

Portuguese Water Dog Foundation, Inc.



2015 SPRING UPDATE

RESEARCH CO-SPONSORED WITH AKC CHF

****Funded in 2014****

2002: Defining the Genetic Basis of Inflammatory Bowel Disease

Principal Investigator: Dr. Karin Allenspach, DVM, PhD; Royal Veterinary College, University of London

Project Abstract:

Inflammatory Bowel Disease (IBD) is a group of disorders in which the intestinal tract has become invaded with the dog's own white blood cells leading to inflammation. Over time, this inflammation causes the intestine to become less efficient at absorbing nutrients from digested food and weight loss, and vomiting or diarrhea often result. IBD can be controlled, but not cured. The cause of IBD is poorly understood, but it appears that genetics, diet, intestinal bacteria, and abnormalities of the dog's immune system all play a role. Dr. Allenspach has recently identified genetic markers known as SNPs (single nucleotide polymorphisms) which she believes contribute to disease susceptibility. Beyond genetics, this research group has mechanistic data showing one of the putative mutations contributes to the inflammation seen in the intestine of dogs with IBD. In order to find all underlying genetic factors that could contribute to disease, they propose to perform a genome-wide association study. This study will lead to the development of new diagnostic and therapeutic avenues for canine IBD as has already been the case in people with IBD.~

(Continued on page 2)

RESEARCH CO-SPONSORED WITH MAF

****Funded in 2014****

D14CA-002 Exploring the Use of a Virus-Based Anticancer Strategy for Lymphoma

Dr. Amy K. LeBlanc, University of Tennessee,

Summary: This study explores the safety and effectiveness of a new virus-based therapy, developed at Mayo Clinic, for dogs with B-cell lymphoma.

Description: Lymphoma is one of the most commonly occurring malignant tumors in dogs. Though treatable, the disease often recurs and spreads. This study will determine the safety, efficacy and prognostic factors of a cancer-killing virus developed by researchers at the Mayo Clinic. Researchers at the University of Tennessee have already determined a safe dosing protocol for this virus in healthy dogs, and this clinical trial will test the dosing in dogs with B-cell lymphoma. Using state-of-the-art cancer imaging, the study team will determine how successfully the virus spreads to sites of cancer. They will also study the dogs' immune responses to the virus. This study provides the first robust assessment of a new anticancer strategy that has the potential to significantly improve quality of life and outcomes for dogs suffering from lymphoma.~

D14CA-047 Testing Strategies to Treat Drug Resistant Hemangiosarcomas - Dr. Erin B Dickerson, University of Minnesota

Summary: Investigators are assessing the potential for a specific tumor-cell population in canine hemangiosarcoma to sequester drugs within their lysosomes as a novel mechanism of drug resistance.

Description: Canine hemangiosarcoma is a common and highly metastatic cancer that affects all breeds of dogs. These tumors are particularly drug resistant, which makes them difficult to treat. The investigators recently identified a more drug-resistant cell population in hemangiosarcoma. These cells appear to be extremely efficient in isolating cancer drugs and preventing them from reaching their targets. The investigators will use several strategies to try to disrupt this process and they will determine whether any of these approaches improves drug responses and diminishes drug resistance. This could lead to more effective treatment of this difficult cancer.~

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MAF CO-SPONSORED RESEARCH (CONTINUED)

D14CA-324 Testing a Potential Therapeutic Target for Lymphoma - Dr. Marie-Eve Nadeau, *University of Montreal, Canada*

Summary: This study investigates a new therapeutic target (valosin-containing protein) for dogs with lymphoma.

Description: A cure for canine lymphoma remains elusive, in part because of the lack of molecular-targeted therapies that can circumvent chemotherapy resistance. The research team's previous findings suggest that valosin-containing protein (VCP) holds particular promise as a therapeutic target. In this study, they will test a known inhibitor of VCP to see if this results in preferential killing of lymphoma cells over healthy cells and to determine the critical mechanisms through which the anticancer effect is achieved. Identifying new therapeutic targets for canine lymphoma is the first step toward developing better treatments for this deadly disease.~

****Funded for 2015****

D15CA-015 Developing a New Tool to Study Viral Infections and Cancer in Dogs, Dr. Paul R. Hess, *North Carolina State University,*

Summary: Researchers will develop a state-of-the-art molecular tool to track and study killer T-cell populations that are responsible for fighting viral infections and cancer in dogs.

Description: In humans, a powerful immunologic reagent called a tetramer is standardly used to visualize changes in the body's killer T-cells. These cells respond to immunologic challenges and are critical to the body's immune system. Current knowledge of T-cell behavior in dogs could be significantly advanced with the development of a dog-specific tetramer. Researchers will work to construct the first canine tetramer, which would then be used in the development of vaccines for infectious diseases and cancer in dogs.~

D15CA-052 Exploring New Medical Treatments for Cushing's Syndrome in Dogs, Dr. Sara Galac, *Utrecht University, The Netherlands,*

Summary: Researchers will investigate potential new medical treatments for canine Cushing's syndrome.

Description: Cushing's syndrome is a hormonal disorder that occurs when the body produces higher than normal levels of the hormone cortisol. Unhealthy levels of cortisol can be triggered by various causes, including pituitary and adrenal gland tumors. Researchers will assess how two novel compounds affect cortisol production and adrenal tumor growth. Identifying novel medical options will help improve treatment strategies for dogs with Cushing's syndrome.

MAF CO-SPONSORED RESEARCH PROGRESS REPORTS

D13CA-033 Determining a More Effective Treatment for Canine Lymphoma

Jaime F. Modiano, VMD, PhD, *University of Minnesota*

Lymphoma is one of the most common cancers in dogs—about 70 percent of lymphomas in dogs result from a subset of lymphoma cells known as B-cells. Unfortunately, the prognosis for dogs with B-cell lymphoma has not changed significantly in more than 30 years. Any dog at any age is at risk of developing lymphoma, but some breeds, such as Golden Retrievers and Boxers, have a historically higher risk for the disease. Other commonly affected breeds include Basset Hounds, St. Bernards, Scottish Terriers, Bulldogs, Airedales, Weimaraners, Doberman Pinschers, Labrador Retrievers, English Setters and Great Danes.

Passive immunotherapy, which uses antibodies to kill cancer cells, has revolutionized lymphoma treatment in humans. Using recently available tools, researchers from the University of Minnesota, funded by Morris

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The Spring and Fall Updates are publications of the Portuguese Water Dog Foundation, Inc.

P. O. Box 203

Parker Ford, PA 19457-0203

www.pwdfoundation.org

The mission of the **Portuguese Water Dog Foundation**

is to generate significant resources to fund research into genetic and other canine diseases that will improve the life and health of all Portuguese Water Dogs.

AKC CHF CO-SPONSORED RESEARCH (CONTINUED)

****Funded for 2015****

01889-G: Innovations in Prevention, Diagnosis, and Treatment of Cancer

Principal Investigator: Dr. Jaime F Modiano, VMD PhD; *University of Minnesota*

Lymphoma and hemangiosarcoma are major health problems in Golden Retrievers, causing both suffering and premature death. Through ongoing collaboration, Drs. Jaime Modiano, Matthew Breen, and Kerstin Lindblad-Toh have identified several regions of the genome that contain genetic heritable risk factors for lymphoma and hemangiosarcoma in Golden Retrievers. They have tumor-specific mutations that occur recurrently in both cancers, some of which are linked to duration of remission when treated with standard of care. Their results indicate that a few heritable genetic risk factors account for as much as 50% of the risk for these cancers. These findings offer the potential to develop tests and strategies for DNA tests that can predict risk for individual dogs, as well as to manage risk across the population as a whole. Indeed, both the inherited risk factors and tumor mutations point to pathways that have been implicated in the pathogenesis of lymphoma and hemangiosarcoma, and thus should inform the development of targeted therapies. In the current study, Drs. Modiano, Breen, and Lindblad-Toh will find the precise mutations for the heritable genetic risk factors and to validate markers (mutations) used to determine risk at the heritable loci in a larger independent population of Golden Retrievers from the United States and from Europe in order to develop robust risk prediction tools and an accompanying DNA test. Further, they will identify and characterize tumor mutations and study their relationship to the heritable risk factors, tumor pathogenetic mechanisms, and disease outcome.

01918-G: Discovery of Biomarkers to Detect Lymphoma Risk, Classify For Treatment, and Predict Outcome in Golden Retrievers

Principal Investigator: Dr. Jeffery N. Bryan, DVM; *University of Missouri, Columbia*

Project Abstract:

Lymphoma strikes 1 in 8 Golden Retrievers, approximately one-third of the cases being B-cell. While T-cell classifications currently inform therapy choices for dogs, B-cell classifications have been investigated little in Golden Retrievers. Dr. Jeffrey Bryan, in collaboration with Drs. Anne Avery and Heather Wilson will focus their efforts on an area of emerging importance in cancer: epigenetics. Epigenetics is defined as stable and heritable patterns of gene expression that do not entail any alterations to the original DNA sequence. Epigenetic DNA methylation changes clearly underlie development of lymphoma in humans, but have been evaluated minimally in dogs. Dr. Bryan and collaborators propose to improve diagnostic, classification, and prognostic ability using flow cytometry paired with biopsy to characterize the B-cell lymphomas of Golden Retrievers. They will identify DNA methylation changes in lymphoma cells not present in normal cells to develop biomarkers of each class of lymphoma and identify new therapy targets for affected Golden Retrievers.

More significantly, because DNA methylation changes occur so early in the process of cancer formation, they hypothesize that they could serve as biomarkers of risk, allowing medicine or diet to prevent lymphoma in Golden Retrievers before it develops. Finally, they propose to identify tumor initiating cells (TIC) in lymphoma biopsies to characterize stem-like cells by surface markers and DNA methylation changes. Identifying these cells will aid therapeutic strategy development. Each project advances a current frontier of research. By performing them in parallel, the markers from each can be combined, correlated, and translated into biomarkers of risk, diagnosis, and prognosis to advance the prevention and management of lymphoma in Golden Retrievers.~

01828: Mapping of Genetic Risk Factors for Canine Hip Dysplasia

Principal Investigator: Dr. Antti Iivanainen, DVM, PhD; *University of Helsinki and the Folkhälsan Institute of Genetics*

Project Abstract:

(Continued on page 3)

MAF CO-SPONSORED RESEARCH PROGRESS REPORTS (CONTINUED)

AKC CHF CO-SPONSORED RESEARCH (CONTINUED)

Animal Foundation, are evaluating whether two antibodies could help treat B-cell lymphoma in dogs. The investigators theorize that because either antibody alone will kill lymphoma cells and delay tumor progression, a combination of these two antibodies will prove to be more effective in treating dogs with this cancer. So far, the research team has confirmed that these antibodies promote the killing and destruction of canine lymphoma cells in a laboratory setting. The researchers are now working to develop a model that will allow them to further test the antibodies against tumor cells in a living system. They expect to complete this model and testing in the next 6 to 12 months.

If proven effective as passive immunotherapy, use of the antibodies in this study could significantly improve treatment for dogs with B-cell lymphoma. These antibodies may also help to provide a more accurate and cost-effective diagnostic tool for dogs with this type of cancer.~

D13CA-044 Developing Ways to Improve Cancer Treatments

Daniel L. Gustafson, PhD, *Colorado State University*

Methods of predicting tumor response to a given chemotherapy protocol have historically focused on a few traits that could be measured in biopsy samples. Recent approaches have looked at the activity of genes within tumors to predict a tumor's sensitivity to a given drug. Because many dogs with osteosarcoma eventually succumb to it in spite of treatment, a method to optimize chemotherapy selection could improve outcomes for dogs with this type of cancer.

Funded by Morris Animal Foundation, researchers from Colorado State University are developing a model to determine whether an osteosarcoma tumor from an individual dog is sensitive to a specific chemotherapy drug. This project is based on the idea that the genetics of human and canine cancers are similar enough that the wealth of data on human cancer genes and drug sensitivity can be mined and applied to canine cancers to aid in chemotherapy selection for dogs.

So far, researchers have collected genetic and drug-sensitivity data from canine cancer cell lines to compare with human data used to evaluate an individual tumor's likely response to specific chemotherapy drugs. Currently, the researchers are looking at several commonly used drugs—doxorubicin, carboplatin and cisplatin—to determine if the human models will be accurate in predicting canine cancer responses to these drugs. Results are promising, and the next step is to use the gene expression-based models to search drug databases for drugs that could be more effective in treating canine osteosarcoma patients than the drugs that are the current standard of care.

If the study is successful, canine patients could be treated with the drug that would be most effective for their particular cancer. Data from this study could also lead to new treatment options for osteosarcoma. This type of approach, known as precision medicine, allows for tailored therapy that better controls cancer growth and spread and minimizes the possibility of using an ineffective drug that may cause unwanted side effects. ~

D13CA-062 Assessing How a Protein Helps Hemangiosarcoma Cells Survive

Erin B. Dickerson, PhD, *University of Minnesota*

Hemangiosarcoma, a highly metastatic and incurable cancer, can affect dogs of any age and breed, though its prevalence is particularly high in Golden Retrievers, Portuguese Water Dogs and German Shepherd Dogs. This cancer is particularly devastating because small ruptures may occur in growing tumors causing blood loss. Often, related clinical signs, such as lethargy and weakness, are so subtle that they may go unnoticed by owners. Eventually, larger or rapidly growing tumors may rupture, resulting in severe bleeding, collapse, shock and death. Despite many attempts to find effective treatments, the survival rate for dogs with hemangiosarcoma has remained relatively unchanged for the past three decades. Current treatments, including surgery followed by chemotherapy, rarely extend life beyond 6 or 7 months from the time of diagnosis.

Recent evidence suggests that populations of cancer stem cells in hemangiosarcoma and other cancers give rise to tumors, promote tumor growth and are the main culprits behind drug resistance and disease recurrence. ~Funded by Morris Animal Foundation, researchers at the University of Minnesota are examining how CSF-1R, a marker present on the

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Canine hip dysplasia is a common developmental disorder of the hip joint that severely affects a dog's quality of life. As the disease has several genetic risk elements and is influenced by environmental factors like diet and exercise, it is of paramount importance that genetic association studies are conducted using adequately-sized cohorts of genotyped diseased and healthy animals. Dr. Iivanainen will sample a large population of dogs (>300-400 dogs) so that contributing genetic loci can reliably be discovered. This research group expects that with such a strongly powered study all major genetic risk factors can be uncovered with a high statistical significance. Investigators expect that identified loci will be discovered across breeds. The identification of genetic risk elements will allow the development of genetic tests that can be used in breeding programs to control the disease incidence, as well as further studies regarding the possible role of diet and exercise in hip dysplasia development.~

02107: Landmark Clinical Trial to Establish the Evidence-Based Use of Regenerative Medicine to Treat Tendon Injury in Dogs

Principal Investigator: Dr. Jennifer G. Barrett, DVM, PhD; *Virginia-Maryland Regional College of Veterinary Medicine*

Project Abstract:

Regenerative medicine is a rapidly developing field with the potential to transform the treatment of canine disease. The ability to repair damaged tissue and treat diseases once believed to be incurable may soon be a reality. However, there are concerns that some techniques are being used prematurely. Due to the lower regulatory barriers in veterinary medicine, company-sponsored regenerative medicine products and techniques are currently used in general practice and specialty hospitals without the benefit of having been preceded by stringently controlled, independently funded clinical trials. As a result, techniques vary widely and the evidence that they work is anecdotal at best. The AKC Canine Health Foundation has made the evidence-based practice of regenerative medicine a major focus within our research portfolio. Through an ongoing commitment to fund research studies that will inform the veterinary community in the use of safe and effective regenerative medicine techniques, we intend to protect dog owners and support veterinarians with innovative technology that will consistently improve outcomes for dogs.

In support of our effort to provide evidence-based regenerative medicine research, CHF is funding this landmark study to evaluate the effectiveness of Platelet-Rich Plasma (PRP) and stem cells in the treatment of the most common sporting injury in dogs: supraspinatus tendonopathy (similar to the rotator cuff injury in humans). Tendon injuries in dogs often progress undiagnosed and result in chronic lameness and pain. Ultimately, unassisted tendon healing results in scar formation and reduced function of the joint and surrounding muscle tissue. PRP and stem cell therapies aim to accelerate and promote healing through tissue regeneration and reduced scarring. Dr. Jennifer Barrett, MS, PhD, DVM, DACVS, DACVSMR, and Dr. Sherman Canapp, DVM, MS, CCRT, DACVS, DACVSMR, propose to conduct the first randomized, placebo-controlled clinical trial evaluating the effectiveness of PRP, adipose-derived, cultured stem cells (ASC) and commonly used stromal vascular fraction (SVF) cells. This will be the first study to directly compare efficacy of intratendinous injection of ASC versus SVF, both of which are currently commercially available despite having limited scientific evidence of efficacy. The study will be conducted at the Veterinary Orthopedic and Sports Medicine (VOSM) Group in Annapolis Junction, MD in order to recruit real-world cases in a clinically relevant, state of the art canine sports medicine environment. Using the gold-standard 'Blinded, Placebo Controlled' clinical trial design, Drs. Barrett and Canapp will not only identify an effective treatment for supraspinatus tendon injury, but their research will have a profound impact on the treatment of a wide array of musculoskeletal conditions affecting dogs and humans.~

01840: Health Implications of Early Spay/Neuter on Canine Health

Principal Investigator: Dr. Benjamin L Hart, DVM, PhD; *University of California, Davis*

Project Abstract:

Most dogs in the United States are spayed or neutered, and the default recommendation has been to perform these elective surgeries prior to physical maturity. However, recent data suggest that early spay and neuter may adversely impact the health and well-being of dogs. In preliminary studies funded by CHF, Dr. Ben Hart of the UC Davis College of Veterinary Medicine found that early spay or neuter, prior to 12 months of age, was related to a significant increase in risk in five diseases of concern: hip dysplasia; cranial cruciate ligament tear;

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MAF CO-SPONSORED RESEARCH PROGRESS REPORTS (CONTINUED)

surface of some hemangiosarcoma stem cells, may contribute to the maintenance and survival of these cells in tumors. Researchers have found two subpopulations of CSF-1R, one of which appears to store the chemotherapy drug doxorubicin. This may explain why it is also more resistant to doxorubicin. They have also found that the two marker subpopulations may depend on each other for survival.

The next phase of this study will involve further evaluation of the two subpopulations of CSF-1R, including how they depend on each other and the drug sequestration properties they display. This information could prove useful in developing therapeutic targets. The researchers will also begin to evaluate how a CSF-1R inhibitor affects survival of this devastating disease.~

The development of new treatment strategies for hemangiosarcoma would be welcome to veterinarians and their patients suffering from this heartbreaking cancer. Studies such as these provide hope that survival rates can be increased, and that pets diagnosed might one day be offered the opportunity for prolonged good quality life with their families. ~

D12CA-033 Evaluating a Novel Drug for Lymphoma

Barbara Biller, DVM, PhD, Colorado State University

Results: Researchers Validate the Safety and Effectiveness of a Candidate Antibody Therapy for Dogs with Cancer

Lymphoma is one of the most common cancers of dogs, accounting for an estimated 25 percent of all canine cancers. In humans with lymphoma, adding therapeutic antibodies to chemotherapy protocols has improved clinical responses. Antibodies are proteins the immune system produces to neutralize foreign substances, such as viruses, but they can be designed to selectively target and kill malignant cancer cells. Although antibodies are widely used to treat lymphoma in people, similar treatments are not currently available for canine patients.

Researchers from Colorado State University, the Garden State Cancer Center and Immunomedics, Inc., recently developed a human-derived therapeutic antibody that effectively kills canine lymphoma cells. Funded by Morris Animal Foundation, the research team conducted a clinical trial to evaluate the antibody's safety and effectiveness in dogs with B-cell lymphoma. Seventeen client-owned dogs, ranging in size from a 19-lb Terrier mix to a 140-lb Great Dane, received antibody therapy and completed the trial.

Data showed that the antibody was well tolerated and had no serious side effects. The therapy successfully targeted B-cells in dogs and decreased the B-cell population up to 50 percent within the first 24 hours after antibody infusion; however, in most patients, B-cells returned to normal values within seven days after treatment.

Although the antibody therapy did not appear to slow down the progression of lymphoma in the study dogs, cancer was temporarily stabilized in three dogs, and the longest period of stabilization was four weeks. This is significant because lymphoma usually progresses very quickly when no chemotherapy is given or when chemotherapy is no longer effective.

Using the information gained in this study, the research team will continue to improve the antibody's design for future clinical trials. This includes making the antibody more canine specific, which would likely improve its effectiveness in dogs with lymphoma. As in human medicine, the researchers hope therapeutic antibodies will one day become part of the standard of care for dogs with lymphoma, improving their quality and duration of life after diagnosis. ~

D12CA-302 Understanding the Role of Specific Cells in Spreading Lymphoma

Daisuke Ito, DVM, PhD, University of Minnesota

Diffuse large B-cell lymphoma (DLBCL), a cancer of the blood and lymph system that is clinically similar to human B-cell non-Hodgkin lymphoma, is among the most common types of lymphoma in dogs. The median survival time for dogs with DLBCL when treated with multiagent chemotherapy is about one year, a number that has not changed for more than 20 years, despite efforts to identify better chemotherapy protocols.

Funded by Morris Animal Foundation, researchers from the University of Minnesota are developing a new strategy to improve survival times for dogs with this cancer. Specifically, they are studying a signaling pathway involved in tumor cell survival. A signaling pathway is a group of molecules that work together to control one or more cell functions, such as cell division or cell death. Overactivation of a particular pathway has been demonstrated in

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AKC CHF CO-SPONSORED RESEARCH (CONTINUED)

lymphosarcoma; hemangiosarcoma; and mast cell tumor.

CHF has now funded the second phase of Dr. Hart's research in which he will expand his work to consider breed differences in vulnerability to joint disorders and risks of various cancers after early or late spay/neuter. Breeds considered will include: Labrador Retrievers, German Shepherd Dogs, and Dachshunds. Rottweilers, Chihuahuas, Standard Poodles, and Miniature Poodles will be included if resources and patient data are available. The expectation is that by inclusion of multiple breeds in phase II Dr. Hart will be able to develop a generalized understanding of the impact of early spay and neuter on disease risk in dogs. This in turn will enable veterinarians and breeders to make data-driven recommendations regarding timing of spay/neuter procedures to reduce the risk of development of multiple devastating diseases. ~

AKC CHF CO-SPONSORED RESEARCH PROGRESS REPORTS

Grant 01849: Filling the Gaps in the Canine Genome

Principal Investigator:

Dr. Shaying Zhao, PhD; University of Georgia Research Foundation, Inc Report to Grant Sponsor from Investigator:

We have finished 85% of the proposed studies. We are integrating our data with others generated by the Broad Institute and other scientists to more accurately identify all forms of dog protein-coding genes. We aim to publish the results in several months. We are performing sequencing analysis to identify canine non-protein coding genes and regulatory regions and will finish the analysis in next few months.~

Grant 01759: Disrupting the Differentiation of Cancer Stem Cells to Prevent the Spread of Hemangiosarcoma

Principal Investigator: Dr. Jaime F Modiano, VMD PhD, University of Minnesota

Report to Grant Sponsor from Investigator:

We continue on track to achieve the milestones laid out for this project. Our data continue to clarify the interactions stemming from inflammation in the tumor environment with regard to cell autonomous growth and survival, reinforcement of traits associated with metastasis, and interactions with other constituents of the microenvironment.

Grant 01822: Beyond the Genome: The Intersection of Genes and the Environment in Canine Cancer

Principal Investigator: Dr. Robert K Wayne, PhD, University of California, Los Angeles

Report to Grant Sponsor from Investigator:

DNA methylation plays a central role in regulating gene expression in both normal and pathological states, with abnormal patterns of methylation having been observed in cancer. However, methylation remains an unexplored dimension of canine disease. Here, we propose to establish the pattern of normal variation in methylomes (the genome-wide collection of methylated sites) from an array-based analysis across a variety of domestic dog breeds. Differences in methylation found between breed lineages will be complemented by the study of gene expression to understand how methylation regulates levels of expression. Our preliminary analysis have focused on a proof-of-principle approach, surveying the differences in methylation of the X chromosome between male and female dogs. Our expectation is that females will display increased levels of methylation due to the inactivation through methylation of an X chromosome for dosage compensation. We do indeed observe this result, and thus are confident that we have the statistical resolution to detect differential methylation across autosomes as well. We focus on methylation as an aspect of gene regulation that could complement gene mapping studies traditionally completed for exploring the genotype-phenotype link. We propose that methylation data can potentially be useful in predicting pathways involved in canine disease risk and progression. We will establish a publically available web-based resource to serve as a repository for the dog methylomes. The collection of methylomes we generate will contribute to the growing resources that are available for investigation of morphology, disease etiology, advancing therapeutic approaches, and overall canine health.~

Grant 01787: Clinical Advancement of a Cancer Vaccine in Dogs

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MAF CO-SPONSORED RESEARCH PROGRESS REPORTS (CONTINUED)

AKC CHF CO-SPONSORED RESEARCH PROGRESS REPORTS

aggressive human DLBCL and is a promising target for DLBCL treatments in dogs.

During the first year of the study, the researchers demonstrated that this pathway is highly active in canine lymphomas, suggesting that it is a potential new chemotherapy target. In the second year of study, the researchers are working to determine which genes in this signaling pathway might be targets for medical intervention. The results will provide new insight about the way lymphoma works in the body and will be used to develop new treatment strategies for DLBCL in dogs, including such high-risk breeds as Golden Retrievers, Labrador Retrievers, Cocker Spaniels, Rottweilers, Boxers, German Shepherds and Doberman Pinschers. This First Award grant also supports the career development of a promising, new canine cancer researcher. ~

DI0CA-501 MADGiC: Making Advanced Discoveries in Golden Cancers

Jaime F. Modiano, VMD, PhD, University of Minnesota

RESULTS: Researchers Identify Two Regions in the Golden Retriever Genome Associated with Lymphoma and Hemangiosarcoma Risk

It is estimated that up to 40% of Golden Retrievers will be diagnosed with hemangiosarcoma or lymphoma in their lifetime. However, the relationship between heritability and susceptibility for these tumors in Golden Retrievers remains unknown.

With funding from Morris Animal Foundation and the Golden Retriever Foundation, a multi-institutional research team from the University of Uppsala & Broad Institute, the University of Minnesota, and North Carolina State University, examined genetic traits that contribute to the risk and progression of hemangiosarcoma and lymphoma in Golden Retrievers. Using recent technological advances to study heritable mutations in the Golden Retriever genome, the research team identified two regions that together explain part of the risk for these two diseases in this breed. They also showed that the disease progression and response to treatment are very heterogeneous in both hemangiosarcoma and lymphoma. At least some of these differences could be related to heritable traits.

The identification of genomic regions associated with these two cancers constitutes a critical step to designing rational strategies to diagnose these cancers earlier and more accurately. The recognition that disease heterogeneity could be due at least in part to genomic alterations is similarly important in the process of developing more effective treatments for Golden Retrievers and for the many other dogs diagnosed with hemangiosarcoma or lymphoma. Continuing research by this team will involve validating these key genomic regions in Golden Retrievers from more families to increase the representation of global pedigrees. If successful, potential outcomes would include tests that could be used to make informed breeding decisions and to guide treatment for dogs diagnosed with hemangiosarcoma or lymphoma. *(Final Report)~*

DI0CA-016 Investigating a Noninvasive, At-Home Diagnostic Technique for Gastrointestinal Disorders

Pedro L. Boscan, DVM, PhD, Colorado State University

Gastrointestinal disorders, such as vomiting, diarrhea, and weight loss, are often associated with a change in the motility, that is, the rate of movement, of food through the stomach and intestines. It is difficult to diagnose and treat motility disorders, but a novel, noninvasive, wireless sensor capsule, called a SmartPill, was recently developed to study gastrointestinal motility disorders in dogs.

Using the SmartPill technology, Morris Animal Foundation-funded researchers at Colorado State University are comparing typical gastrointestinal transit times for dogs in a nonstressful home environment with transit times in an often stressful veterinary hospital environment. So far, the researchers have given the SmartPill to 37 client-owned dogs, about 75 percent of their planned patient enrollment. They expect to complete the clinical trial this summer and have final data analysis available by year end. Preliminary data analysis appears to confirm that gastrointestinal transit times vary greatly between dogs and may correlate with body size. For example, the time for food to move through the gastrointestinal tract in a Chihuahua

Principal Investigator: Dr. Nicola J Mason, BVetMed, PhD

University of Pennsylvania

Report to Grant Sponsor from Investigator:

The goal of this proposal is to build on our previous work developing a cell-based vaccine that aims to stimulate potent tumor-specific immune responses that will kill lymphoma cancer cells. Our previous work has shown that white blood cells known as B cells found in the peripheral blood can be activated and grown outside of the body using special "feeder cells" that express an important molecule known as CD40L. The stimulated B cells (known as CD40-B cells) can be loaded with genetic material (RNA) that has been extracted from the patient's tumor. When re-injected back into the patient, the CD40-B cells are able to present the tumor material to the body's immune system and stimulate an anti-tumor immune response. We have shown in a phase I clinical trial that this approach has produced promising results with respect to prolonging overall survival in dogs with lymphoma. We have now improved the generation of this vaccine by generating canine specific feeder cells that are moderately more efficient at inducing canine B cells to grow from PBMCs. We have started to evaluate ways to improve the immune stimulatory function of these vaccine cells but have not yet identified an immune stimulant that can significantly augment the CD40-B cell capacity to stimulate canine T cells in vitro beyond that already achieved. Our studies in this area continue.

Our current methods of generating the CD40-B vaccine from lymphoma patients are labor intensive and require specialized laboratory equipment that is not available in most facilities. Therefore, we have made second-generation feeder cells that stably express the canine form of CD40L (we previously used the human CD40L molecule in our feeder cells) and have evaluated a non cell-based technique for canine B cell culture. We found that our second generation canine CD40L expressing feeder cells work well in our culture system and are much simpler to maintain in the laboratory than the previously used transfected cells expressing human CD40L. We also performed several experiments to evaluate whether these second-generation feeder cells can be irradiated, frozen and then thawed prior to their effective use in B cell generation. This would enable these cells to be distributed to other centers that do not have ready access to an irradiator and enable those centers to generate CD40-B cell vaccines on site. We have found that canine B cell expansion using thawed, previously irradiated KTcCD40L feeder cells is possible however it is sub-optimal when compared to freshly irradiated feeder cells. Unfortunately, we found that a soluble form of CD40L was not effective at activating and expanding canine B cells from peripheral blood lymphocytes in culture. Therefore, we will continue to generate CD40-B cell vaccines using freshly irradiated feeder cells.

We have also tested the hypothesis that the ability to stimulate anti-tumor immunity can be improved through the addition of a potent immune adjuvant (CpG DNA) to our CD40-B cell cultures. Our preliminary results indicate that while the addition of CpG may induce a mild increase in the number of CD40-B cells generated we have not identified any significant increase in expression of B cell surface molecules over and above a negative GpC control. These experiments are currently being repeated.

Regulatory approval for our second clinical trial using our improved CD40-B cell technology is being sought and we expect to begin recruitment for the second phase of this proposal in the near future.~

Final installment of the Rabies Challenge was paid in 2014. We hope to have available the results of first challenge phase in spring of 2015.

(Continued on page 6)

MAF CO-SPONSORED RESEARCH PROGRESS REPORTS (CONTINUED)

may be different from that of a Great Dane. Transit time may also be dependent on the environment in which dogs are evaluated. The researchers showed that hospitalization decreased gastrointestinal transit times in some, though not in all, dogs.

The research team has not encountered any major complications during clinical trials; however, they have found that smaller dogs weighing between 20 and 40 pounds have a higher chance of not passing the SmartPill beyond the stomach. For this reason, the researchers are focusing the remainder of the study on larger dogs.

This study will help veterinarians evaluate gastrointestinal motility problems associated with such diseases as bloat, gastritis, inflammatory bowel disease, enteritis and colitis. This diagnostic method may also provide a less stressful and more accurate alternative to hospitalization for testing motility disorders in dogs. This is important as gastrointestinal function varies depending on such factors as stress levels. ~

D13CA-400 Searching for Ways to Control Hemangiosarcoma Cancer Cells in Dogs

Jong Hyuk Kim, DVM, PhD, University of Minnesota

Hemangiosarcoma is a highly metastatic and incurable cancer that can affect dogs at any age. It is particularly prevalent in certain breeds such as Golden Retrievers, German Shepherds and Portuguese Water Dogs. Unfortunately, hemangiosarcoma is a disease that is poorly understood and for which there are currently no good treatment options.

One of the main reasons why therapies fail may be due to the existence of cancer stem cells. These cells are responsible for initiating and maintaining the cells within the tumor and they are also highly resistant to most chemotherapy. Researchers at University of Minnesota, funded by Morris Animal Foundation, are examining the role of small molecules that may serve as signals in the regeneration of hemangiosarcoma stem cells. Specifically, investigators are evaluating the potential to control the activity of hemangiosarcoma stem cells by altering these molecular signals in a way that stops stem cell regeneration and enhances sensitivity to chemotherapy.

So far, preliminary data show that one signaling pathway under study has distinct effects on the regeneration of hemangiosarcoma cancer stem cells in the lab. This signaling pathway seems to affect the stem cells' efficiency in forming a sphere as part of the self-renewal process. If this signal can be altered, it may reduce the stem cells' ability to renew and maintain the tumor. Further research is needed to confirm the results.

This study is providing valuable insight into the properties of cancer stem cells that could help in the development of tests to predict a patient's outcome and appropriate therapies to treat canine hemangiosarcoma. Funding for this project is also supporting the training of a promising, new canine cancer researcher.~

D12CA-026 Developing a New Delivery System for Lymphoma Treatment

Nicola J. Mason, BVetMed, PhD, University of Pennsylvania

Lymphoma affects approximately 30 out of every 100,000 dogs. Although the current treatment regimen of multiple chemotherapy drugs induces remission in about 75 percent of patients, most dogs ultimately relapse within six to nine months of diagnosis.

Rituximab, an antibody-targeting drug, has substantially improved survival times for people with various types of B-cell lymphoma. However, rituximab cannot be used in dogs because it does not recognize canine B cells and is rapidly destroyed by the dog's immune system.

Funded by Morris Animal Foundation, researchers at the University of Pennsylvania are developing a novel system to generate canine-derived antibody fragments similar to rituximab that will specifically target canine lymphoma cells and not be rejected by the dog's immune system. So far, they have successfully generated "target" cells that stably express canine CD20, the same molecule that rituximab targets in humans. Researchers are now screening canine antibody fragment libraries for fragments that will bind tightly to these cells.

Development of a canine-derived antibody fragment will provide a system that allows for targeted delivery of cell killing agents to the malignant B-cells thereby allowing for increased chemotherapy doses, reduced side effects and improved outcome for dogs with B cell lymphoma.~

D09CA-913 Studying Tumors That Cause Cushing's Disease

Miriam J. Kool, DVM, University of Utrecht

Results: Researchers Identify New Targets for Therapy in Malignant Adrenal Tumors

Adrenocortical carcinoma, a rare but highly malignant type of cancer affecting the adrenal glands, causes approximately 20 percent of cases of naturally occurring Cushing's disease in dogs. Dogs with Cushing's disease have increased levels of cortisol, which can lead to excessive drinking and urination, exercise intolerance, muscle breakdown, skin and urinary tract infections, hair loss on the flanks and enlarged amounts of abdominal fat. Disease-related complications can be severe, and in the case of a malignant tumor, the prognosis is poor. Unfortunately, few treatment options are available for dogs with adrenal gland tumors, largely because the mechanism by which they release excessive cortisol is poorly understood.

Funded through a Zoetis-Morris Animal Foundation fellowship training grant, Dr. Miriam Kool worked with researchers from Utrecht University to study adrenocortical tumors. Specifically, they used molecular laboratory techniques to compare the genes and proteins in adrenal glands with tumors to those in healthy adrenal glands. They also looked for defects in genes that may lead to tumor development.

The researchers have identified two different proteins that might be strong candidates for future therapies. These proteins are particularly exciting because specific drug inhibitors are already available for them.

The researchers have also made a surprising discovery: 30 percent of the adrenocortical tumors studied harbored a defect in a gene that is involved in cortisol secretion. This defect may partially explain the increased cortisol production found in canine adrenal tumors.

New and improved treatments are needed to enhance the prognosis and quality of life of dogs suffering from adrenocortical tumors. Knowledge of how these tumors form and function is an essential first step toward developing such strategies. *(Final Report)*~

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- Nicole Bearman in memory of Kalista's Even Keel, Elton – you will be missed
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- Annie Herberholz in memory of Stargazer's One For The Money 'Lisbon' loved & missed by Jon and Carol Lesch. Happy Heavenly Birthday, Lisbon!
- Annie Herberholz in memory of "Skye" Portolano Helms Alee Rainmaker CD CWD – blessed, are all those you visited and supported
- Linda K. & Krista K. Hunt, Kalista in honor of Lauren McDermott/Amarinhar for 1st Place PWDCA OBAA 2012
- Linda K. & Krista K. Hunt, Kalista in honor of "Breaker" HIT Kalista's Icebreaker UD RE OA AXJ WWD GROM on his UD!!
- Linda K. & Krista K. Hunt, Kalista in honor of "Lucy" Kalista's I'm Gilded Two CDX RN NA NAJ WWD SROM for her CDX & High In Trial at the PWDCTC Specialty
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- Linda K. & Krista K. Hunt, Kalista in memory of "Rudy" Kalista's Freedom Reign loved and missed by Stephen Larson & family
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- Linda K. & Krista K. Hunt, Kalista in honor of "Y" Kalista's Now What BN RA MX MXB MXJ MJB NAP MJP XF WWD CAA for his CA and CAA
- Linda K. & Krista K. Hunt, Kalista in congratulations to "Winnie" Kalista's Mahtomedi Mermaid NA NAJ on her new Agility titles!!
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- Linda K. & Krista K. Hunt, Kalista in congratulations to CH Kalista's Quite The Tale "Moby" on your AKC Championship
- Linda K. & Krista K. Hunt, Kalista in memory of "Sparky" CH Kalista's Curious Charmer loved and missed by the Boland family
- Linda K. & Krista K. Hunt, Kalista in congratulations to CH Kalista's Quite The Catch "Keeper" on her AKC Championship
- Linda K. & Krista K. Hunt, Kalista in congratulations to CH Kalista's Natational

Nuni "Nuni" on her AKC Championship

- Linda K. & Krista K. Hunt, Kalista in congratulations to CH Kalista's Ready to Rock BN CGC "Jagger" on his AKC Championship
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- William M. Lyons in memory of Pogo for Dave and Marta
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- Southern California Portuguese Water Dog Club in memory of Int/Mex/US CH Seadream Satin Doll CDX WWD GROM – "Dolly"
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- Kathleen Souza in memory of CT Dreamstar Femme Fatale-VCD3 RE MX MXJ MXP MJP2 OF CWDX GROM
- Kathleen Souza in memory of Int Am CH Vindouro's Once In a Lifetime RN AWD
- Nancy Vener
- Kendra & Matthew Yociss Happy Birthday Taylor Belle, a ClasSea dog, who turns 3 on July 2nd!
- Stacy, Dante, Chase, & Bella and Allison, Scott & Brook wishing a Happy 70th Birthday to Jolene Rissman

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- American Egg Board in memory of Rudy Pierce
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- Ann Benninger in memory of CH Cortereal Seakiss Of Starview VCD1 RE OAP AJP (Gaby)
- Valerie Berry in honor of the PWD PSG
- Benita Bottom in honor of TagAlong PWDs first litter turning 6!
- Eliot & Mary Brown
- Mary Jo Burgess
- Linda Carey, Rockmere in memory of Suzanne Garcia

(Continued on page 9)

- Ellie Chiampa & Tim Lohrenz in memory of sweet and gentle Lilly
- Nigel Clark/OreoBay PWDs in honor of the PWD PSG
- Bob and Jean Combs – Baymist PWD in memory of CH Belavista Alegria “Ali” De Alto Mare 7-26-98 to 1-27-2013
- Barb Crowther in memory of CH Abeja Manoel Pardal Rivero CD RE NAP NJP THD, “Bandit”
- Anat Cunha in honor of the PWD PSG
- Tom, Michelle & Lucky in memory of Webster “Walter” Shear
- Libby & Nick Devlin wishing a Happy 14th Birthday to Ragmop
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- Roslyn Eskind and John H. Brown in memory of Rudy Pierce
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- Fred Forman in honor of Lilly
- Fred Forman in honor of Annie
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- Richard A. Frankel in memory of Bernie, Izzy and Sophie
- Terry Freeman in memory of Bombardier
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- Frank & Gillian Goldschmidt in honor of the PWD PSG
- Gillian Goldschmidt in honor of the PWD PSG
- Patrick Gross & Stacy Sola in memory of “Nora” – Amarinhar Nora of Kalista CD AX AXJ NAP NJP AWD SROM
- Kimberly Hanson in memory of Brutus, loved by Karen Moench
- Kimberly Hanson in honor of Chuck Bubert & Jack
- Angela Harding in honor of the PWD PSG
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- The Janeczeks as a Thank You to Kris Cofield for introducing them to PWDs & Speedy
- Morgan Jennings in memory of Regan
- Morgan Jennings for Fawks
- Morgan Jennings in memory of Sweet Byng
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- Randy and Karen Latham in memory of Bella, CH Falcor-Hytide’s Cruzin’ Seaward owned & loved by James & Vanessa Ward
- Debbie Lauer
- Sarah Leatherman in memory of Jaime, beloved companion of Judy Newton, Ellen Gale & Erin
- Sarah Leatherman & Luna in memory of Hoke, beloved companion of Jennifer & Eric Wilmoth
- Sarah Leatherman in memory of Erin – beloved companion of Ellen Gale and Judy Newton
- Sarah Leatherman in memory of “Gabby” beloved companion of Twyla and George Buzick & “Doc”
- Sarah Leatherman in memory of “Lilli” loved and missed by Diane & Jeff Brundage & Chloe
- Sarah Leatherman in memory of “Gussie” and “Kip” beloved companions of Sandee Arndt & “Eddy”
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- Kathie Peightal, Rain Dawg PWD in memory of CH Kao De Blackjack CD RE TDI CGC, my Heart Dog and my first PWD
- Sally & Tony Perez in memory of CH Bramblecreek Combahee “Kirby”
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- Heather Shilo in honor of the PWD PSG
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- Laura Voelker in memory of Lucky
- Laura Voelker in honor of Karhu
- Claire P. von Meysenbug in honor of all Portuguese Water Dogs

(Continued on page 10)

- Mary & Jim Walker in memory of Captain Morgan's sister Abbie
- Janet Warnsdorfer & Galaxy PWDs in memory of Molly - Galaxy's Face of Venus
- Janet Warnsdorfer Galaxy PWDS in memory of Maya
- Liz Weidner
- Marvin & Fran Weinberger in memory of Jake
- Barbara & Ed Weisman-Geibert as congrats on "Sea Angels" new CH Marty
- Sue Wilcox-Hall in honor of the PWD PSG
- Elana Winsberg & Mike Barber as a Thank You to Susan & Don Myrick
- Jerry & Kim Wolcoveick in memory of Alice Vicha & all the "Norvic" PWDs
- Diane Workman in honor of the PWD PSG
- Deb Zorn in memory of Schatzie, Kizmet, Wooster, Karma, Marina and Phoenix

Boatswain \$100-\$249

- Tim Abbott & Mariana Palacios in memory of Archie
- Alberta Portuguese Water Dog Club in thanks to Lauren McDermott
- Anonymous – One who loved Mia and loves Max, Sandra & Bob – in memory of "Mia" GCH CH Imagine's La Regina Del Castello
- Chris Avery in honor of Cammi Avery. "Cammi, thank you for your thoughtfulness & generosity in support of the Portuguese Water Dog Foundation!"
- Diana Bailey
- Belouro Reg'd PWD group in memory of Melanie M. Williams, DVM, DACVO
- Ann Benninger in memory of CH Dacher's Winsome Reuben CDX RE AX MXJ MJB OF CGC WWD GROM
- Ann Benninger in memory of "Clio" CH Azeitona Rosa Do Preta
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- Rhea & Glen Case in memory of Molly, Galaxy Face of Venus: We'll miss you & love you always
- Rhea & Glen Case in honor of Janet Warnsdorfer, Thank you for our beautiful Molly, Face of Venus
- Scott & Annette Castiglione as a Happy Birthday to Bella
- Cathy Cates & Charles White in honor of the PWD PSG
- Joanne Chilton in memory of Trinket
- Kay Clark in memory of CH Del Sur Sail Away Color Me Pimenta "Pepper" 3/24/02 - 7/21/13
- Julie Conger in honor of the PWD PSG
- Susan & Carl Craig in memory of "Reuben": CH Dacher's Winsome Reuben CDX RE CGC AX MJX OF WWD GROM
- J. Matthew Eggemeyer in memory of Buoy Strunk
- Amanda Ford as a Thank You to the United Sunshine State Portuguese Water Dog Club for the opportunity to judge my first solo water trial
- Cecilia S. Ford in memory of Tess, beloved PWD 1994-2004
- Fred & Susan Forman in memory of Cypress Bays Aviator Magellan
- Friends of Pesa in memory of "Pesa" UCD UAGI Robel Wind at Rivergate UD RE MX MXJ CWDX ONYX
- Cheryl & John Frye in memory of Hy Berg
- John & Linda George, Karl Ryder and Kevin Burkum in memory of Rudy C. Pierce
- Yogi & Cinder Gerber in memory of our friend,
- Helen, CH Marshview Call Me Helen Wheels RA OA OAJ CGC WWD SROM
- Nancy Gills in memory of "Squid" Voyager's Squid at Anchor RA NA NAJ NF
- Vicki & Ken Goldberg as a Thank You to Ginnie Santoli for unconditional support & Guidance day or night!
- Vicki & Ken Goldberg as a Thank You to Kathy Ferrandino "Fair Skies PWD" for an adorable & loving puppy
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- Jenn & Michael Greene in memory of Windruff Nautica
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- Melanie Groth in memory of Zoltar - A Gentleman For Life – CH Vindouro's Your Wish Is Granted RN WWD
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- Brad & Belinda Harvey in memory of Lakecrew Copernicus' Sun "Cooper"
- Melinda Hatton in honor of Lilly
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- Dr. Cheryl Hoofnagle in memory of "Darcey" Blue Run's Doce Mora
- Sandra Holden in memory of all PWDs that have crossed over the Rainbow Bridge in 2014
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- Marion Krupka in memory of Razzi who died Feb 14, 2013 of hemangiosarcoma (spleen)
- Otto A. Kuehne & Cynthia A. Kuehne in honor of Joan Bendure for cancer research
- Otto & Cynthia Kuehne in memory of Kolbe 12/05/2006 – 07/01/2014
- Nancy & Michael Kurkjian in memory of Amigo's Mars MX MXB MXJ MJB XF CGC - Jazz - always in our hearts
- Nancy & Michael Kurkjian in memory of "Jazz" Amigo's Mars MXB MJB CGC
- Bobbe Kurtz in memory of my precious "Siren" of Starview
- Haven Lane in memory of Tejo forever
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- Stephen Lawroski & Monica Hadrian in memory of Onyx
- Susan Lefebvre & Joan Perriello in memory of CH Driftwood C Deuce Nativewind CD RE CWS
- Arthur & Roberta Levin in memory of our beloved PWD Bissa Levin
- Rhonda Levinowsky & Gale Erskine in memory of "Bacchus" CH MACH9 Cutwater Wine and Dine Me MXS3 MJS3 XF AD WWD TDI SROM
- Thomas & Linda Majcher
- Tom & Tina March
- Pam Marshall
- Pamela Marshall in thanks to Sandy & Ken Melson for hosting the 6th Vista do Lago's Water Camp, and trainer, Judy Murray
- Jere McInerney in memory of Stella – she was a special pup – we miss her
- Gale Meadow in honor of the PWD PSG
- Judy & Mike Modugno in memory of Seaport Vanuatu (Annie) AKC #WR078274102 - April 13, 2013 of hemangiosarcoma
- The Morrissey Family in memory of Bella
- The Morrissey Family in memory of Bella
- Nathalie Moyen in thanks to Jodi Whetzel
- Maryanne Murray in honor of the PWD PSG
- Donald D. Niemann
- The Nutmeg Portuguese Water Dog Club in memory of Jim Bell
- Pacific NW PWD Club in honor of the Seattle Seahawks and their 2014 Superbowl win
- Pacific NW PWD Club in honor of Yeager, Abby and Tradewind's PWD Kennel
- Robert & Lois Pierce in memory of Ruch C. Pierce
- Denise & Sandy Ratner in memory of Madsen, a very special PWD
- Linda Reimen in memory of Bo'sun (Armada's Boatswain Helm's Alee)
- Janine Richter
- Janine Richter and Tony Paton
- Monica & Peter Rowsom in honor of Caly – Windruff PWDs Puppy Pledge
- Judy & Barbara in memory of Pi
- Stan & Milarie Rude in congratulations to GCH CH Bayswater's Bodacious Brio RN BN NAJ TT WWD CGC on his new title

(Continued on page 11)

- Robert & Virginia Santoli for Addison's research
- Lynne J. Schwartz, Rivendell Kennel in memory of Susan McMahon
- Cheri Scofield in memory of Sun Joys Magnus & CnSands Hanes lost to cancer & loved forever
- Joan Sennett & Dick Tewes in memory of our beloved Schooner
- Joan Sennett in memory of Dick Tewes & our beloved Schooner
- Southern California Portuguese Water Dog Club in memory of Karen Begin
- Donna E. Shalala in memory of Cheka
- Southern California Portuguese Water Dog Club as a Thank You to Janis Watts for judging our 2013 Independent Specialty
- Southern California Portuguese Water Dog Club in memory of:
- CT Dreamstar Femme Fatale-VCD3 RE MX MXJ MXP MJP2 OF CWDX GROM – Carmen
- CH. Sunnyhill Jovial Jagador – Cody
- Mariner Happy Scamper WWD – Flora
- Multi BIS BISS BOSS CH Deleao's Amadeus of Kimlyn CD NAP NAJP NA NAJ WWD TDI SROM – Mo
- Seashells Malibu Rum – Raley
- MBISS BIS CH. Questar's Thunder and Lightning CGC RN NA NAJ AOMs CSLI-F - Rascal Starview's Sea Siren - Siren
- Southern Connecticut Agility Team, Inc. in memory of Melinda Harvey's Pi
- Wendy Spradlin in memory of Rascal
- James & Phyllis Stanton in memory of Augusto 1997 - 2011
- Kersten & James Terry in memory of Kika, Nautiques Neptonian Nymph OA AXJ NAF
- In honor of Pam Marshall to thank her for all her help & continued support of Vista do Lago's Water Camp
- Claire P. von Meysenbug in honor of all Portuguese Water Dogs
- Brad & Daphne Wagnon in memory of Katie
- Tom & Peggy Weissenborn in honor of Baron, Ripley & Jeremy
- Todd & Christine Williams in memory of Molukie
- Connie & Hale in memory of CH Sunnyhill Morgan
- By The Lee RE BN WWD MX MXJ SROM

First Mate \$250-\$499

- Chris C. Avery
- Donna Boles in honor of my beautiful dog Munson
- Friends of Lisa and Ace in memory of CH MACH5 Freestyle Best Of Three CDX RAE MXB3 MJS3 MXF MFB T2B WWD GROM
- Marietta Castellano & LaPescheria
- Colorado Portuguese Water Dog Club
- The Sesame Place VP Staff, Moses, Marikate & Sue in memory of "Mia Caruso" GCH CH Imagine's La Regina Del Castello
- John & Susan Cucura
- Cheryl Hoofnagle, Blue Run PWDs in honor of Ancient Mariner Aarion George Washington "Dante"
- Sue Hopkins
- Sue Hopkins in honor of the PWD PSG
- Jim and Judy Kamman
- Jim & Judy Kamman
- Carolyn M. Knutson
- Ken & Cynthia Kongorski
- Michael and Linda McLean
- Sarah Leatherman wishing a Happy Birthday to Luna my sweet girl
- Overboard Portuguese Water Dog Club in memory of our members dogs who have passed on.
- Overboard PWD Club in memory of our members dogs: past, present and future
- Sherry N. Rady in memory of Afortunado Cassiopea by The Lake "Cassi"
- Marilyn Rimmer, Questar in memory of Thomas F. McDonnell
- Marilyn Rimmer, Questar in memory of Fizz
- Marilyn Rimmer, Questar in memory of Isabella
- Marilyn Rimmer, Questar in memory of Snickers
- Marilyn Rimmer, Questar in memory of Nantucket
- Marilyn Rimmer, Questar in memory of Casper
- Marilyn Rimmer, Questar in memory of Karen Begin

- Dr. Mark & Jill Roudebush
- Bob & Ginnie Santoli in memory of "Teaky" Windruff Teak Tack Tow BN RA WWD CGC
- Elaine Suter, Hunter PWDs in memory of Rudy Pierce

Captain \$500-\$999

- Roberta Capuano & Tom Heffernan in honor of Jane & Stu Freeman, Freestyle
- Shirley Coleman & Allan Barnes
- The Keystone Portuguese Water Dog Club
- Robert Knight in memory of Slippers & Lillian
- Annette Claire Konga
- Sarah Leatherman in honor of "Luna's" Happy 7th Birthday
- Jan & Norm Mosher in memory of BISS CH Watermark Toby's Black Jack AOM AWD POM CGC TDI
- Francie & Peter Newfield in honor of Melanie Groth & Lucy
- Nutmeg Portuguese Water Dog Club in appreciation of Lauren McDermott's superb Water Workshops
- The Nutmeg Portuguese Water Dog Club in memory of Ed Whitney
- Sauder's Eggs in memory of Rudy Pierce
- Portuguese Water Dog Club of the Twin Cities in memory of our beloved PWDs who left us in 2012
- Nancy Schlemmer in memory of Buddy – Tesouros Emerald Cs Buccaneer
- United Sunshine State Portuguese Water Dog Club
- Bob & Charlene Wolfe in memory of our beloved girl "Rainy" Roughrider's Glacier Rainbow CD TDI

Commodore \$1,000+

- 2012 PWDCA National Specialty hosted by the Portuguese Water Dog Club of Greater Chicagoland
- From all of the participants at the Ailsby/McDermott May 2014 Arizona Camp
- Deb Bender/Calimel
- John & Sarah Brock, Brock Company Inc. in memory of Seaworthy's Magic Rope Trick CGC JWC
- Sue Hopkins, Chulsa Kennels
- Friends of Sarah Kahn & Ace
- GOOGLE
- Gary Gottlieb & Derri Shtasel
- Keystone Portuguese Water Dog Club
- Nutmeg Portuguese Water Dog Club, Inc. sincerely thanks all the PWD lovers who purchased the Nutmeg 2013 calendar
- James D. Northway, MD in memory of Ann Northway and PWDs Cassie & Splash
- Susan Paulini as a Thank You to Rolli Grayson and to Curtis Parham
- Pouch Cove Portuguese Water Dogs
- Rio Salgado Portuguese Water Dog Club & the volunteers & participants at the May 2013 Ailsby/Mc Dermott Water Workshop
- Peggy Weissenborn in memory of CHs Baron & Ripley
- Geri Zuckerman, Seadream Portuguese Water Dogs

Super Bowl XLVIII Challenge**Is Gambling a Good Habit or a Bad Habit?**

-Challenge between members of the PNWPWDC and COPWDC in honor of the Seahawks v. Broncos in the 2013 Super Bowl.

- Anne M. Andritsch – Go Seahawks
- Karen Kirby Ash & Saltydawg PWDs in honor of the Denver Broncos
- Janet Lee Boyd in honor of the Seattle Seahawks
- Charles F. Bubert in honor of the Colts
- Janice L. Butler in honor of the Broncos
- Pamela L. Coffeen in honor of the Seahawks
- DevOcean PWDs – Brisa & the Denver Broncos.....GO ORANGE!
- Donna Fisher in honor of Seattle Seahawks!!
- Suzanne Stone Foisie in honor of the Seattle Seahawks – Go Hawks!
- Amanda Ford with sympathy to Bronco fans
- Fred Forman in honor of Denver Broncos (COPWDC)
- Susan B. Forman in honor of Denver Broncos (COPWDC)
- Laurie Hardman in honor of the Seahawks – GO HAWKS!

(Continued on page 12)

- Anne Herberholz in honor of the Seahawks
- Katrina Jackson Jewel de Agua PWDs – betting on the Seahawks
- Judith James – Go Seahawks
- Lisa Kinney – PNWPWDC Super Bowl challenge
- C. Kratz & M. Koenigsberg in honor of Peyton Manning
- Corine Knudsen in honor of the Seattle Seahawks
- Mary Lane in honor of the Seahawks
- Sonia Manejkowski in memory of the Seahawks and in honor of my sweet loving Perry
- Lucinda Miner in honor of the Broncos!!!
- Gloria Morris in honor of Broncos win Super Bowl
- Leigh Pelc in honor of the Denver Broncos
- Pickwick Hollywood Litter: Seahawks 6, Broncos 4
- Sandy Pond – Go Seahawks
- Linda Reimen in honor of the Seahawks
- Valerie Tangen in honor of Seattle Seahawks – Go Seahawks!
- Martha J. Thomas – COPWDC Denver Broncos
- Marsha K. Tintes in honor of the Broncos
- Go Broncos!!!! From Hudson and Reggie Totten
- Anne Colston Wentz, MD in honor of the Broncos
- Anne Colston Wentz, MD in honor of the Seahawks
- Sue Zgol – COPWDC Denver Broncos

**“Groomed For A Cure” campaign
for hemangiosarcoma research**

Up to \$50

- Benita Bottom-Svitchen in honor of Galileo's new clip
 - Chuck & Candi Bubert in honor of Chugger
 - Chuck & Candi Bubert in honor of Jack's MACH I I
 - Chuck & Candi Bubert in memory of our buddy Porter
 - Sigrid Bundy in memory of CH Benhil's Kahlua 'n Cream & Cornus Kessi (Wh Dachshund), both victims & missed dearly
 - Mr. & Mrs. Fredric C. Burton, II in honor of Oreo & Pesci
 - Nigel Clark-Dixie is shave down to honor Sloopy who we lost to hemangiosarcoma in December 2012
 - Barbara & Joseph Connolly in honor of our new Karma puppy
 - Roberta Croce
 - Auntie Anat Cunha in memory of Marley Nickerson - Beira-Mar Bom Bom, July 27, 1999 - April 8, 2013
 - Mommy & Daddy Cunha - Karma PWDs wishing a Happy 3rd Birthday to our sweet Eight!!
 - Sandra Fournies
 - Fred & Susan Forman in memory of Magellan
 - Ann Harrison & Minx
 - Kara Kolster DVM
 - Jo-Anne Kruzynski
 - Nancy Kurkjian in honor of Jeter's first PACH points & first 2 MXP legs
 - Nancy Kurkjian in honor of Jeter as the Courier "Cover Boy"
 - Nancy Kurkjian in honor of Roxie
 - Nancy Leon
 - Pam Marshall
 - Jane & Art McEwen in memory of Delilah & Sampson
 - Mary Beth McManus
 - Judith Miller & Richard Corman in honor of Huck Finn (Navio's Born To Run AX AXJ OF)
 - Michelle & Jim Montgomery
 - Angie Robinson
 - Jerry Rollyson in honor of CH Glenwood Chuva's Doroteia CD RE WWD BROM
 - Elizabeth Shannon
 - Valerie Tangen
 - Deborah Totten in honor of my sweet boys Hudson & Reggie
 - Barbara Williams & Joyce Polak
 - Lisa & Tom Wolford
- \$50-\$99**
- Carlos & Kim Aguiar
 - Karen Ash in memory of Sierra, Whiteny & Lucy

- Leanne, Spice & Ivy Bertino
- Ann Camp
- Roslyn Eskind in memory of Mindy's Samson
- Bill & Sue Evans – Merlin & Sophie groomed in memory of Meia, Sculler & Fred, all lost to hemangiosarcoma in the last 3 years
- Melinda Harvey in memory of Redwoods Infinite Sea, Pi
- Karma PWD Family in honor of Karma's Poetic Justice litter pup "Prince" – service dog in training
- Karma PWD Family in honor of Karma's Poetic Justice litter pup "Pheonix"
- Karma PWD Family in honor of Karma's Poetic Justice litter pup "Schooner"
- Karma PWD Family in honor of Karma's Poetic Justice litter pup "Valentino"
- Karma PWD Family in honor of Karma's Poetic Justice litter pup "Gracie"
- Karma PWD Family in honor of Karma's Poetic Justice litter pup "Phelps"
- Karma PWD Family in honor of Karma's Poetic Justice litter pup "Raven"
- Karma PWD Family in honor of Karma's Poetic Justice litter pup "Willow"
- Karma PWD Family in honor of Karma's Poetic Justice litter pup "Hunter"
- Karma PWD Family in honor of Karma's Poetic Justice litter pup
- Karma PWD Family in honor of Karma's Poetic Justice litter pup "Lucy"
- Karma PWD Family in honor of new Karma puppy
- Bob & Adina Mathewson as congrats on your new championship Sweetpea
- Connie Millard
- Constance Rego
- Saltydawg PWDs in memory of CH Friendship N Northstar's Lilly CGC

\$100-\$249

- Florbela Allen in honor of Karma Tickle The 88 Imagine "Lennon" – Karma PWDs Puppy Pledge
- Karen Kirby Ash in memory of CH Saltydawg N Driftwood Lets Do It "Comet"
- Chelsea & Cooper Benson in memory of their wonderful OreoBay parents UGRAC4H/AKC CH Deewal Oreo Speedwagon AriesCD RN OA OAJ BROM TDIA "Oreo" & UKC ACHX AKC CH Marshviews My Girl Sloopy RN CD OA NAJ CGC TDIA "Sloopy"
- Barbara & Joe Connolly in honor of Karma Tickle The 88 Set Fire To The Rain "Adele" – Karma PWDs Puppy Pledge
- Yvonne & Dennis Ferreira in honor of Karma Tickle The 88 Great Balls of Fire "Jerry Lee" – Karma PWDs Puppy Pledge
- Robin & Tracy Gagnon in honor of Karma Tickle The 88 Skyfall "Adele" – Karma PWDs Puppy Pledge
- Kate & Steve Gerencser in honor of Karma Tickle The 88 Bennie And The Jets "Fisher" – Karma PWDs Puppy Pledge
- Vicki & Ken Goldberg in honor of "Buoy" loved & missed by Carol Mattingley & Ann Bowley
- Kimberly Hanson
- Susan Kingon in honor of Karma Tickle The 88 This Girl Is On Fire "Teal" – Karma PWDs Puppy Pledge
- David & Gina Maloney in honor of Karma Tickle The 88 Piano Man "Billy" - Karma PWDs Puppy Pledge
- Melinda Miller in memory of Reese 2004-2013
- Kit Murphy & Phil Arensburg in memory of Nico & Bruxa
- Richard Oberndorf & Michelle Broido in memory of Raven
- Tabby Thompson in memory of Dutch
- Sandra Pond
- Tabby Thompson in memory of UKC HIT UACH AKC CH Marshview's My Girl Sloopy CD RN OA NAJ
- Lisa Wilkinson

\$250-\$499

- Chuck & Candi Bubert in memory of our very special girl Gertie
- Betsy Schimpff in memory of Questar's Moon Over Mandela

\$500-\$999

- Tina Cross Morse Thanks to the many supporters of Made Especially for Dog fundraiser to support Groomed for a Cure