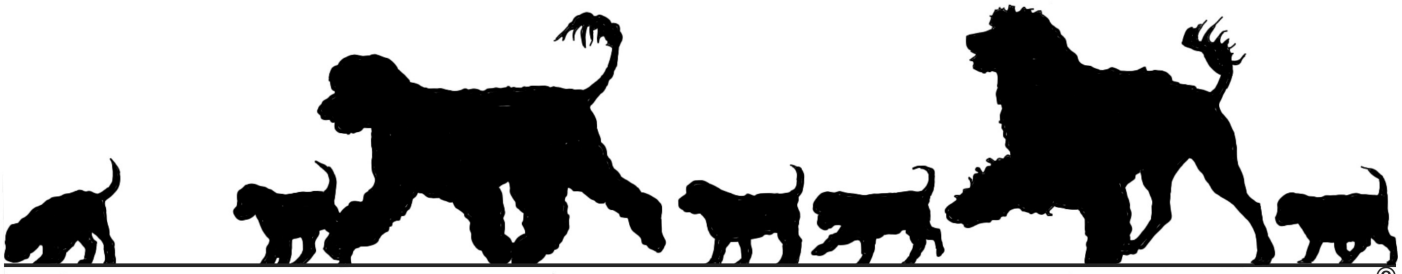

The Portuguese Water Dog Foundation, Inc.©

We are dedicated to funding canine medical research focused on issues that affect the health and well-being of Portuguese Water Dogs everywhere.

Inside this issue

A Novel Approach for the Prevention of Hemangiosarcoma: **The Medtech Study**

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

The Problem: Hemangiosarcoma is the cause of death for one out of every five Golden Retrievers in the United States. Portuguese Water Dogs and Boxers also have an especially high risk for this disease. One feature that makes hemangiosarcoma incurable is the fact that it is almost always detected at a very advanced stage when it is resistant to conventional therapies. This insidious cancer almost always grows out of sight without causing pain or obvious symptoms, so it is diagnosed late in the course of disease or after death.

This status quo is unacceptable.

A method to detect hemangiosarcoma in its earliest stages and an effective mechanism for prevention would be a giant leap forward in the management of this disease.

The Opportunity: Solving the cancer problem is a long-term challenge. However, we believe we have reached a point where we can diagnose hemangiosarcoma in the early stages and treat this disease before it reaches the clinical crisis point. Our goal is to continue development of two technologies that will allow us to achieve this.

First: a test and patented process to detect hemangiosarcoma cells in the circulation (blood).

Second: a novel drug, heretofore called "bispecific EGF angiotoxin," or BEAT, which attacks the hemangiosarcoma cancer stem cells that are responsible for establishing and maintaining the disease. BEAT effectively kills the cancer cells or makes the environment inhospitable for their growth. The results from our initial clinical trial have been highly promising. BEAT acts differently from conventional chemotherapy, so it does not pose the risks that make chemotherapy unacceptable for otherwise healthy patients. At the end of this project, we expect to have created tools to guide further development, licensing and deployment of these paired technologies in the community setting.

Approach: We will recruit cases with a confirmed or presumed diagnosis of hemangiosarcoma and dogs with no evidence of hemangiosarcoma of any age and any breed, as this will accelerate the process of validation.

Summary: The anticipated result will be preliminary tests for detection of circulating hemangiosarcoma cancer stem cells that will be in a "beta"- ready format, along with a safe and reliable treatment option for prevention of disease.

Note: Although this research project is pending peer review, the opportunity to make this research a reality is the result of unprecedented collaboration between the PWD Foundation, the Golden Retriever Foundation, the American Boxer Charitable Foundation, and the AKC CHF. Together, we are ready to promise a total of $472,000 over a three-year period to help researchers detect and treat hemangiosarcoma. Dubbed the "Shine On Challenge" thanks to an initial GR donor challenge, you can read the full story at our website, including details about the Foundation's support of earlier research into the development of the diagnostic test that will be an integral part of this exciting new project. ***Go to www.pwdfoundation.com***

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AKC CHF Research Progress Reports

Grant 01787: Clinical Advancement of a Cancer Vaccine in Dogs

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

Grant Objectives:

To optimize our cell-based vaccine approach to induce functional, long lasting tumor-specific immune responses that aim to prevent relapse and prolong survival in dogs with non-Hodgkin's lymphoma.

Report 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 cells (known as T cells) 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. Since then we have been working to further improve this vaccine in 2 important ways: firstly we aim to generate a more robust system that induces greater B cell proliferation and produces B cells that have improved capacity to stimulate the patient's T cells against the cancerous cells; and secondly to generate a more user-friendly system of B cell activation and expansion that would only require basic laboratory equipment to make these vaccines for canine patients. This is an important step towards potential commercialization of the product enabling its use for many more dogs.

Our work has focused on optimizing the feeder cells that are used to stimulate B cells from the patient's blood. Our proposed clinical trial involves repeat vaccination of dogs with lymphoma (rather than just 3 initial vaccines as per our first, phase I trial). Thus generation of a robust system that allows for optimal B cell activation and expansion aims to supply more B cells that can be cryopreserved for repeat vaccinations.

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 now made second-generation feeder cells that stably express the canine form of CD40L (we previously used the human CD40L molecule in our feeder cells). We found that our second-generation canine CD40L expressing feeder cells 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. Therefore, we will continue to generate CD40-B cell vaccines using freshly irradiated feeder cells. Interestingly, although our second-generation feeder cells are easier to maintain, we found that they appeared to only support B cell expansion for one round of stimulation and then the B cells died in the culture. After interrogating the system, we believe that the reason for this is that the new feeder cells support robust proliferation of B cells that requires altered culture conditions (diluting the rapidly expanding B cells out to lower concentrations than before and re-stimulating the B cells at shorter time intervals). We are now finalizing these important changes to our standard operating procedure.

Regulatory approval for our second clinical trial using our improved CD40-B cell technology has been given and we have recruited 2 dogs to date. Unfortunately both dogs failed to achieve clinical remission with chemotherapy and we were ineligible for the clinical trial. The difficulties we have had with identifying the reason for the apparent "failure" of our B cell culture system has significantly delayed our progress on the clinical part of this trial and we have had to temporarily halt trial recruitment. However, following 2 more confirmatory experiments we hope to start enrollment again.

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

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

Grant Objectives

We will examine the potential to use the multipotency of hemangiosarcoma cells to our advantage by forcing them to differentiate into lineages with reduced malignant potential.

Report from Investigator:

We have made substantial progress. Our results confirm and extend the notions that interactions between the tumor and its local environment regulate hemangiosarcoma progression. Yet, variability in cells within tumors can reduce the predictability of hemangiosarcoma behavior, and possibly contribute to therapy resistance. For example, hemangiosarcomas respond to the degradation of their supporting matrix by recruitinginflammatory cells and blood vessels. But the magnitude of this effect is variable among different hemangios, which requires us to consider that these tumors might adapt efficiently to very different microenvironments. The hemangiosarcoma microenvironment also tends to be rich in a molecule called CXCL12, which is used as a means of communication between the tumor cells and the normal supporting cells. Only some of the tumor cells have the receptors that transmit the signals from CXCL12. These cells help to support the tumor, and also can be efficient mediators of metastasis. But in their absence, other mechanisms might perform these functions. This suggests that blocking this pathway and others like it could have a positive therapeutic effect, but managing this disease will require combining strategies that lower the capacity of cells to simply switch their behavior to use alternate pathways to survive and thrive.

Grant 01828: Mapping of Genetic Risk Factors for Canine Hip Dysplasia

Dr. Antti Iivanainen, DVM, PhD
University of Helsinki and Folkhälsan Institute of Genetics

Grant Objectives:

1. To establish an accurately phenotyped primary study cohort for genetic studies. We aim to sample at least 300 cases and 300 controls.

2. To perform a GWAS for 144 cases and 144 controls using canine high density SNP arrays.

3. To replicate the associated loci in independent multinational cohorts of dogs in different breeds.

Report from Investigator:

The overall objective of our study is to perform a genome wide association study (GWAS) of canine hip dysplasia (CHD) in German Shepherds using a large sample cohort (200 cases and 200 controls). CHD is a common problem in many breeds. The dysplasia phenotype is graded from radiographs. In this study, we use the standards of Fédération Cynologique Internationale (FCI) ranging from A (healthy) to E (severely dysplastic). Each hip joint is graded individually. As the disease progresses also the risk for hip joint arthrosis – a painful and incurable condition – increases. The identification of genetic risk factors would enable the development of genetic tests to aid the breeders in controlling the disease. Four hundred animals consisting of carefully matched pairs of healthy and affected individuals should provide enough power for the association study to uncover the major genetic risk factors for this degenerative disease.

Since last report at the end of 2014, we have not performed larger genome-wide association studies but have instead concentrated our efforts on expanding the study cohort. The preliminary GWAS during 2014 was done using the cohort of 286 genotyped individuals and uncovered a promising association signal from one canine chromosome. This chromosomal region is currently under further analysis.

At present, we have collected a study cohort of 1141 dogs including 411 cases and 730 controls. 171 cases (with FCI-graded "D" or "E" hip joints) and 171 controls (with bilateral "A" hip joints) have been extracted from the study cohort. This subset is balanced with regards to working, show or mixed line subpopulations. From the balanced cohort, we have thus far genotyped 143 cases and 143 matched controls.

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

Dr. Benjamin L Hart, DVM, PhD
University of California, Davis

Grant Objectives:

To develop a generalized understanding of the impact of early spay and neuter on disease risk in dogs.

Report from Investigator:

The long-term goal of this project is to evaluate, using one consistent and uniform data base at our large veterinary medical center, the breed-specific effects of neutering at different ages on joint disorders (hip dysplasia, cranial cruciate ligament tear and elbow dysplasia) and some cancers (lymphosarcoma, hemangiosarcoma, mast cell tumor) that can be increased by neutering. The effects of neutering at various ages are also examined with regard to mammary cancer, urinary incontinence, and pyometra in females.

Our previous studies on the Golden Retriever and Labrador Retriever (supported by CHF), and published in two papers in the open-access journal, PLOS ONE, found a 3- to 4-fold increase in the incidence of one of more joint disorders (hip dysplasia, cranial cruciate ligament tear and elbow dysplasia) with early neutering in males and females. In female Golden Retrievers, neutering at any age resulted in a 3 to 4 fold increase in the occurrence of at least one of the three cancers followed. This effect on cancers was not seen in males. The research over the past year covers the popular German Shepherd Dog, the most important military and police canine, and the Rottweiler and Boxer, two breeds known for over-representation in death rates due to cancer.

Grant 01889-G: Innovations in Prevention, Diagnosis, and Treatment of Cancer - Goldens Lead the Way

Dr. Jaime F Modiano, VMD PhD,;Matthew Breen PhD, CBiol, FSB; Kerstin Lindblad-Toh, PhD
University of Minnesota, North Carolina State University Broad Institute of MIT and Harvard

Grant Objectives:

To determine whether newly identified risk loci harbor key genes or regulatory elements that contribute to and/or lower the threshold for initiation of lymphoma (LSA) and hemangiosarcoma (HSA), and furthermore, if they cooperate with acquired mutations that are necessary for clinical progression of these two diseases.

Report from Investigator:

This project has completed the first eighteen months. For analysis of the DNA sequence mutations we identified previously, whole genome sequencing of DNA from many golden retriever blood samples from dogs affected by lymphoma and hemangiosarcoma has been performed.

Continued
Thousands of DNA sequence variants have been detected and are being carefully analyzed. Collection of blood samples from golden retrievers across the US and Europe is complete and over the coming months we will assess the frequency of the risk haplotypes within these two populations.

For evaluation of DNA sequence changes in lymphoma and hemangiosarcoma tumor samples, we have compiled cases providing sufficient material for multiple analytical approaches and the first manuscript is in the pipeline for publication. More than 100 genes were found to have multiple mutations in lymphoma tumors. These genes and mutations are now being further studied to find the mechanisms involved in tumor progression, and to investigate shared and distinct genomic features within and between breeds and tumor subtypes.

We have made new and exciting discoveries that will help us to understand mechanisms of tumor progression and response to therapy, and we have created the infrastructure to integrate clinical performance and outcome data with the molecular properties of the tumors.

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

By Dr. Jeffery N. Bryan, DVM
University of Missouri, Columbia

Grant Objectives:

1. Characterize the types of B cell lymphoma in Golden Retrievers by flow cytometry.

2. Define the methylomes of B cell lymphomas in Golden Retrievers.

3. Identify and characterize subpopulations of cells within types of B cell lymphoma in Golden Retrievers with TIC phenotype.

Report from Investigator:

Progress continues at all 3 institutions. The proposed immunohistochemical evaluations and flow cytometry techniques are developed and are being refined as cases are added to the series. The population of B cell lymphomas appears to be a diffuse large B cell variety (DLBCL) similar to the aggressive form in humans. An immunohistochemistry panel is now functional to identify these and flow cytometry is being optimized. Sequencing experiments have identified a growing list of genes that are hypermethylated in B cell lymphomas of Golden Retrievers that is similar to those in human lymphoma. TAMU has successfully generated tumor initiating cell populations from cultured lymphocytes and has optimized the procedures to be performed on fine-needle aspirates of lymphoma nodes. It appears that multiple aspirates will be necessary to get all the material needed for characterization. Sufficient TIC cells can be generated for sequencing with the protocol in place at MU. The personnel for the studies are now in place. Further sequence generation is pending that will allow us to move forward with the diagnostic PCR panel.

Grant 02002: Defining the Genetic Basis of Inflammatory Bowel Disease

Dr. Karin Allenspach, DVM PhD
Royal Veterinary College, University of London

Grant Objectives:

The objectives of the present study are to identify single nucleotide polymorphisms (SNPs), which may confer genetic susceptibility or resistance to IBD using a genome-wide association study (GWAS).

Report from Investigator:

This study is investigating the genetics of Inflammatory Bowel Disease (IBD) in German Shepherd Dogs (GSD) from the UK and the USA by using a new technique called Genome—Wide Association Study. The results of this study will reveal important factors that contribute to the disease and that could in the future help to find novel treatment options. Owners and veterinarians of the cases and control dogs were contacted to ascertain that their group allocation is still correct. We have started extracting DNA from GSD with IBD and controls from the UK and are well underway to get the samples analyzed by Genome---Wide Association Study.

For the US part of the study, we are looking for help from owners and breeders of GSD. We will need DNA from GSD that have either been diagnosed with IBD at their vets or are healthy, over 8 years of age and have never had significant bouts of diarrhea in their lives. If you own a GSD that could fit one of these criteria, please get in touch with us to see if you could contribute to this important study by donating DNA from your dog.

Contact information: Atiyeh Peiravan, Department of Veterinary Clinical Sciences and Services Royal Veterinary College (RVC), University of London, Hawkshead Lane, North Mymms, AL9 7TA, UK. apeiravan@rvc.ac.uk.

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

By Dr. Jennifer G. Barrett, DVM, PhD
Virginia-Maryland Regional College of Veterinary Medicine

Grant Objectives:

To evaluate the effectiveness of stem cell and platelet therapy for the treatment of naturally occurring tendon injury in dogs and to compare efficacy of two different types of regenerative therapies.

Report from Investigator:

We are actively recruiting canine patients (all breeds and mixed breeds) with unilateral lameness originating at the shoulder, with no signs of osteoarthritis on radiographs.

If you or someone you know has a dog with primary unilateral lameness originating at the shoulder please consider participating and contact CHF or the contacts below for more information.

In this study, platelet rich plasma (PRP) will be combined with one of two types of cell therapy: concentrated adipose stem cells (ASC) and a mixture of adipose-derived cells called the stromal vascular fraction (SVF). This clinical trial is blinded and placebo-controlled.

Contact information: Jayme Schatz, Surgical Coordinator Email: jschatz@vosm.com | Phone: (240) 295-4400 Ext. 210 Katie Cox, Research Technician Email: kcox@vosm.com | Phone: (240) 295-4400 Ext. 215

Please note: This study takes place at Veterinary Orthopedic & Sports Medicine Group, Annapolis Junction, MD. Cell processing will be done off-site at the Equine Medical Center in Leesburg, VA.

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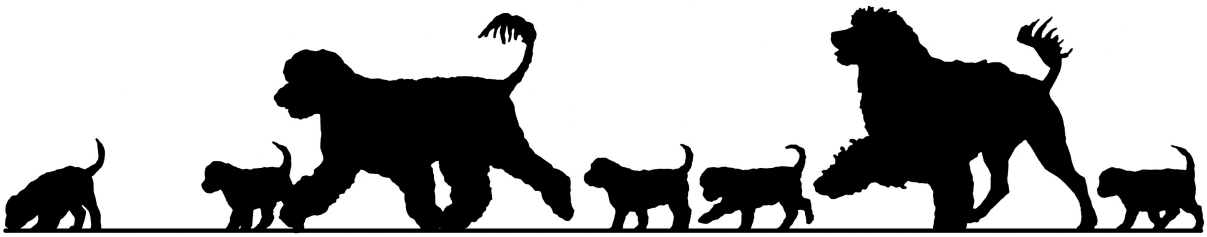

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Diane & John Burke wishing Happy Birthday to Jake & Allie

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Marian Ormont

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Kristi & Mike Portugue in memory of our first two PWDs, Neptune and Ocea. We carry your memory in our hearts forever.

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Helene & Allen S. Rothman, MD in memory of Stuart B. Freeman

Jaime Rubinson

Ellen Sard & Richard Salus in memory of Stu Freeman

Cheryl Sears

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Michelle Stone in memory of CH Driftwood's Cheers to Sea Girt

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Sharon Verley in memory of my first PWD, Kita, great dog!

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Corine Knudsen in memory of Jewel De Agua Joey

Sarah Leatherman in memory of Radar, mighty & beloved companion to Pat, Jim, Jed and C.J.

Sarah Leatherman in memory of Angus, a gentle dog and dear friend missed by Zander & Janice

Sarah Leatherman in memory of Maisy, sweet, dear companion to Suzanne, Shawn, Aiden and Flip

Michael & Dona Lee

Warren & Sandra Lloyd in honor of the PWD PSG

Kathy Maguire in memory of Stu Freeman

Mayflower Pembroke Welsh Corgi Club in memory of H. Edward Whitney

Mayflower PWDC in memory of Stu Freeman

Paul & Sandy Novicki in memory of our beloved PWD, Sailor – we miss her

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Marilyn Rimmer, Questar PWDs, in honor of the PWD PSG

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Rio Salgado PWD Club in memory of Legado Estou Enamarada WWD "Leal"

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Virginia Santoli in honor of the PWD PSG

Sandra Saybolt in honor of the PWD PSG

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Patricia Snyder in honor of the PWD PSG

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Elana Winsberg in memory of Tinker and Ella

Jerry & Kim Wolcoveick in memory of all Alice Vicka's Portuguese Water Dog

Kendra & Matthew Yociss wishing Happy Birthday to Taylor Belle and Sasha Rayne, two fabulous Classea dogs!

Theresa Zorad in honor of the PWD PSG

Deck Hand up to $49

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Linda K. & Krista K. Hunt, Kalista in honor of "Hydro" Kalista To Splash-n-Dash BN RN NA NAJ for his Novice Agility titles

Linda K. & Krista K. Hunt, Kalista in honor of "Moxie" Kalista Hitherandthithering CD RAE MX MXJ MJB OF AWD and Moe Flaherty for Moxie's MX!!

Linda K. & Krista K. Hunt, Kalista in memory of MACH2 Agua Viva's Princess Bela RN MXC MJS2 MXP MJP3 MJPB NF loved and missed by Sandy Kott

Linda K. & Krista K. Hunt, Kalista in honor of "Mira" Kalista Miranda Writes A New Chapter RN CGC for her RN

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Linda K. & Krista K. Hunt, Kalista in memory of CH Amarinhar Espiralado MX MXJ AXP MJP XF NFP CWDX loved and missed by Lauren McDermott

Linda K. & Krista K. Hunt, Kalista in honor of Kalista's Serendipity RN NA NAJ CGC "Sprite" for her NA and NAJ!!

Linda K. & Krista K. Hunt, Kalista in honor of Kalista To Splash-n-Dash BN RN OA OAJ CGC "Hydro" for his OA & OAJ!!

Linda K. & Krista K. Hunt, Kalista in honor of Kalista's Queen Of Everything OA OAJ "Nettle" for her OA & OAJ!!

Linda K. & Krista K. Hunt, Kalista in honor of AM/Can CH Kalista's Sun Star Traveler RN "Sunni" and Judy Cheguis on Sunni's AKC Championship, owner handled!!

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Linda K. & Krista K. Hunt, Kalista in memory of Kalista's Batedor "Scout", loved and missed by Heidi, Dan, Desmond and Isabel McKeown

Linda K. & Krista K. Hunt, Kalista in honor of Kalista's Signature Blend "Kona" for his AKC Championship

Linda K. & Krista K. Hunt, Kalista in memory of "Jet" Kalista's Bold Endeavour CD OA NAJ loved and missed by Pat Belliveau & family

Dale and Kathleen Jose in memory of Ashbe Legado Somente Uma

The Kerk Family in honor of Olive and Leah

Nancy Kurkjian in thanks to Diana & Noah for their hemangio research efforts

Carol Mattingley and Ann Bowley, Windruff PWDs, in memory of Hunter's Ginsan Que Sera Sera RE OA AXJ WWD "Casey"

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Karen and Walter Paulick in honor of Misty Rose our beloved Portie!!!

Kristi & Mike Portugue in memory of "Lucy" Kalista's I'm Guilded Two, for all you & Eileen taught us in puppy class

Kristi & Mike Portugue in memory of John Bruch, dog lover & dad to Porter, Iggy and Ziva

Kathleen Skeels

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The Southern California PWDC in memory of CH Ashbe Legado Somente Uma BN RN TD CGC AWD "Uma"

The Southern California PWDC in memory of Legado Faladora De Fenix CDX RE OA OAJ AXJ CWDX SROM "Sarah"

Kathleen Souza in memory of Lorraine Carver in appreciation for introducing me to the world of PWDs

Scott & Debbie Totten in memory of Misty

Terri Watkins in honor of CH Horizon I'd Rather Be Fishing BN RN NA NAJ CGC "Eli"

Jamie & Perry Weiner in honor of Dori – Holly Lanes adorable girl

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MAF Final report:

Determining a More Effective Treatment for Canine Lymphoma

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

Lymphoma is one of the most common types of malignant cancer. Several types of lymphoma are diagnosed in the dog, but the majority (approximately 70 percent) arise from transformed B-cells. Unfortunately, in spite of many advances in chemotherapy protocols, the prognosis for dogs with B-cell lymphoma has not changed significantly in more than 30 years.

Result: Immunotherapy may revolutionize B-cell Lymphoma treatment in dogs

Passive immunotherapy, which uses molecules called antibodies to kill cancer cells, has revolutionized lymphoma treatment in humans. Morris Animal Foundation-funded researchers at the University of Minnesota, in collaboration with researchers at Idexx Laboratories, Elanco Animal Health, and Stanford University, investigated whether or not this type of treatment could translate into helping dogs with cancer. Using recently developed canine-specific antibodies, the research team evaluated the safety and efficacy of these antibodies in the treatment of canine B-cell lymphoma. Antibodies were directed against two different targets on the cancer cells, and investigators believed that a combination of these two antibodies would be effective in treating dogs with B-cell lymphoma.

The researchers confirmed that the antibody combination promoted the killing of canine lymphoma cells in a laboratory setting. The research team then used a pre-clinical model to test the combination. Highly encouraging data suggested that this immunotherapy combination is both safe and effective in treating diffuse large B-cell lymphoma. The next research step is to shepherd these antibody therapies through regulatory approval and into canine clinical trials.

Lymphoma is a serious and common canine cancer, affecting dogs of any age or breed. Some breeds, such as golden retrievers and boxers, have a historically higher risk for the disease. Other commonly affected breeds include basset hound, Saint Bernard, Scottish terrier, bulldogs, Airedale, Weimaraner, Doberman pinscher, Labrador retriever, English setter and Great Dane. If proven effective in clinical trials as passive immunotherapy, use of the antibodies in this study could significantly improve treatment for all dogs with B-cell lymphoma. (D13CA-033)