



# 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.*

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*The Foundation is proud to have supported the Rabies Challenge since its start in 2007.*

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## 2016 GRANTS FUNDED

### Is Defective Secretion of Antimicrobial Peptides Associated with Reduced Microbicidal Effects in Atopic Keratinocytes?

This study investigates whether the complications resulting in skin infections experienced by dogs with allergies can be better treated by increasing the secretion of the body's natural antimicrobial defenses. In this particular case, much work in this direction has been done in human medicine and the researchers at the University of Florida will determine how this work in human medicine can benefit dogs with complications from skin allergies.

Antimicrobial peptides (AMPs) are small proteins produced by many organisms. They have multiple functions, the most important of which is the defense against pathogens. The antimicrobial activity of such proteins has been demonstrated against multiple microorganisms. Recently, a lack of secretion of AMPs, after exposure to bacteria in human skin cells harvested from allergic patients, has been hypothesized as a possible cause of recurrent infections in allergic skin conditions. Allergies are common in dogs and frequently associated with recurrent, antibiotic-resistant skin infections. Thus, the identification of ways to boost ability to fight bacteria is important. The

investigation of possible changes between healthy and atopic skin cells is fundamental in order to be able to intervene, and make such secretion more effective without the use of synthetic antimicrobials. Thus, the goal of this study is to determine if, like in people, lower AMP secretion is present in skin cells harvested from allergic dogs after stimulation with common cutaneous pathogenic bacteria. The hypotheses to test are 1) whether a lower amount of AMPs are secreted by allergic skin cells compared with healthy ones, and consequently, bacteria are not effectively killed; and 2) if a higher amount of AMPs is retained within the allergic cells. This study has the potential to open the way for a revolutionary approach to treating skin infections that occur secondary to allergies in dogs by increasing the secretion of natural antimicrobial defenses, and thus reducing the use of synthetic and expensive antimicrobials with potential side effects.

*Co-sponsored with the AKC Canine Health Foundation, Grant Number: 02182-A*

### Measuring Chemotherapy Drug Resistance in Dogs with T-cell Lymphoma

Human cancer treatment is becoming more and more tailored to each individual case and tumor characteristics. This study will be conducted by researchers at North

Carolina State University and will follow a small number of cells that evade chemotherapy agents, providing information on the effectiveness of particular agents against T-cell lymphoma. The data from this study will be used to personalize treatment protocols for dogs on an individual basis in hopes of improving outcomes, survival rates, and quality of life.

Description: Since the use of combination chemotherapy was first reported in 1968, little progress has been made in improving the survival of dogs with T-cell lymphoma. Effectively monitoring chemosensitivity – the number of tumor cells killed by chemotherapy – of individual lymphoma cells exposed to multiple agents over many treatments remains a challenge. Following the small numbers of cells that evade chemotherapy would provide information on the effectiveness of a particular chemotherapy agent. In this clinical trial, researchers will use state-of-the-art DNA technology to measure changes in this small population of resistant cancerous T-cells in client-owned dogs with lymphoma. Data will be used to personalize treatment protocols for individual dogs in hope of improving survival and quality of life.

*Co-sponsored with the Morris Animal Foundation, Grant Number: D16CA-056*

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PROGRESS REPORTS**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:**

Examining the potential to use the multipotency of hemangiosarcoma

**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 recruiting inflammatory cells and blood vessels. But the magnitude of this effect is variable among different hemangiosarcomas, 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 01787: Clinical Advancement of a Cancer Vaccine in Dogs***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:**

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 previously 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 aimed 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 and distribution of the feeder cell 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 explored ways to enable use of these feeder cells without the need for special equipment. To this end, we have developed protocols that utilize KTCD40L cells that have previously been irradiated (using a Cs-137 irradiator) and cryopreserved. This eliminates the need for irradiation and maintaining a fresh stock of KTCD40L cells and provides a more off-the-shelf product that could be used by standard laboratories to stimulate canine PBMCs.

We have performed several experiments to evaluate whether these pre-irradiated, cryopreserved feeder cells can effectively expand canine B cells. 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. However, the moderate drop in B cell expansion efficacy is off-set by the easy of use and potential for commercialization enabling any facility with standard laboratory to equipment to generate autologous CD40-B cells.

We have now re-opened our clinical trial and have since enrolled 13 dogs following optimization of our B cell culture system. Over the next year, we will perform trial follow up, continue to generate and administering autologous CD40-B cells every 2 months to patients remaining in remission and will perform anti-tumor immune function testing to determine whether our approach is able to induce and maintain clinically relevant, anti-tumor immunity.

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

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

#### **Grant Objectives:**

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. 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.

#### **Report from Investigator:**

At present, we have collected a study cohort of 1141 dogs including 411 cases and 730 controls. From the study cohort, we have thus far genotyped 167 severe ("D" or "E" hip joints) and 38 mild ("C" or "D" hip joints) cases and matched controls ("A" hip joints) for all of them.

Since last report in June 2015, we have collected an independent second cohort (833 dogs, four breeds) and performed larger genome-wide association study with 526 dogs. Several clusters of SNPs suggestive of association were detected in eight chromosomes. The validation of these

SNPs will take place in 2016 using the independent second cohort.

#### **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:**

This report of unpublished research since this earlier work, will give a rough idea of the findings. (*Large breed results only; small breed findings are available at the PWDF website.*)

#### **German Shepherd Dog**

Using a database for this breed of 1,250 cases, we found that 7 percent of gonadally intact males and 5 percent of intact females were diagnosed with one or more joint disorders. But neutering in the first year increased the incidence of one or more joint disorders by 3-fold over that of the intact dogs. The occurrence of the cancers we followed in this breed was at a low 3 percent in the intact males and females, and was not affected in either sex by neutering. The take-home message is that delaying neutering until the dogs are at least 1 year old markedly reduces the likelihood of one or more disabling joint disorders.

#### **Rottweiler**

Using the database for this breed of 696 cases we found that 9 percent of intact males and 16 percent of intact females had at least one joint disorder. Neutering during the first year increased the likelihood of a joint disorder in both sexes by up to 3 times. The occurrence of one or more of the cancers in the intact dogs was relatively high, 12-13 percent, but neutering at any age did not increase the rate. Delaying neutering in this breed until the dog is well past a year of age should avoid increasing the already high risk of a joint disorder.

#### **Boxer**

With a database for this breed of 646 cases, we found that the occurrence of one or more joint disorders in intact males and females was just 2 percent, and there was no indication of an effect of neutering at any age. We are tentatively attributing this to the somewhat wide stance of this breed. The underlying occurrence of at least one of the cancers in intact dogs was high; 12 to 16

percent respectively in males and females, but there was no indication of an increase in this measure for any neuter period with the exception of a higher cancer occurrence in the 1- year neuter period of males. Until the statistical analyses are done, it seems like avoiding neutering males at the age of 1 to 3 years would be advised.

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

*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 monomorphic group of 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. Because the lymphoma samples are so similar across the board, we are changing Aim 1 slightly to evaluate gene expression in these tumors as it relates to the methylation profile.

# MAF FINAL REPORT: ASSESSING HOW A PROTEIN HELPS HEMANGIOSARCOMA CELLS SURVIVE

Erin B. Dickerson, PhD, University of Minnesota

Hemangiosarcoma, an aggressive and uniformly fatal cancer, affects dogs of any age and breed. Its prevalence is noticeably higher in some breeds, including golden retrievers, Portuguese water dogs and German shepherds. This cancer is particularly devastating because the growing tumors can rupture, causing rapid and significant blood loss which often is fatal. Although some dogs exhibit no signs of disease prior to tumor rupture, some dogs have clinical signs, such as lethargy and weakness. However, these signs can be so subtle they may go unnoticed by owners. Despite many attempts to find effective treatments, the survival rate for dogs with hemangiosarcoma has remained relatively unchanged for several decades, measured in days, weeks or months. Tumor persistence, due to chemotherapy drug resistance, is a major barrier to successful treatment of this cancer. Recent evidence suggests that populations of cancer stem cells give rise to tumors, promote tumor growth, and are the main culprits behind drug resistance and disease recurrence. To explore this theory, Morris Animal Foundation-funded researchers at the University of Minnesota examined how CSF-1R, a marker present on the surface of some hemangiosarcoma stem cells, contributes to the maintenance and survival of these cells.

The team found that one subpopulation of CSF-1R expressing cells uses a novel mechanism to evade the effects of the chemotherapeutic agent doxorubicin. The researchers discovered hemangiosarcoma stem cells with high numbers of CSF-1R on their cell surface trap doxorubicin in a cell structure called a lysosome. Cancer cells rely on lysosomes to break down cell components to regenerate nutrients needed for tumor growth. By using this novel strategy to trap doxorubicin, hemangiosarcoma tumor cells are able to avoid the damaging effects of the drug and survive. In continued foundation-funded research, the team is looking at strategies to interrupt this novel cellular strategy of isolating drugs.

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Vicki Goldberg in memory of Blue, Maggie and all the beloved PWDs who have touched the lives of my friends  
Vicki Goldberg in honor of my mother – I am the woman I am today because of you

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Pam Marshall as a thank you to Sandy & Ken Melson for hosting the 7<sup>th</sup> Vista do Lago's Water Camp, and trainer, Judy Murray  
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Norm & Jan Mosher in honor of Chester (CJ) Favre for his love and dedication to PWDs  
Marian A. Ormont  
Tony Paton & Janine Richter in memory of Puddles, passed September 1, 2015 at age 14 years  
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WWD AOMs GROM "Spencer"

Cheri Scofield in honor of the PWD PSG

Kenneth Sears

Katherine Klein Shaffer in memory of Immaculata  
Pearl Bingham "Immie"

James & Phyllis Stanton in memory of Augusto  
1997 - 2011

Ken & Kathy Tallering

Sigrid Tiedtke

William Varr & Victoria Moreo in memory of  
Stuart Freeman

Judge & Mrs. Thomas Wallitsch in memory of  
Reggie

Barbara Weisman & The SeaAngel puppies in  
memory of NorthStar's Fudge-A-Mania

Kendall & Melinda Wellman

Anne & Dennis Wentz

Bob & Charlene Wolfe in memory of our darling,  
"Rainy"

Paul Zoschke & Marcie Wallace for Cheers' 15<sup>th</sup>  
Birthday

## Sailor \$50-\$99

Anonymous in honor of Bean Klipp

Karen Ash in honor of the PWD PSG

John & Geri Azevedo in memory of CH Aviators  
Anchors Aweigh "Anchor"

Fabiana Bazzani in honor of the PWD PSG

Ann Benninger in honor of the PWD PSG

Eliot Brown

Paul Byrum in honor of Meg DeFore, Torrid Zone

Barbara & Roy Cawley in memory of "Jeremy"  
Makitso's Jeremy Rose - The sweetest El  
Diablo!

Barbara & Roy Cawley in memory of "Vino" -  
The Commodore of Makitso

Clarice De Christina, DVM & Curtis Reif

Colorado PWD Club in memory of Jim Carpenter

Paul Croce in memory of Bobbi Croce

Barbara Crowther in honor of the PWD PSG

Warren P. Davis & Elizabeth C. Belden

Libby & Nick Devlin in memory of Roxy &  
Ragmop

Angelika Ebenig in honor of the PWD PSG

Fran & Paul Fehling in honor of Daisy and Calvin

Barbara Floch in honor of the PWD PSG

Laura Fosholt and Bea Jennings in memory of  
CH Timbermist Halley's Sweetheart, who had 5  
wonderful pups continuing to give joy to their  
people

Fred & Susan Forman in honor of the PWD PSG

Jane Freeman in honor of the PWD PSG

Debbie Giewont in memory of Independence  
(Inde)

Dale Goldheim in memory of Raven

Christine Harris in honor of the PWD PSG

Claire Hart

Martin Hatlie in honor of the PWD PSG

Anne Herberholz in memory of Beatle, loving  
companion to Sandy & Jim

Anne Herberholz in memory of Chili. A girl like  
no other - sweet, cuddly & loving, yet wired to  
work and excel! Beloved companion to Suzanne  
& Jan Malick, but missed by so many.

Anne Herberholz in memory of "Cheeto"  
Stargazer Trip the Light Fantastic. Loved and  
adored by the Dipperys. Gone too soon.

Silke Hirtz-Schmidt in honor of the PWD PSG

Pat Hogan in honor of the PWD PSG

Linda K. & Krista K. Hunt, Kalista in  
congratulations on your new titles to Am/Can  
CH Kalista's Sun Star Traveler RN NA NAJ JWC  
"Sunni" for her NA NAJ and JWC!

Linda K. & Krista K. Hunt, Kalista in memory of  
BIVS CH DownEast Claudia Shipper CD RN  
WWD from granddog "Teddy the Felon" and the  
rest of the Kalista "L" litter

Sue Mueller Jobe in memory of John A. Jobe  
and our babies "Tug" and "Jazz", and in honor  
of "Star"

Mary Beth Johnson in loving memory of Emmie,  
Ben, Hobie & Nina

The Kerk family in memory of Olive

Ralph Klump & Family in memory of Abby

Alan Lane

Candace Lawhorne in memory of "Andy"  
Amigo's Cookies & Cream

Warren & Sandra Lloyd in honor of the PWD  
PSG

Lone Star PWD Club in memory of Mary Jane  
Dill

Lone Star PWD Club in memory of Praia de  
Milfontes do Vale Negro "Marouta"

Lone Star PWD Club in memory of HIT Am/Can  
CH Eauchien's Boca Chica TD UD NA CWDX  
Platinum ROM "Boca"

Lone Star PWD Club in memory of CH  
Driftwood's Texas Sound Off UD CWDX  
Versatility "Sounder"

Lou A. Lindquist

Judy Malstrom in memory of our love bug  
Cutter's Aureole. He changed the lives of 2  
young girls forever.

Sonya Manejkowski in memory of Joanne  
Forsythe's "Tru" - beloved angel

Sandy Myers in honor of the PWD PSG

Michael Mobley in honor of the PWD PSG

Sonia & David Novick

Jim & Deb Nungesser in memory of Abby  
Cooper Potter

Karen & Walter Paulick in honor of our PWD,  
Misty Rose

Kathie Peightal, Rain Dawg in memory of Kao &  
Moosey

Kathie Peightal, Rain Dawg in honor of the PWD  
PSG

Marilyn Rimmer in honor of the PWD PSG

Rio Salgado PWD Club in memory of Legado  
Faladora De Fenix CDX RE OA AXJ CWDX  
"Sarah"

Rio Salgado PWD Club in memory of Neocles LL  
Been CGC Therapy Dog "Bean"

Martha Ruskai in honor of the PWD PSG

Julie & Ty Rust in honor of the PWD PSG

Sandra Saybolt in honor of the PWD PSG

Lauren & Tony Showers in memory of Oreobay  
Get in the Game "Splash"

Linda Shultz

Kathy Souza in honor of the PWD PSG

Barb Stanek in memory of Rudy

Barbara Stanek in honor of the PWD PSG

Nancy B. Stewart

Elaine Suter in honor of the PWD PSG

Cindy Tanenbaum & Ezra Simon in honor of Rita  
O. Sylvia

MaryBeth Turner in honor of the PWD PSG

Nancy Vener

Rosemary Walmsley in honor of the PWD PSG

Janis Watts in memory of "Sarah" Legado  
Faladora de Fenix CDX RE OA OAJ AXJ CWDX  
SROM

Sian Wilstrup in honor of the PWD PSG

Kimberly & Jerome Wolcoveick in memory of  
Curly, Catie, and all of Alice Vicha's PWDs

## Deck Hand up to \$49

Jim & Laurie Boland in honor of Kalista - and all  
the work you do with, and for, PWDs!

Francine Brown

Michael & Susan Burke

Judy Cheguis in memory of Jiggy

Ordean & Dorothy Finkelson in memory of Osha  
& Neptune

Valerie Grahn

Linda K. & Krista K. Hunt, Kalista in  
congratulations on your new title to MACH 2  
Kalista's Harley Marley RN MXS MJG OF T2B  
THD CWD "Marley" for his CWD!!

Linda K. & Krista K. Hunt, Kalista in  
congratulations on your new title to CH Kalista's  
Ready To Rock BN CGC AWD "Jagger" for his  
AWD!

Linda K. & Krista K. Hunt, Kalista in  
congratulations on your new title to MACH  
Kalista's Now What BN RA MXS MXG MXF NAP  
MJP T2B CAA CWD "Y" on his CWD!!

# HONOR ROLL OF DONORS

## Deck Hand up to \$49 (continued)

Linda K. & Krista K. Hunt, Kalista in honor of MACH2 Kalista's Just What The Dr Ordered UD RAE MXG MJG MXF MFB T2B CWDX SROM "Queixo" for her 2015 Versatility Q!

Linda K. & Krista K. Hunt, Kalista in congratulations to CH Kalista's Um, You Rang? "Maynard" for his AKC Championship!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to CH Kalista's Ready To Rock BN CGC WWD "Jagger" for his WWD!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to Am/Can CH Kalista's Sun Star Traveler RN NA NAJ AWD "Sunni" for her AWD!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to Kalista's Vixen JWC for her JWC!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to Kalista's Queen of Everything AX AXJ AWD "Nettle" for her AWD!

Linda K. & Krista K. Hunt, Kalista in memory of Kalista's Heart of Gold "Higgins" loved and missed by Signe & Doug Pagel

Linda K. & Krista K. Hunt, Kalista in memory of GCH CH Praia Norte Agua Julius loved and missed by Beryl Nord & Tom Kaiser

Linda K. & Krista K. Hunt, Kalista in memory of Kalista's Feelin' Jazzy "Satchmo" loved and missed by Joel and Vida Peskao

Linda K. & Krista K. Hunt, Kalista in honor of HIT Kalista's Icebreaker UD RAE OA AXJ WWD "Breaker", for his RAE!

Linda K. & Krista K. Hunt, Kalista in memory of "Maddie" Kalista's Batata Doce OA NAJ

Logan Keay in memory of Lucie Goosey. To know her was to love her. May she be swimming in the biggest pool only her wildest dreams could have ever imagined.

Kara A. Kolster, DVM in memory of CH Ebb Tide's Too Hot to Handle

Kara A. Kolster, DVM in memory of GCH CH Ebb Tide's Up Country Titan CD AX MXJ WWD

Carol Mattingley and Ann Bowley, Windruff in memory of "Spencer" MBIS MBISS CH Freestyle Ocean Breeze OA OAJ NF WWD AOMs GROM

Mary Morehouse

Karen & Walter Paulick in memory of Rosie Nolan

Jayne Phillips in memory of "Windy"

Marilyn Rimmer, Questar in memory of Primo

Marilyn Rimmer, Questar in memory of Casey

Marilyn Rimmer, Questar in memory of Lovey

Marilyn Rimmer, Questar in memory of Stu & Spencer Freeman

Marilyn Rimmer, Questar in memory of Ed Whitney

Marilyn Rimmer, Questar in memory of Patrick

Marilyn Rimmer, Questar in memory of Lorraine Carver

Marilyn Rimmer, Questar in memory of Sam Assam

Marilyn Rimmer, Questar in memory of Trudy

Marilyn Rimmer, Questar in memory of Phil Young, MD

Southern California Portuguese Water Dog Club in memory of BISS CH Roseknoll's Perfect Storm "Stormy"

Southern California Portuguese Water Dog Club in memory of Del Sur's Mandalay Bay "Ruby"

Kathleen Souza in honor of Cortereal Ginger Rogers CWD2

## Board of Directors

Carol Mattingley — *President*

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Janet Boyd — *Director*

Cindy O'Connor — *Director*

Anne Paige — *Director*

## SUMMARY OF THE LATEST RABIES CHALLENGE FILE:

*Kris L. Christine, Founder, Co-Trustee, The Rabies Challenge Fund*

The Rabies Challenge Fund's latest trial conducted with the USDA's new rabies challenge virus is 6 weeks post-challenge and, so far, has yielded results meeting our interim goals as well as partially satisfying the Title 9 Code of Federal Regulations (CFR) requirements for rabies vaccine licensing.

Fifteen dogs were included in this trial. Only one of the 5 dogs vaccinated in 2007 showed protection against rabies, while 4 of 5 dogs vaccinated in 2009 (80%) demonstrated protection against a rabies virus challenge. Once all 5 of the unvaccinated control dogs showed the very early clinical signs of rabies virus infection, they were humanely euthanized.

Per CFR's Title 9 recommendations, the surviving 5 vaccinates will remain under observation for another 6 weeks to detect late development of any clinical signs of rabies.

Whole blood mononuclear cells collected at post-challenge days 4 and 10 were tested by flow cytometry to determine kinetics of immune memory cell responses. Tissue samples from the euthanized dogs are currently undergoing rabies virus detection testing. Serum samples collected at various time points in the study are being evaluated by serum virus neutralization and ELISA testing methods.

One remaining challenge with 15 dogs is planned to fulfill the USDA Title 9 requirements for rabies vaccine licensing and to establish a canine rabies titer standard as confirmed by challenge data.