



2005 Grant Awards
George and Phyllis Miller Trust
San Francisco Foundation
Seven studies funded for a total of \$96,427

The Winn Feline Foundation is pleased to announce the recent award of seven grants funded by the George Sydney and Phyllis Redman Miller Trust. The Trust designated the Winn Foundation as one of its advisor organizations in their desire to “support medical research to investigate the causes, prevention and development of cures for diseases of . . . domestic cats.”

RNA interference of the glycoprotein-D and DNA polymerase genes of feline herpesvirus by synthetic siRNAs. Rebecca P. Wilkes, Stephen A. Kania; University of Tennessee; \$10,863

Feline herpesvirus 1 (FHV-1) is a DNA virus that produces mainly upper respiratory tract disease in cats. Despite available vaccines and the labile nature of the virus, FHV-1 is wide-spread in the feline population. Approximately 80% of FHV-1 infected cats develop a latent infection and serve as reservoirs for the virus. Currently available antiviral treatments for herpesvirus infections have limited efficacy, and there is no effective therapy that specifically targets FHV-1. RNA interference therapy would specifically target FHV-1. RNA interference is a RNA-guided gene regulatory mechanism that is found in a variety of eukaryotic organisms, including yeast, plants, and mammals. One of its biological functions is anti-viral immunity in plants. This defense mechanism is triggered by double stranded RNA, and small interfering RNAs can be chemically produced and delivered to cells to silence specific genes of interest. RNA silencing has recently been used for prevention of various mammalian viral infections both in vitro and in vivo. The purpose of this study is to determine the feasibility of using RNA interference to prevent or treat FHV-1 infections in vitro and to lay the ground work for a new therapeutic approach for the treatment of FHV-1 infected cats.

Tramadol: a new analgesic for use in cats. Bruno H. Pypendop, Jan E. Ilkiw; University of California-Davis; \$11,492

Drug options to provide analgesia in cats are limited, especially when oral administration is required. Drugs that are commonly used in other species, such as opioids, non-steroidal anti-inflammatory agents, alpha-2 agents, the dissociative, ketamine, and local anesthetics often produce side effects in cats. Tramadol is an orally administered, centrally acting analgesic drug that interacts with opioid, adrenergic and serotonin receptors. It has been shown to be efficacious and well tolerated in people and dogs. Although tramadol is currently used in cats, no scientific data support this use. Its

pharmacokinetics are unknown in that species, making rational dosing recommendations impossible. Its duration of action is also unknown. Although it appears to improve pain clinically, no objective data on efficacy in cats are available. In this study, we propose to determine the pharmacokinetics of tramadol in cats. We will then characterize its analgesic effects, the dose-dependence of these effects, and their duration using a thermal threshold model. This study will allow the recommendation of optimal dosing regimens, both in terms of dose and dosing interval.

Incidence of occult heart disease in apparently healthy mixed breed cats. Sonya G. Gordon, Risa Roland, Matthew W. Miller, Ashley Saunders, Lori Drourr; Texas A& M University; \$12,700

Cardiomyopathies are the leading cause of symptomatic feline heart disease. The incidence of occult heart disease in apparently healthy mixed-breed cats with and without auscultatory abnormalities such as murmur, arrhythmia or gallop rhythm is not well documented in the veterinary literature. Additionally, current clinical recommendations include an echocardiogram for all cats with auscultatory abnormalities. One recent study with a small sample size suggested that as many as 86% of clinically asymptomatic cats with a murmur have echocardiographic evidence of heart disease. A second report cited the presence of cardiac disease in approximately 20% of cats undergoing post mortem evaluation for sudden death. Together these findings suggest that clinically significant occult heart disease may be relatively common and represent a risk factor for an adverse clinical outcome. We propose to evaluate 100 apparently healthy mixed-breed cats of various ages to further describe the incidence of occult cardiomyopathy in cats with and without auscultatory abnormalities. Evaluation will include auscultation, indirect systolic blood pressure, thoracic radiographs, heartworm antibody, total T4, blood urea nitrogen, creatinine, packed cell volume and total solids. This study will, in addition, generate normal ranges for two dimensional echocardiographic left atrial and left ventricular dimensions and a variety of tissue Doppler parameters, as well as determine any significant correlation with a radiographic vertebral heart score. Cats with auscultatory or echocardiographic abnormalities will have annual follow-up evaluations for a total of 2 years. Data generated by this study will be entered into a new web-based feline cardiac registry.

Identification of the mutation for an inherited feline craniofacial defect. Leslie Lyons; University of California-Davis; \$15,000

The Burmese breed of cat originated in the 1930's. A phenotypic change occurred in the 1970's when the "Eastern", "new look", "contemporary", or "more extreme" style became prominent. These cats differed from the "traditional" or "less extreme" cats with a higher frontal prominence, more rounded head, more prominent eyes and a more demarcated nose break. After this phenotype became more widely distributed, some breeding lines produced kittens with a severe congenital craniofacial defect. This disease follows an autosomal recessive mode of inheritance and is characterized by a duplication of the upper maxillary, whisker pads and incomplete formation of the cranium. This condition is fatal although kittens must occasionally be euthanized post parturition.

Research has established linkage to three markers with the disease phenotype. One of the developmental gene clusters, HOXC, lies on the edge of the linked region. This cluster of genes has been studied extensively in mouse, frog and avian models, which have elucidated genes in pathways of development. Therefore, HOXC may contain the causative mutation for the defect, and is a strong candidate. This research will focus on the sequencing of this cluster of genes from affected and unaffected cats to identify a mutation causing this inherited defect.

Phase II Studies on the Heritability of Resistance/Susceptibility in Feline Enteric Coronavirus Infection in Randomly-bred, Colony-reared, Domestic Cats. Niels Pedersen, Leslie Lyons; University of California-Davis; \$15,000

The present proposal outlines phase II of a three part long term study involving feline enteric coronavirus (FECV) immunity. The goal is to create two small breeding colonies of cats selected specifically for either extreme resistance or extreme susceptibility to the virus. These colonies will serve as a basis for future studies on determining the genetic basis for resistance/susceptibility and to ultimately apply genetic selection for the control of feline infectious peritonitis (FIP) in pedigreed cats. FECV is not a significant pathogen by itself. However, mutations in the small envelope (3c) gene result in a novel virus with tropism for macrophages rather than gut epithelial cells. This novel virus causes a highly fatal disease known as feline infectious peritonitis. The FECV → FIP virus (FIPV) mutation occurs in about 5-10% of FECV infected cats, and one half or more of animals exposed to this mutant virus will fail to mount protective immunity and die. FECV immunity in most cats is either non-existent (about 10-20% of cats become persistent shedders) or short-lived (70-80% of cats undergo constant reinfections). Only 10-20% of cats develop strong immunity. Genetic factors are the best explanation for these differences in infection outcome. This phase II study is to determine whether the resistance/susceptibility to FECV infection is under genetic control. Phase I infection studies yielded a breeding colony of 7 animals. Kittens produced by these breedings, and not naturally infected with FECV, will be infected at 16 weeks of age with FECV and fecal FECV shedding monitored for 8 months. If heritability can be demonstrated, phase II studies will be extended to preliminary studies on a genetic basis of resistance and susceptibility. Phase III would be to apply specific genetic tests to detect resistance pedigreed cats and to see if FIP can be controlled by selective breeding.

Repair of Feline Corneal Ulcers with Mesenchymal Stem Cells. P. Richard Vulliet, David Maggs; University of California-Davis; \$8,672

This project will investigate if mesenchymal stem cells (MSCs) or adult bone marrow stem cells can be used for treatment of refractory ulcerative disease of the feline cornea. We will investigate various growth conditions to direct the MSCs into a corneal epithelial phenotype. Differentiation of the stem cells will be demonstrated by immunocytochemical techniques and PCR. We will also grow MSCs from the bone marrow of selected cat patients in fibrin gels and place the gels on ulcerated corneas under a protective contact lens. After three days, the contact lens will be removed and the

eye examined for engraftment and differentiation of the fluorescently-labeled cells. The cats will then be examined on a regular basis to assess engraftment and differentiation of the MSCs and healing of the ulcer. We will continue the differentiation studies, enroll additional patients in the clinical arm of this study, and optimize methods of cell administration. We expect to demonstrate grafting of the MSCs, differentiation into corneal stromal keratinocytes, an improvement in corneal clarity and decreased recovery time. Similar or identical studies to this project are currently being performed in humans with remarkable success.

Expression and Pharmacologic Inhibition of Anti-Apoptotic BCL-2 Family Members in Feline. David M. Vail, Douglas H. Thamm, E.J. Ehrhart III; Colorado State University; \$22,700

Vaccine-associated sarcoma (VAS) and oral squamous cell carcinoma (OSCC) are two of the most common and troubling feline neoplasms encountered in veterinary practice. A better understanding of the biology of these diseases is necessary to facilitate the discovery of novel efficacious therapies. Anti-apoptotic proteins such as those in the Bcl-2 family are commonly expressed in neoplasia, and their pharmacologic inhibition may be an important novel treatment modality for veterinary cancers. The hypothesis we intend to test is that anti-apoptotic Bcl-2 family members will be detectable in feline VAS and OSCC, and their pharmacologic inhibition with the small molecule ABT-737 will result in significant potentiation of chemosensitivity *in vitro* and *in vivo*. This hypothesis will be tested by the completion of the following specific aims: 1) Determine the expression of Bcl-2, Bcl-X_L and Bcl-w in a panel of feline VAS and OSCC cell lines and archived paraffin embedded tissues; 2) Determine the effect of ABT-737 on the growth and chemosensitivity of VAS and OSCC cells *in vitro*; 3) Determine the effect of ABT-737 on the growth and chemosensitivity of VAS tumors in a murine xenograft. Successful identification of anti-apoptotic Bcl-2 family members and their pharmacologic inhibition with ABT-737 will provide important proof of principle and proof of target, justifying the clinical evaluation of Bcl-2 family inhibition in cats with VAS and OSCC.

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