not tumor-specific and frequently cause adverse side effects which limit the dose that can be given and the therapeutic efficacy of the agent. It is the aim of this proposal to develop a method to generate synthetic antibodies that will in the future be used to identify canine cancer targets and deliver cytotoxic agents directly to canine tumors, resulting in reduced systemic toxicity and increased therapeutic efficacy of the agent against the tumor. White blood cells known as B lymphocytes produce antibodies and are part of the body?s immune defense system which is involved in the recognition and destruction of invading infectious agents and tumors. B cells recognize different targets via different B cell receptors (antibodies) displayed on their surface and the specificity of each B cell receptor/antibody is determined by the genetic code located within the B cell itself. Molecular techniques performed on a peripheral blood sample can be used to clone these receptors and generate synthetic antibodies that can be screened to determine what their target molecules are. Recent data using this technology have shown that human cancer patients generate antibody responses against their own tumors and this has led to the identification of tumor-associated molecules that can be targeted by the synthetic antibodies. In this proposal we aim to take advantage of the recently sequenced canine genome in order to develop molecular techniques that will allow us to examine the immunological responses that canine caner patients make to their own tumors. In the future, these techniques will be used to identify tumor molecules present in canine cancer patients and to generate synthetic canine antibodies that could be used therapeutically in vivo to target them.

Canine Health Foundation Project 837-A:
Prognostic Significance of Ezrin Expression in Canine High-Grade Soft Tissue Sarcoma
Grant Status: Closed
Dr. Kathryn Vickery, VMD, Colorado State University
September 1, 2006 - August 31, 2007

"Soft tissue sarcomas (STS) are a group of malignant cancers arising from the skin and structures below the skin (connective tissues, muscles, and other structures), in predominately large breed dogs. These tumors tend to infiltrate through surrounding structures and destroy the normal tissue architecture. Low and intermediate grade STS are unlikely to spread to other organs whereas 44 percent of the high grade STS may spread to organs such as the lungs and lymph nodes. It is currently impossible to predict which high grade STS will go on to spread and therefore which patients might benefit from treatment (such as chemotherapy) to attempt to delay or prevent spread. Ezrin is a protein found on cell surfaces that has been shown to promote the spread of cancer. Recent studies have demonstrated the correlation between ezrin expression and cancer spread in canine osteosarcoma (bone cancer) as well as in human STS. The purpose of this study is to evaluate the expression of ezrin and its usefulness as a prognostic marker in canine high grade STS. This information may provide a more accurate prediction of tumor behavior and help determine the need for post-surgical therapies to address potential spread."

Canine Health Foundation Project 826-A:
Percutaneous Cryoablation in Canine Osteosarcoma
Grant Status: Closed
Dr. Dale Bjorling, DVM, University of Wisconsin, Madison
August 1, 2006 - July 31, 2007

Osteosarcoma is the most common primary bone tumor of dogs. This cancer most often affects long bones (radius, humerus, femur, and tibia) creating significant pain and lameness in affected dogs. The most common treatment for this disease is limb amputation that results in average survival times of 4 months without, and 12 months with, postoperative chemotherapy. Owners are often reluctant to have their dogs undergo limb amputation due to the relatively short postoperative life expectancy, especially in giant breed dogs and dogs with concurrent orthopedic problems. Elimination of the primary tumor is not curative, and 98 percent of these dogs have some form of metastatic disease at the time of diagnosis. In special circumstances, a limb-sparing procedure may be performed during which the tumor-laden bone is removed and replaced with an implant. While this surgery often creates a functional limb, it has significant limitations. It is only suitable for a subset of cases, infection rates are high, it is relatively expensive, and tumors grow back locally in up to 30 percent of dogs. Palliation and pain reduction may be achieved by administration of 1-4 doses of radiation therapy for dogs not treated with surgery. About 74 percent of dogs receiving radiation experience pain reduction, with a median response time of 2.5 months. Unfortunately, radiation may only be given once and duration of pain relief is relatively short. Thus, while there are current treatment options that are tailored to individual patients based on owner preference and individual patient factors, including arthritis, body conformation, anticipated ability to walk on 3 legs, etc., none of these are optimal. Radiation is the most common therapy for humans with painful metastatic bone cancer, and response rates are similar to those observed in dogs. Cryoablation of tumors has recently become a viable minimally-invasive treatment option for malignant and benign bone tumors in humans. This technology uses a small (1.7-2.4 mm) probe insert

Canine Health Foundation Project 00591:
In Vitro Effects of the Milk Thistle Extract Silibinin in Canine Tumor Cells
Grant Status: Closed
Dr. Douglas H Thamm, VMD, Colorado State University
April 1, 2006 - March 31, 2007

Many forms of canine cancer are currently incurable, and novel treatments are needed. The phytochemical silibinin, the bioactive constituent in the herb milk thistle, has been shown to induce growth arrest and apoptosis, inhibit angiogenesis, and decrease tumor cell invasion in various human tumor cells, and inhibit tumor growth in a variety of mouse models of cancer. In parallel with phase-I dose escalation and pharmacokinetic studies currently ongoing in tumor-bearing dogs at the Animal Cancer Center at Colorado State University, researchers propose to evaluate silibinin in vitro against a panel of canine tumor cell lines, including hemangiosarcoma, osteosarcoma and transitional cell carcinoma. Silibinin will be evaluated for its ability to: 1) induce growth arrest; 2) induce apoptosis; 3) enhance chemosensitivity; 4) inhibit transwell migration and Matrigel invasion. Successful demonstration of in vitro antitumor activity in these currently incurable cancers will provide important preliminary data justifying further clinical evaluation of silibinin in canine cancer.

Canine Health Foundation Project 00593A:
Mapping Genes Associated with Canine Hemangiosarcoma
Grant Status: Closed
Dr. Kerstin Lindblad-Toh, PhD, Broad Institute
April 1, 2006 - September 30, 2008

Hemangiosarcoma (HSA), a malignant tumor of vascular endothelial cells, is a significant health concern in dogs, with an incidence of ~2% of all tumors. A national heath survey of golden retriever reported that neoplasia accounted for >60% of all reported deaths and HAS was the most common malignant tumor affecting >15% of golden retrievers. A particularly high disease incidence of hemangiosarcoma in golden retriever suggests a genetic susceptibility. The purpose of this study is to identify the mutations causing the increased risk for hemangiosarcoma in golden retriever. To do this, we have proposed to compare the genotypes of dogs diagnosed with HSA with healthy older dogs using a statistical analysis. To date, we have collected 125 blood samples from golden retrievers diagnosed with HSA and more than 400 healthy golden retrievers over 8 years old. We have identified six regions in chromosomes associated with HSA and are have narrowed these to precise regions (a few hundred thousand base). We now need to find the precise mutations that cause the disease and link them to functionality. In the long term, this work should allow the development of specific genetic tests for carriers of HSA. Ultimately, understanding of the disease biology, which will lead to identification of target genes for prevention, early detection and novel treatments of this malignancy.

Canine Health Foundation Project 678:
Generation and Analysis of Canine Bone Marrow Derived Mast Cells
Grant Status: Closed
Dr. Cheryl London, DVM PhD, Ohio State University
April 1, 2006 - March 31, 2007

Disorders of mast cells, particularly mast cell tumors (MCTs), are common in dogs and there is now evidence that many of these exhibit breed specific tendencies, suggesting underlying genetic causes. This is particularly important for MCTs in which certain breeds (e.g., Pugs) develop benign disease while others (e.g., Chinese Shar-Pei) develop malignant disease. In comparison to humans and mice, very little is known about the biology of normal canine mast cells. Such knowledge is critical for understanding how mast cell disorders develop. In other species, large numbers of mast cells can be generated from purified bone marrow cells (termed BMMCs) and these are used to study normal mast cell biology. We have recently been successful in generating canine BMMCs from canine bone marrow. The purpose of this proposal is to fine tune the process of canine BMMC generation and to study the functional properties of mast cells in detail. These studies will supply important new information regarding the biology of normal canine mast cells and provide a platform for future work investigating the genetic basis of mast cell disease in dogs.

Canine Health Foundation Project 00390: Extended Medical Surveillance of Dogs Deployed to the World Trade Center and the Pentagon
Grant Status: Closed
Dr. Cynthia M. Otto, DVM PhD, University of Pennsylvania
January 1, 2006 - December 31, 2008

We have been monitoring the health and behavior of the search and rescue dogs deployed on 9/11/01 to the World Trade Center and Pentagon disasters since shortly after those events took place. During the first year of surveillance, significant changes were identified in the blood work of the deployed dogs versus the control dogs. These changes proved temporary however, and in year two of the study, blood work values mostly returned to normal. These initial changes suggest that deployed dogs were exposed to more hazardous substances during deployment and ultimately these substances are likely to cause long-term health changes. Additionally, ten deployed dogs and two control dogs have died since surveillance began. This rate of mortality, while not completely unexpected in this size population, is important considering the major cause of death in deployed dogs was cancer. Another four deployed dogs have been diagnosed and are currently living with cancer. It is essential that we evaluate these dogs throughout their lifespan to determine whether the 9/11 deployment is a factor in the rate and onset of cancer in these dogs.

Canine Health Foundation Project 22101T:
Development of Anti-Canine Il-2R? Antibodies Using CpG Oligodeoxynucleotide Vaccination
Grant Status: Closed
Dr. Stuart Helfand, DVM, Oregon State University
January 1, 2006 - June 30, 2006

Despite progress in treating canine lymphoma, most affected dogs eventually develop resistance to chemotherapy and succumb to their disease. In human medicine, the a subunit of the interleukin-2 receptor (IL-2Ra) has been developed as a target for immunotherapy of chemoresistant lymphoma patients. Intensively studied as a molecule present on normal lymphocytes that are activated, IL-2Ra has recently been developed as a target on malignant lymphocytes that have been found to express this protein inappropriately. This has been accomplished using anti-IL-2Ra monoclonal antibody (Mab) to deliver cellular toxins or radioisotopes directly to the cancer cells, a strategy that overcomes drug resistance. We would now like to develop such an antibody against the canine Il-2Rasubunit that could benefit dogs with lymphoma. Our rationale for pursuing this approach stems from molecular data we generated indicating that a high percentage of canine lymphomas from Golden Retrievers and Rottweilers express Il-2Ra Having synthesized canine recombinant (cr) IL-2R?a using the gene sequence we cloned, we are now ready to produce and validate Mabs against it. To accomplish these goals, 1) we will employ a novel immunization strategy to prepare murine anti-canine IL-2Ra Mabs, 2) develop a test using anti-canine IL-2Ra Mabs for the detection of IL-2R?ashed into the blood of Golden Retriever and Rottweiler lymphoma patients, and 3) screen lymphoma biopsies from Golden Retrievers and Rottweilers for IL-2Ra. Mabs generated to Canine IL-2R?a will be a powerful tool in the diagnosis, prognosis, and treatment of canine lymphoma.

Canine Health Foundation Project 00632:
MicroRNAs and Canine Lymphoma
Grant Status: Open
Dr. William C Kisseberth, DVM PhD, Ohio State University
October 1, 2005 - September 30, 2010

Lymphoma is one of the most common cancers in the dog. The current classifications of lymphoma do not explain or predict its changing clinical behavior. Much of the progress in diagnosis, prognosis, and

treatment of lymphoma and other cancers in people has been the result of advances in "genomics." Recently the canine genome has been sequenced, providing the opportunity to apply new genomic approaches to better understand and treat cancer in the dog. MicroRNAs (miRNA) are small non-protein coding molecules that have been linked in humans as having an important role in cancer and a variety of other diseases. In this study, the researchers will identify miRNAs using bioinformatic methods. The researchers will then use miRNA microarrays to study normal canine tissues and canine lymphoma biopsies. These results (miRNA expression profiles) will be linked with previous diagnosis and clinical restrictions. The goals of this study are to identify canine miRNAs and their normal patterns of expression and to determine if specific subtypes of lymphoma are characterized by unique miRNA expression profiles, if specific miRNAs have predictive importance, and to identify potential goals for future investigation and therapies. This study will also generate new tools for future miRNA investigation in the dog.

Canine Health Foundation Project 00372:
Determination of Breed-Specific Reference Ranges for Assessing Thyroid Function in Several Breeds
Grant Status: Open
Dr. Rebecca L. Davies, PhD, University of Minnesota
July 1, 2005 - June 30, 2010

Background: The thyroid gland secretes hormones which are very important for development, growth, reproduction and metabolism. Sometimes the thyroid gland does not produce enough thyroid hormone and hypothyroidism occurs. Hypothyroidism is very common in dogs and many are treated with thyroid hormone supplementation. This disease occurs frequently in Alaskan Malamutes, English Setters, Golden Retrievers, Keeshonden, Samoyeds, and Siberian Huskies. Hypothyroidism is generally diagnosed by measuring the level of thyroid hormones in serum. This level is then compared to a reference interval made from measurements of thyroid hormone in samples taken from large groups of normal dogs. Generally this works very well, however, in some breeds, the true reference range is lower than the range determined when dogs of various breeds (or mixed-breeds) are analyzed. Breed-specific thyroid hormone reference intervals have only been determined in a few breeds. Without breed-specific intervals, inappropriate use of general values may result in healthy dogs being misclassified as hypothyroid. These animals may be incorrectly placed on thyroid supplementation, and unnecessarily removed from breeding programs.

Objective: The researchers wish to establish breed-specific normal thyroid reference intervals to improve the diagnosis of true hypothyroidism in the Alaskan Malamute, English Setter, Golden Retriever, Keeshond, Samoyed, and Siberian Husky breeds.

Publication(s)
- Sharkey L, Gjevre K, Hegstad-Davies R, Torres S, Munoz-Zanzi C. Breed-associated variability in serum biochemical analytes in four large-breed dogs. Veterinary Clinical Pathology ISSN 0275-6382.
  - Manuscript preparation (Breed- Spedific Reference Intervals for Assessing Thyroid Funcation in Seven Dog Breeds. RL Hegstad-Davies, SMF Torres, LC Sharkey, CA Mu?oz-Zanzi, SC Gresch

Canine Health Foundation Project 349:
Genetic Epidemiology of Cancer in the Golden Retriever
Grant Status: Closed
Dr. K. Ann Jeglum, DVM, The Wistar Institute
July 1, 2005 - June 30, 2008

We have collected pedigrees from Golden Retrievers with cancer and constructed a large pedigree map of the affected dogs. As reported in the 1998 GRCA National Health Survey, the most common tumors have been lymphoma and hemangiosarcoma as well as other soft tissue sarcomas. These were aggressive cancers and many occurred at a young age. These are clinical indicators of a possible genetic predisposition. We propose to conduct a statistical evaluation known as segregation analysis to determine a possible pattern of inheritance. Additionally, we will gather epidemiological data through owner questionnaires to evaluate the role of environmental and socioeconomic factors in the diagnosis of these cancers. There is great concern amongst breeders concerning non-genetic factors such as diet, vaccinations, passive smoking, flea and tick control, and stress and their role in developing cancer. Another question is whether the overall high incidence is related to the owners' financial ability and willingness to diagnose and treat. Blood and tissue samples have been and will continue to be collected from affected and unaffected relatives and will be made available to investigators conducting DNA based analyses.

Canine Health Foundation Project 336A:
Mapping of the Gene for Malignant Histiocytosis in the Bernese Mountain Dog
Grant Status: Closed
Dr. Elaine Ostrander, PhD, National Human Genome Research Institute
April 1, 2005 - March 31, 2007

We are interested in finding the genes that predispose the Bernese Mountain Dog (BMD) to malignant histiocytosis (MH) for three reasons. First, we are interested in improving the health of this increasingly popular breed, which is severely affected by this deadly disease. Finding the underlying disease gene will lead to the development of genetic tests that can ultimately generate improvements in breeding programs. Second, once we know the underlying cause of MH we can begin to work toward targeted therapies that improve both life, quality and duration for affected dogs. Finally, we are interested in the genetics of similar disorders affecting humans. We propose two complementary strategies: 1) Family based linkage analysis to track the disease gene in a large BMD family affected with MH; and 2) An association-based linkage study encompassing a minimum of 119 affected and at least 100 unaffected control dogs. Both approaches can be expected to highlight regions of the genome where a gene of interest may lie. In summary, these approaches will allow us to eliminate false positive results, increase statistical power for fine linkage mapping, and provide a large resource for mutation screening.

Canine Health Foundation Project 373A:
Mapping Genes Associated with Osteosarcoma in Large Dog Breeds
Grant Status: Closed
Dr. Kerstin Lindblad-Toh, PhD, Broad Institute
January 1, 2005 - December 31, 2006

Eight thousand to ten thousand cases of osteosarcoma, a malignant bone tumor, are reported in dogs in the United States annually, representing a significant health concern. In the majority of cases, spread of the tumor throughout the body and death follows within a few years. Osteosarcoma affects all dogs, but the disease frequency is considerably higher in large and giant breeds, including the long-limbed hounds (Irish Wolfhound, Great Dane, Greyhound, Scottish Deerhound, Rhodesian Ridgeback, Great Pyrenees and Borzoi) and Mastiff-type breeds (Rottweiler, Labrador Retriever, Flat-Coated Retriever, Golden Retriever, Mastiff, Bullmastiff, Saint Bernard, Irish Setter, and Newfoundland). It is clear the genetics play an important role. We propose to identify the genetic risk factors for osteosarcoma in two breeds: Greyhound and Rottweiler. While certain characteristics of these two breeds make them ideal to study, we expect that the genes identified in these breeds may also be associated with osteosarcoma in related breeds. This study should lead to the development of genetic tests for osteosarcoma that could be used to eliminate carriers from breeding populations, eventually reducing the frequency of this devastating cancer. Ultimately, it could also lead to improvements in treatment of osteosarcoma.

Canine Health Foundation Project 249:
Genomics of Canine Brain Neoplasia
Grant Status: Closed
Dr. Matthew Breen, PhD, North Carolina State University
October 1, 2004 - September 30, 2006

Genetic abberations underlie many different types of cancer. Identification of these aberrations provides important information on the malignancy of different cancers, but until recently it has been extremely labor intensive to screen for such anomalies. Gene expression profiling is a technique that provides information on the level of expression of thousands of genes in a single assay, greatly improving the efficiency of screening for genetic aberrations. We hypothesize that there are tumor-specific differences in gene expression in canine brain tumors, which will correspond to and be predictive of clinical outcome. As certain breeds of dog (e.g. the Boxer, Boston Terrier and Golden Retriever) are predisposed to developing brain tumors, we may find breed specific genetic aberrations that are associated with the development of brain tumors. In this study, we will apply gene expression profiling to tissue taken from naturally occurring brain tumors (obtained during routine diagnosis and treatment) to identify tumor specific differences in gene expression. We will correlate these differences with tumor type and the clinical course of disease to identify prognostic factors for survival. This study may enable us to identify prognostic factors and novel therapeutic targets applicable to many different types of canine cancer.

Canine Health Foundation Project 415:
Anti-HLA-DR Antibody Therapy in Canine B-cell Lymphoma: Preliminary Clinical Evaluation
Grant Status: Closed
Dr. Rodney Page, DVM, Cornell University
October 1, 2004 - September 30, 2005

Canine lymphoma is a frequently occurring, temporarily controllable form of cancer that is similar to high-grade non-Hodgkin?s lymphoma in people. The best conventional chemotherapy results in rapid improvement, but ultimately relapse and progression occur. Adjustment of current chemotherapy protocols is unlikely to result in substantial gains in survival due to development of multiple mechanisms of drug resistance occurring during treatment. Therefore, new strategies that have demonstrated efficacy in humans are worth developing for dogs. An antibody that recognizes cancer cells and stimulates the patient's immune system to eliminate the cancer is an example of such a strategy. We have determined that an antibody made against human lymphocytes cross-reacts with canine lymphoma and causes cell death. This antibody has been confirmed to be safe in normal dogs. We propose to optimize the administration of this antibody in dogs that have already failed chemotherapy for lymphoma. We will evaluate the safety and potential efficacy of this antibody as a prelude to more extensive testing in dogs with lymphoma. This antibody also recognizes cells from dogs with malignant histiocytosis and may be useful for management of this disorder as well.

Canine Health Foundation Project 267:
Investigation into Combined Molecular Approaches to Treat Hemangiosarcoma
Grant Status: Closed
Dr. David J. Argyle, PhD, University of Wisconsin, Madison
July 1, 2004 - June 30, 2005

Hemangiosarcoms (HSA) is a common and fatal cancer in dogs for which there is no effective treatment. Despite surgery and intensive chemotherapy, the median survival time for dogs diagnosed with HSA is little more than six months. From our own studies on canine cancer, expression of the enzyme telomerase allows cancer cells to become immortal and has emerged as a central and near universal marker of cancer, making it a candidate target for novel therapies. In this study we will explore the value of telomerase inhibition to treat HSA using the novel mechanism of RNA interference (RNAi). Our

hypothesis is that potent inhibition using this technique will inhibit the immortal phenotype of the cancer cells and cause them to die. However, it is possible that a combined approach, targeting two molecular pathways, may offer greater therapeutic benefit. Consequently, we will also explore the potential synergism of combining telomerase inhibition with an alternative inhibitor of a further mechanism involved in cancer (receptor tyrosine kinase inhibition) on targeting this highly malignant tumor. In this we will use in vitro cell culture techniqus for initial inhibition studies followed by studies in a novel canine HSA model system to ascertain the potential clinical merit of this approach for dogs with HSA.

Canine Health Foundation Project 305:
Histocompatibility Alleles Conferring Susceptibility to Canine Diabetes, Immune-Mediated Thyroiditis and Immune-Mediated Hemolytic Anemia
Grant Status: Closed
Dr. Wayne Potts, PhD, University of Utah
July 1, 2004 - June 30, 2006

Autoimmune diseases cause significant amounts of mortality and debilitating disease in dogs. In humans many autoimmune diseases occur only in individuals expressing one of the few predisposing histocompatibility genes. For example, all cases of type I diabetes in humans are associated with only a few of the many alleleic forms of class II histocompatibility genes. Consequently, if the frequencies of these few alleles were reduced by half, the incidence of diabetes would be reduced by half. Here it was proposed to characterize histocompatibility susceptibility alleles for three major, heritable canine autoimmune diseases - diabetes, immune-mediated thyroiditis and immune-mediated hemolytic anemia. If any of these three debilitating (or lethal) autoimmune diseases have a restricted number of susceptibility alleles it will allow: (1) development of diagnostic tests for identifying individuals at risk for prophylactic therapy and research and (2) reduction of he incidence of the disease by selective breeding of individuals carrying the predisposing histocompatibility alleles. For each of the three autoimmune diseases, the researchers proposed to collect DNA samples from approximately 100 purebred dogs diagnosed with the disease and clone and sequence the histocompatibility genes. From the data collected, no genetic association could be detected between the affected and unaffected samples.

Canine Health Foundation Project 237:
Molecular Epidemiology of Ehrlichia and Bartonella spp. Infection in Golden Retrievers with Lymphoma
Grant Status: Closed
Dr. Edward B Breitschwerdt, DVM, North Carolina State University
July 1, 2004 - June 30, 2006

Bartonella spp. are a group of related bacteria, most of which have only been discovered within the last 10 years. They are able to infect and survive inside cells, causing persistent infections in mammals. Infection with Bartonella spp., however, does not always cause disease manifestations and for this reason, a positive blood test documenting infection with Bartonella spp. does not necessarily mean that Bartonella is the cause of an animal?s disease. However, in people, there is growing evidence implicating Bartonella spp. as a cause of a broad spectrum of disease syndromes, and there is some evidence to support the potential that chronic Bartonella infection may contribute to the development of cancer. The purpose of this study is look for evidence of Bartonella infection in Golden Retrievers with lymphoma, as compared to a healthy control group. We will use standard serologic tests which are currently available for Bartonella spp. testing of dogs, but we will also use a newer, more broadly reaching method of molecular testing. This will allow us to test for a larger number of Bartonella spp., and may potentially provide greater test sensitivity. As previous work from our laboratory has documented co-infection with B. vinsonii (berkhoffii) and Ehrlichia canis, another tick transmitted bacteria, we will test for both of these organisms in this study.

Canine Health Foundation Project 272:
Oligonucleotide Microarray Gene Expression Profiling of Canine Lymphoma
Grant Status: Closed
Dr. William C Kisseberth, DVM PhD, Ohio State University
July 1, 2004 - June 30, 2006

Lymphoma is one of the most common cancers seen in the dog. Current methods of classifying lymphoma neither explain nor predict its variable clinical behavior. While the majority of canine lymphomas appear microscopically similar and affected dogs show similar clinical signs, the clinical course of the disease can vary significantly in patients with microscopically identical tumors with identical clinical signs. This heterogeneity in behavior is particularly evident with respect to response to chemotherapy. Although the majority of patients initially respond well to chemotherapy, some are disease-free for a few months, while others remain disease-free over two years. Clearly, microscopic and initial clinical appearances inadequately explain the variable clinical behavior. In order to better understand and explain these differences, we will create and develop a specialized dog lymphoma gene microarray; a new tool that can be used to determine which groups of genes are important in different sub-types of lymphoma. Ultimately, by identifying these important groups of genes, we hope to 1) provide better prognostic information regarding individual tumor clinical behavior, 2) identify important groups of genes that characterize unique lymphoma sub-types, and 3) identify new molecules or genes that can be targets for development of new drugs to treat lymphoma

Canine Health Foundation Project 212A:
Development of a New Resource for Positional Cloning of Hip Dysplasia Genes: A High Density SNP Map of Canine Chromosome One
Grant Status: Closed
Dr. Elaine Ostrander, PhD, Fred Hutchinson Cancer Research Center
April 1, 2004 - March 31, 2006

Genome maps are essential for identifying disease genes. The current canine map is composed of several thousand markers, and as a result, has proven useful for localizing several disease genes. A much more highly refined map is necessary if we are to actually clone disease genes of interest (not just identify their location) and, subsequently, develop appropriate genetic tests. This proposal aims at developing the technology to do that, focusing on a test case on chromosome one, where two genes associated with hip dysplasia in the Portuguese Water Dog have been mapped. The goal is to determine the location and linear order of many hundred small variants called ""SNPs"" throughout chromosome one. The resulting SNP map can then be used 1) by ourselves to identify the culprit gene(s) in PWD and 2) by anyone studying hip dysplasia in any breed of dog to determine if the same chromosomal region that is mutated in the Portuguese Water Dog is similarly responsible for disease in other breeds.

Canine Health Foundation Project 2667:
Cellular Genomics - Molecular Cytogenetic Investigation of Canine Soft Tissue Sarcomas
Grant Status: Closed
Dr. Matthew Breen, PhD, North Carolina State University
April 1, 2004 - March 31, 2006

It has been established that non-random chromosome aberrations are characteristic of specific types of many different human cancers. The knowledge of such aberrations has identified areas of the human genome to be targeted for further research. In the dog the extent and identity of chromosome aberrations associated with specific cancers is still largely unknown. In certain breeds, such as the Flat-Coated Retriever and Bernese Mountain Dog, soft tissue sarcomas account for up to 50 percent of all malignant tumors and thus represent a serious health and welfare issue for those breeds. These tumors are difficult

to classify by conventional means and so attention is required to develop alternative approaches. Human soft tissue sarcomas have been demonstrated to be associated with specific chromosomal aberrations that have been shown to have both diagnostic and prognostic significance. This proposal will make use of major recent advances in canine molecular cytogenetics to identify recurrent chromosome aberrations associated with canine soft tissue sarcomas, in particular those of histiocytic origin. This project will identify areas of the canine genome associated with such cancers for further investigation at the sub-chromosomal level.

Canine Health Foundation Project 2616:
Molecular Analysis of Contributory Factors of Osteoarthritis in Canine Hip Dysplasia
Grant Status: Closed
Dr. Alpana Ray, PhD, University of Missouri, Columbia
April 1, 2004 - March 31, 2006

Hip Dysplasia is a common disease of dogs that ultimately leads to osteoarthritis (OA), a serious debilitating condition, which at present, is treated by symptomatic management of pain. Accidental injuries also lead to the development of OA. Cartilage degeneration is fundamental to the pathogenesis of OA. We propose to study the transcriptional control of MMP-1, a major enzyme involved in the degradation of articular cartilage. Expression of MMP-1 gene and the corresponding protein is markedly increased under osteoarthritic condition. Because cytokines like IL-1 and TNF-a increase expression of MMP-1 and biomechanical factors also influence its expression in osteoarthritic, unstable joints, the objectives are to understand what components of the promoter region of canine MMP-1 gene are influenced by these factors. At present no data is available on canine MMP-1 gene regulation. This proposal is aimed towards understanding the regulation of canine MMP-1 gene expression in response to biomechanical stress and cytokines by isolating canine MMP-1 gene, identifying the regulatory elements in the promoter responsive to biomechanical stress and cytokines, and analyzing MMP-1 expression in chondrocytes of articular cartilage from normal and osteoarthritic dogs with the intent to develop novel therapeutic drugs to combat this disease.

Canine Health Foundation Project 2434:
Recombinant Thyrotropin (TSH): Standard for the Next Generation of Canine TSH Immunoassays with Improved Sensitivity
Grant Status: Closed
Dr. Duncan Ferguson, DVM PhD, University of Georgia
January 1, 2004 - December 31, 2006

Overall, this research project completed the genetic phase of the project and are reproducibly producing reasonable quantities of recombinant canine TSH in vitro and purifying it with high recovery to high purity. In the future, this standard can serve as a reproducible standard for all available canine TSH assays. Although the researchers did not succeed with the development of a permanent cell line expression canine TSH, the transient expressions in PEAK cells and purification procedures have resulted in hundreds of micrograms of pure canine TSH devoid of contaminating LH and FSH. The data support the importance of the lack of standard cross-contamination with these more abundant but structurally related pituitary glyoprotein hormones. The research also pursued the promising development of antibody titers against TSH in 2 immunized mice. Unfortunately, neither of the animals resulted in a successful result: the positive hybridomas from one mouse were contaminated in the UGA monoclonal antibody facility, and the positive clones from the other mouse did not result in permanently positive clones.

In summary, using the currently available pairing of the 14H9 monoclonal antibody with a limited quantity of anti-cTSH polyclonal antibody, the researchers have developed a canine TSH immunoassay with an IRMA format, but the assay in this format does not appear to be more sensitive than the current commercial canine TSH assay. However, the use of highly purified recombinant canine TSH does provide

an improvement over the use of pituitary source TSH inevitably contaminated by LH and FSH. Such contamination provided by pituitary source glycohormones could theoretically be more problematic for accurate measurement of TSH in the cycling bitch.

Publication(s)

- Ferguson DC. Testing for Hypothyroidism in Dogs. Veterinary Clinics of North America: Small Animal Practice 2007;37:647-669.

Canine Health Foundation Project 2629:
Clinical and Immunological Outcomes in Dogs with Osteosarcoma Treated with Intratumoral Interleukin-12 Microspheres
Grant Status: Closed
Dr. Stuart Helfand, DVM, University of Wisconsin, Madison
January 1, 2004 - December 31, 2005

Appendicular osteosarcoma, or bone cancer of the limbs, is an important tumor in dogs representing nearly 10 percent of all canine cancers. Despite progress in treating canine osteosarcoma using a combination of limb amputation and chemotherapy, life expectancy is not usually extended by more than 6-10 months compared to amputation alone. Death is due to dissemination of cancer cells beyond the leg and it is estimated that the cancer has already spread in at least 95 percent of dogs when it is initially diagnosed. Novel treatment regimens are urgently needed to improve the lives of large breed dogs such as Golden, Labrador and other Retrievers, Rottweilers, Irish Wolfhounds, Great Danes, German Shepherds and others that are at greatest risk for developing this cancer. Stimulating the immune system of dogs with cancer has been a goal of veterinary cancer researchers for more than 20 years and osteosarcoma is a tumor that has shown positive responses to some of these interventions. This research proposes to add a potent new form of immunostimulation to the standard treatment for canine osteosarcoma. This strategy uses a powerful stimulant of the immune system called interleukin-12 (IL-12) that has been shown to induce strong antitumor responses in experimental animal models. Stimulated by IL-12, immune cells tolerant of cancer can be triggered to specifically kill cancer cells throughout the body. What?s more, the cells have long-term memory for the specific cancer. Our laboratory has shown that IL-12 enhances killing of osteosarcoma cells by immune cells from dogs. We propose that injection of IL-12 directly into limb osteosarcoma using a novel (microsphere) formulation resulting in slow IL-12 release within the tumor environment will promote active antitumor immunity in dogs with osteosarcoma and lengthen their survival time. A number of pertinent immunological questions will also be addressed.

Canine Health Foundation Project 2447:
Genetic Determinants of Susceptibility to Hypothyroid Disease in Dogs
Grant Status: Closed
Dr. George Happ, PhD, University of Alaska, Fairbanks
October 1, 2003 - September 30, 2004

Canine hypothyroid disease is very similar to Hashimoto's disease in humans, which has been shown to be associated with human MHC genes. This study proposed to determine if hypothyroid dogs has a similar association with canine MHC genes, these could provide useful genetic markers for selective breeding to reduce disease incidence in dogs. Hypothyroid disease is the most common endocrinopathy of dogs, and represents a significant veterinary problem. Definitive diagnosis is difficult since good clinical diagnostic tests are not available. The disease is characterized by low levels of thyroid hormones, but these may result from other diseases and it appears that primary hypothyroid disease is characterized by the presence of autoantibodies to thyroglobulin. There is a clear genetic component to canine hypothyroid disease, and a number of breeds are thought to be more susceptible. This study generated data on the incidence of hypothyroidism in a variety of breeds. Identified in which breeds (Dobermans, Rhodesian

Ridgebacks and English Setters) that a higher frequency of the DLA-DQAI *00101 allele was associated with hypothyroidism. The increase in risk was estimated as 1.97.

Publication(s)
- Kennedy LJ, Quarmby S, Happ GM, et al. Association of canine hypothyroidism with a common major histocompatibility complex DLA class II allele. Tissue Antigens 2006;68:82-86.
- Kennedy LJ, Huson HJ, Leonard J, et al. Association of hypothyroid disease in Doberman Pinscher dogs with a rare major histocompatibility complex DLA class II haplotype. Tissue Antigens 2006;67:53-56.
- Kennedy LJ, Barnes A, Short A, et al. Canine DLA diversity: 3. Disease studies. Tissue Antigens 2007;69:292-296.

Canine Health Foundation Project 2646:
Characterization of Receptor Tyrosine Kinase Dysfunction in Malignant Histiocytosis
Grant Status: Closed
Dr. Cheryl London, DVM PhD, University of California, Davis
October 1, 2003 - September 30, 2005

Malignant histiocytosis (MH), while rare in people, occurs frequently in certain breeds of dogs including Rottweilers, Golden Retrievers, Flat-Coated Retrievers and Bernese Mountain Dogs. There is no effective therapy for this disease and nearly all patients die within two to four months of diagnosis. The prupose of this proposal is to evaluate MH tumor specimens for mutations in genes that may contribute to the development of this devastating cancer. The genes of interest are those that code for proteins known as growth factor receptors. These proteins are present on the surface of the cell and when stimulated by growth factors, signal into the cell promoting cell survival and growth. Dysregulation of growth factor receptors is a common mechanism through which normal cells undergo transformation into cancer cells. Significant research has been directed toward the development of inhibitors capable of blocking the function of dysregulated receptors. Recent success of this approach has been realized with the inhibitor Gleevec in the treatment of chronic myelogenous leukemia in people. The purpose of this proposal is to identify growth factor receptors that are dysregulated in MH to provide the foundation for future clinical application of growth factor receptor inhibitors in the treatment of MH.

Canine Health Foundation Project 2465:
Identification and Characterization of Genetic Mutations in Canine Mast Cell Tumors
Grant Status: Closed
Dr. Cheryl London, DVM PhD, University of California, Davis
April 1, 2003 - March 31, 2005

The most common malignant tumor in dogs is the mast cell tumor (MCT, a form of skin cancer), occurring with an incidence of close to 20 percent in the canine population. MCTs range from relatively benign to extremely aggressive, leading to tumor spread and eventual death. Particular breeds of dog are at risk for the development of this tumor, indicating a role for genetic factors. We have previously identified mutations in the gene c-kit in 30-50 percent of dog MCTs. c-Kit plays a critical role in regulating the growth and function of normal mast cells, and as the mutations we discovered cause uncontrolled function of c-kit, it is likely they influence MCT development in dogs. This proposal will establish a prospective tumor registry of dog MCTs to be used for investigation of the true incidence of c-kit mutations within specific dog breeds. Moreover, the studies outlined in this grant will identify additional genetic mutations present in dog MCTs that can be used for the development of new targeted therapeutics. In summary, this work will provide a much more detailed understanding of dog MCTs, thereby building a framework for the development of new therapies and strategies for disease prevention.

Canine Health Foundation Project 2038T:

The Molecular Cytogenetics of Canine Lymphosarcoma: Correlating Chromosomal Changes with Clinical Disease
Grant Status: Closed
Dr. Matthew Breen, PhD, North Carolina State University
September 30, 2002 - September 30, 2003

Cancer kills. Twenty years ago, the diagnosis of lymphosarcoma (a tumor of the lymph glands) in humans was almost invariably fatal. However, with the development of improved means to sub-classify this neoplasm and the tailoring of therapies that are subtype-specific, more and more forms of lymphosarcoma are treatable. One of the most important means of sub-classification of human tumors is based on the identification of chromosome abnormalities. In the dog, lymphosarcoma comprises one in five malignancies; however, the extent and identity of chromosome aberrations is still unknown. This is largely because the chromosomes of dogs were extremely difficult to identify with confidence. Recently, we have developed a set of canine chromosome-specific reagents that allow us to identify conclusively every dog chromosome. We propose to use these reagents to identify the chromosome aberrations associated with dog lymphosarcoma and to investigate the correlation between these aberrations and the clinical disease. Such an approach offers a means to potentially sub-divide this diverse disease in dogs, thereby offering new information of diagnosis, prognosis and therapy. Identification of specific chromosome aberrations will also help to investigate the correlation between the genetic etiologies in dogs with those in humans.

Canine Health Foundation Project 2254A:
Heritable and Sporadic Genetic Lesions in Canine Lymphoma and Osteosarcoma
Grant Status: Closed
Dr. Jaime F Modiano, VMD PhD, AMC Cancer Research Center
May 1, 2002 - June 30, 2005

Lymphoma (cancer of lymph glands) and osteosarcoma (bone cancer) are two common cancers of dogs with remarkable breed predisposition. Lymphoma accounts for approximately 20 percent of all canine tumors, and > 80 percent of cancers originating from blood cells. Osteosarcoma is the most common bone tumor in dogs, accounting for 85 percent of skeletal cancers. All cancers have a genetic basis, and in effect, these conditions represent various diseases, each sharing one or more genetic abnormalities that contribute to overall risk and treatment response. However, a method does not exist to identify individuals at risk, or whether a dog that develops a tumor is likely to respond to conventional therapy. We have identified individual genes and larger regions within the genome that appear to be important in some canine cancers. For this project, we propose to confirm the frequency and significance of these genetic anomalies in lymphoma and osteosarcoma of Golden Retrievers, Rottweilers, Irish Setters, and Bernese Mountain Dogs. This work will begin to determine which of these anomalies may be heritable and which may be sporadic, and pave the way to apply this knowledge for clinical benefits by providing potential targets for treatment, and tools to define individual risk to develop these types of cancer or produce cancer-prone progeny.

Canine Health Foundation Project 2336:
Medical Surveillance of Dogs Deployed to the World Trade Center and the Pentagon
Grant Status: Closed
Dr. Cynthia M. Otto, DVM PhD, University of Pennsylvania
January 1, 2002 - December 31, 2004

September 11, 2001, will live on in the memory and psyche of the American people. These events may also alter the lives of the hundreds of dogs and their handlers that served in a time of disaster. Federal (FEMA) Urban Search and Rescue Teams, police and private search dog handlers were involved in the search mission. The dogs were exposed to numerous hazardous materials. Although the acute medical problems were limited, it is impossible to predict the long-term effects of this disaster on the health and

behavior of the dogs and the mental health of their handlers. In order to identify problems, we will perform intensive surveillance of the FEMA Team dogs and survey monitoring of the remainder of dogs. The intensive monitoring will include blood work (for evidence of infection, toxic injury and cancer) and chest radiographs over the next three years. Behavior and activity information will be collected at each time period for all dogs. The medical and behavior changes in the search dogs will be compared to controls to determine the lasting effects of this disaster and its response. Psychological effects on the FEMA dog handlers will also be monitored and interactions with the medical and behavioral effects of the dogs will be evaluated.

Canine Health Foundation Project 2277:
Identification of the Genetic Cause or Causes for Cataracts in Several Breeds
Grant Status: Closed
Dr. George J. Brewer, MD, University of Michigan
January 1, 2002 - December 31, 2004

Inherited cataract is a major health problem in dogs, with a significant frequency in 66 breeds. In some breeds the puppy is born with cataract (congenital) while in others it develops at six months of age, and in others at 12-18 months, long after the animal has been placed. Obviously, inherited cataract places a large emotional and financial burden on the dog fancy. As the first step in our attach on inherited canine cataract, we will look for linkage between a cataract gene and a DNA marker in nine breeds, selected because they show clinical variability in cataract type. Choosing multiple types of cataracts in various breeds increases our chances of multiple hits with our list of candidate genes. The nine breeds are Labrador Retrievers, Golden Retrievers, Cocker Spaniels, Poodles, German Shepherds, Miniature Schnauzers, Boston Terriers and West Highland White Terriers. Other breeds will also be helped because th discovered causative mutations will likely be shared by many other breeds. As a backup approach, we'll screen ""pre-hoc"" candidate genes for causality to the extent that time permits. In the end we will have developed DNA tests for many if not most of the inherited canine cataracts.

Canine Health Foundation Project 2232:
Cricopharyngeal Dysphagia in the Golden Retriever
Grant Status: Closed
Dr. Margret L Casal, DVM, PhD, University of Pennsylvania
November 7, 2001 - December 31, 2003

Cricopharyngeal Dysphagia is a swallowing disorder in dogs that is apparent at the time of weaning. It may result in failure to thrive, regurgitation, nasal discharge, pneumonia or even death resulting from recurrent pneumonia. Cricopharyngeal dysphagia has been described in several dog breeds and is suspected to be an autosomal recessive disease in these breeds. It has come to our attention that the disease has been recently described in Golden Retrievers, where the disease is suspected to be a dominant trait with variable expression. The objective of this study is to determine the mode of inheritance and to collect DNA samples to develop a genetic test in the future. To determine the mode of inheritance, careful diagnosis of the presence of absence of cricopharyngeal dysphagia using fluoroscopy is required, as there may be some dogs with a very mild, almost undetectable swallowing disorder. The ultimate goal is to eradicate this devastating disease from the Golden Retriever population.

Canine Health Foundation Project 2044:
Ultrasonic and Cytologic Evaluation of the Thyroid Gland in Golden Retriever Dogs
Grant Status: Closed
Dr. Richard Nelson, DVM, University of California, Davis
September 1, 2000 - September 30, 2001

Ultrasound evaluation of the thyroid gland was performed in healthy, hypothyroid, and euthyroid Golden Retriever dogs with nonthyroidal illness (NTI) to determine the diagnostic usefulness of ultrasound for differentiating between euthyroid and hypothyroid dogs. Thirty-six healthy, 11 hypothyroid, and 35 euthyroid dogs with NTI were evaluated. Each thyroid lobe was examined ultrasonographically for size, shape, echogenicity, and homogeneity. Thyroid lobe volume was estimated by using the equation for an ellipsoid: pi/6(length X height x width). No differences were found between healthy dogs and euthyroid dogs with NTI. In the majority of euthyroid dogs, the thyroid lobes were fusiform and triangular in shape in longitudinal and transverse planes, respectively. The thyroid capsule appeared smooth. The thyroid parenchyma had a homogeneous echogenic pattern and usually was hyperechoic or isoechoic compared with the surrounding musculature. Ultrasound findings in hypothyroid dogs were more variable, including a greater frequency of round to oval-shaped thyroid lobes in the transverse imaging plane ($P < .05$), hypoechogenicity of the thyroid parenchyma compared with surrounding musculature ($P < .001$), and a decrease in the size and volume of the thyroid lobes and total volume of the thyroid gland ($P < .05$) compared with euthyroid dogs. Other findings in hypothyroid dogs included an irregular surface to the thyroid capsule, a heterogeneous pattern to the thyroid parenchyma, and differences in the echogenic pattern between the left and right thyroid lobes. Results suggest that determination of thyroid size and volume by ultrasound may be a useful adjunctive test for differentiating between hypothyroid and euthyroid dogs with NTI.

Publication(s)

- Brmel C, Pollard RE, Kass PH, et al. Ultrasonographic Evaluation of the Thyroid Gland in Healthy, Hypothyroid, and Euthyroid Golden Retrievers with Nonthyroidal Illness. Journal of Veterinary Internal Medicine 2005;19:499-506.

Canine Health Foundation Project 2025:
Growth Signaling Pathways in the Pathogenesis and Treatment of Canine Cancer
Grant Status: Closed
Dr. Stuart Helfand, DVM, University of Wisconsin, Madison
August 1, 2000 - September 30, 2002

Hemangiosarcoma (HAS) is a common cancer in dogs that originates from cells lining the blood vessels. HAS can affect any dog, but is seen more often in German Shepherds, Skye Terriers, and Golden Retrievers. This suggests that this disease has a heritable component. Tumors arise when cells respond inappropriately to growth factors, allowing them to divide continuously in an uncontrolled fashion. Tumor suppressor genes contain or eliminate these rapidly dividing cells, but mutations in these genes can disable their ability to function correctly. Our laboratory is examining the idea that the loss of function of one of these tumor suppressor genes, PTEN, leads to the increased production of tumor growth factors. In our studies, we will examine the frequency of the mutations in the PTEN gene from dogs with HAS, and the relationship of these mutations to increased production of a specific tumor growth factor, VEGF. The results of our research could lead to tests for screening dogs for mutations in PTEN, and information could have an immediate and long-lasting impact on canine health when used judiciously for breeding decisions. We will also test the ability of a novel therapeutic approach to restore normal function within these cells as a treatment for HAS. Such work may lead the way for the further development of novel therapies for the treatment of canine hemangiosarcoma.

Canine Health Foundation Project 2012:
Development of PCR Multiplexed Canine Marker Panels for the Purposes of Genome Screening and Linkage Analysis
Grant Status: Closed
Dr. Marcia Eggleston, PhD, University of California, Davis
July 19, 2000 - September 30, 2002

A number of tests have been developed to screen for affected or carrier animals with genetic diseases. The development of many of these tests was possible due to the availability of information regarding similar disorders in humans. The majority of disorders affecting purebred dogs have no known counterpart in humans and therefore no candidate gene. However, an indirect test that screens for a strong association of a microsatellite DNA marker with a disease phenotype can be incorporated in a simple diagnostic test for carriers of the trait. The ultimate goal of the research is to design marker panels containing a minimum of 300 microsatellite markers covering all the linkage groups or chromosomes of the canine genome. The Veterinary Genetics Laboratory has completed panels containing 100 markers with known map locations. This project will incorporate at least 200 additional markers that will then represent the entire canine genome and allow effective screening for linkage of markers to disease gene(s).

Canine Health Foundation Project 2035:
Transferrin Receptor Expression by Canine Brain Tumors
Grant Status: Closed
Dr. Natasha J Olby, VetMB PhD, North Carolina State University
July 17, 2000 - September 30, 2001

Dogs that are not treated following diagnosis of a brain tumor survive an average of only two weeks. Treatment with either surgery and/or radiation can extend their survival to about ten months. Clearly there is a need for new approaches to treating brain tumors in dogs if survival is to be improved. In humans, malignant brain tumors are being treated successfully with toxins targeted specifically to a marker expressed by tumor cells: the transferrin receptor. In preliminary work, we have shown that untreated canine brain tumors also express this marker. The primary aim of this project is to establish whether brain tumors that have recurred following treatment with radiation or surgery still express the transferrin receptor. This information will allow us to determine whether transferrin receptor targeted toxins should be used as the primary form of treatment of brain tumors, or can be used to treat recurrent tumors following more conventional therapy. We hypothesize that brain tumors will express transferrin receptors at high levels after treatment and therefore that transferrin-linked therapy can be instituted in combination with more conventional therapy.

Canine Health Foundation Project 1626:
Significance of Tumor Suppressor Genes in Canine Cancer
Grant Status: Closed
Dr. Jaime F Modiano, VMD PhD, Texas A&M University
September 1, 1998 - August 31, 2000

The research conducted in this study will provide the basis for future research that may, ultimately, lead to scientists being able to provide a better assessment of individuals? risks for cancer (or for cancer in progeny), as well as determine whether a given dog is a good candidate for a given therapy. This project has helped to broaden the understanding of why tumors happen, so that the abnormalities can be targeted and better therapies devised. Researchers developed and tested gene therapy for melanoma. In a clinical trial involving five dogs with facial or oral melanoma, they found that the gene therapy, in which tumors were injected with modified genes, was both free of adverse effects and effective.

Canine Health Foundation Project 1419:
Inheritance and Molecular Genetic Evaluation in Newfoundlands and Golden Retrievers with Subvalvular Aortic Stenosis
Grant Status: Closed
Dr. Kathryn Meurs, DVM PhD, Ohio State University

September 15, 1997 - September 14, 2000

Analysis of blood samples and pedigrees of Newfoundlands and Golden Retrievers indicates that subvalvular aortic stenosis (SAS), a thickening of a specific part of the heart wall, is likely inherited as an autosomal dominant pattern with variable penetrance (varying degrees of symptoms and signs). SAS can be hard to study because the hearts of affected dogs can sound normal when listened to with a stethoscope. Researchers in this study used Doppler echocardiography to evaluate 215 Golden Retrievers and 175 Newfoundlands. They found that 72 of the Goldens and 86 of the Newfoundlands were affected with SAS; 56 and 20, respectively, were equivocal (open to interpretation). Researchers observed that the dogs classified as equivocal also produced severely affected offspring. The information and genetic samples obtained for the study are being used to perform genetic linkage studies.

Canine Health Foundation Project 2155E:
Genes, Dogs and Cancer: Emerging Concepts in Molecular Diagnosis and Therapy;
Scientific Conference, May 20-22, 2001; a white paper with presentation summaries is available from the Canine Health Foundation for $5

Genes, Dogs and Cancer: 2nd Annual Canine Cancer Conference; Scientific Conference
September 18-19, 2002: a white paper with presentation summaries is available from the Canine Health Foundation for $5