Protocol 39403 Application 1.1

Approval date	08/03/2022
Expiration date	08/03/2025

1. Basic Information

1. Elements ID

For existing protocols, enter the ID assigned to this protocol in Topaz Elements.

9688

2. eACUC Number (Automatically Assigned)

39403

3. Principal Investigator

Whiting, Rebecca Elizabeth Hannah

Job title
PROF, AST
Department
Ophthalmology
Division
Medicine
Business unit
University of MO-Columbia

4. Protocol Title

Gene and stem cell therapies for neurodegenerative disease

5. Triennial Re-write

Is this protocol a triennial re-write of a protocol that was previously approved at the University of Missouri?

● Yes ○ No

A. Historical Protocol Number

What is the ACUC number this protocol was previously approved under?

Protocol 21440

B. 3 Year Progress Report

Give a brief summary of the work completed on the historical protocol listed above.

In the past 3 years, we finalized our study with BioMarin pharmaceutical that demonstrated efficacy of periodic intravitreal injection of TPP1 in preventing and slowing progression of retinal degeneration in the dachshund model of CLN2 disease. This study was published and is currently being investigated in

clinical trials to treat children with this disease. We have also shown that an AAV2.TPP1 gene therapy vector injected into the vitreous is capable of slowing progression of retinal degeneration and are working with partners to bring this therapy to clinical trials as well. We also began studies to investigate therapies to treat the CNS symptoms of CLN2 disease including an AAVrh10.TPP1 gene therapy vector and the use mesenchymal stem cells; these studies are ongoing.

2. Species Section

1. Please note, the total number of animals requested is the amount of animals you will need for a 3 year period. This number should include all experimental animals plus animals used for colony maintenance (breeders and offspring produced that are not used for experiments). These numbers should match the amounts in the Justify Animal Numbers section. If this is a triennial re-write these amounts should also include any animals on the previous protocol that will be transferred to the new protocol.

Species	Strain/Stock/Breed	Age/ Weight	Pain/ Distress Category	Authorized	Ordered	Received	Adjustment	Available
Dog	Dachshund,Donations	birth to 10 years; 100 grams to 80 pounds	USDA Category B	200		30		170
			USDA Category D	100				100
Total Dogs:				300	0	30	0	270

2. Phenotypic consequences

Describe any phenotypic consequences of the genetic changes to the animals and the outcome of these consequences (e.g. whether or not any change in animal welfare or husbandry is anticipated).

No Phenotypic consequences...

3. Wild Animals

Are WILD ANIMALS to be used or studied?

○ Yes ● No

4. Client-Owned Animals

Are CLIENT-OWNED animals to be used or studied?

○ Yes ● No

3. Proposal Overview

1. Purpose

Purpose of the study:

We are working to develop and test therapies for a group of inherited diseases in humans and dogs called the neuronal ceroid lipofuscinoses (NCLs) caused by genetic mutation in one of 13 genes (CLN1 - CLN13).

These diseases cause progressive degeneration of the central nervous system and the retina and are ultimately fatal. We are currently conducting studies to test 1) direct gene therapy and 2) stem cell based ex vivo gene therapy in Dachshunds with a naturally-occurring null mutation in the CLN2 gene.

We would also like to establish research colonies of dogs with other inherited neurological diseases for preclinical therapeutic studies. We do genetic screening of privately-owned dogs as a service to identify dogs that have mutations that are responsible for the diseases in which we are interested. Periodically, the owners of affected dogs offer to donate them to us for research purposes. We would like to accept donations of reproductively intact dogs that could be used for breeding to establish a means of producing affected dogs that could be used for therapeutic studies with the corresponding disease. There are funding agencies that would support pre-clinical therapeutic studies on affected dogs if we had the means to breed them. All dog donations will meet the USDA requirements for animal acquisition and be approved by the Office of Animal Resources prior to receiving the animals at MU.

2. Value

Please provide the information necessary to allow the ACUC to evaluate the objectives of the study against potential animal welfare concerns.

Development of effective treatments using dog models will serve as the basis for treating serious inherited diseases in humans and dogs that cause degeneration of the retina and nervous system. Dachshunds that are homozygous for a naturally-ocurring mutation in the CLN2 gene suffer from a progressive fatal neurodegenerative disease that is very similar to the disease affecting children with mutations in the same gene. Successful development of an effective treatment for the dogs using gene therapy will serve as the basis for human clinical trials that could lead to a cure for this debilitating disease. We currently offer a DNA test that can identify affected dogs and prevent further breeding of dogs with the disease in order to prevent additional cases in the pet community. For dogs with other inherited diseases, being able to generate affected dogs by breeding would enable us to conduct preclinical therapeutic studies that would provide the safety and efficacy data required to support human clinical trials for treatment of the corresponding diseases in people.

3. Lay Term Description of Experimental Design

To put something in layman's terms is to describe a complex or technical issue using words and terms that the average individual (someone without professional training in the subject area) can understand. This section should be written so that someone with a **10th grade science education can easily understand the project.**

We are conducting studies to develop treatments for a fatal childhood disease called CLN2 that causes degeneration of the nervous system. Our studies are being conducted using a naturally-occurring Dachshund dog model. The disease results from inherited genetic mutations in the CLN2 gene. Dogs that are homozygous for this mutation develop progressive degeneration of neurons and corresponding neurologic symptoms of the disease such as seizures, loss of motor control, and cognitive deficits. In addition to neurological symptoms that develop as a result of degeneration of the brain, children and dogs with this disease lose their vision due to degeneration of both the retina of the eye and the regions of the brain responsible for vision.
In dogs with CLN2, disease signs first become apparent at approximately 5 months of age and progress to a stage requiring humane euthanized at 10 to 11 months of age. Due to the inherited genetic mutation, dogs with this disease are missing a lysosomal enzyme called TPP1 that is important for normal turn-over of proteins in the body. Without TPP1 enzyme, neurons in the body cannot function normally and gradually degenerate over time. We are using gene therapy and stem cell therapy approaches to replace this missing TPP1 enzyme in order to slow or stop the disease progression.

A number of approaches to deliver TPP1 enzyme to the brain and retina are being evaluated including a direct gene therapy approach and a stem-cell based approach. Direct gene therapy relies on a vector, which is

composed of a copy of the normal TPP1 gene (missing in CLN2 affected dogs) packed in a virus coat that helps the TPP1 gene get into the cell nucleus where it can direct production of the TPP1 protein. The newly made TPP1 protein can then be used normally by the brain or retina cells, and this results in slowing or halting of disease progression. Direct gene therapy relies on some cells in the brain or retina to make a large amount of TPP1 protein to share with the entire brain or retina, in addition to continuing to perform all of their other normal functions. An alternative approach uses stem cells (isolated from the bone marrow) to produce the needed TPP1 enzyme. These stem cell "TPP1 factories" have only a single function and can be implanted near the brain or retina tissue that needs TPP1, thereby allowing the native brain and retina cells to perform their normal functions without the extra burden of excessive TPP1 protein production.

We will test the efficacy of these approaches in preventing or slowing the progression of neurologic disease signs and/or deficits in the retina of the eye. Therefore, dogs will receive some combination of the following (1) Injection of direct gene therapy vector into the cerebrospinal fluid (CSF) that circulates throughout the brain and spinal cord to deliver TPP1 protein; (2) Injection of direct gene therapy vector into the vitreous of the eye for TPP1 delivery to the retina; (3) Injection of TPP1-producing stem cells into the CSF; (4) Injection of TPP1-producing stem cells into the vitreous of the eye, and 5) We may also employ periodic injection of pure TPP1 enzyme into the vitreous of the eye (enzyme replacement therapy) or into the CSF. While enzyme replacement therapy is more direct, it requires regular repeat injections every few weeks, while the gene therapy and stem cell approaches should offer a one-time treatment option and remove the need for repeat treatment. For all approaches, some dogs will be treated using green fluorescent protein (GFP) as a control protein in place of the therapeutic TPP1 protein so that we can visualize which cells in the brain and retina have been reached by the treatment.

Dogs will each receive the chosen treatment combination between the ages of 3 and 6 months; dogs may either receive eye treatment only, brain treatment only, or a combination eye and brain treatment. The dogs are then monitored weekly to monthly with a panel of tests to evaluate neurological disease progression including behavioral testing, neurological and ophthalmic exams, electrophysiological testing, and MRI imaging. Blood and CSF samples are also collected periodically for analysis. When the disease progresses to end-stage, the dogs are euthanized for humane reasons and their tissues are collected for evaluation. We anticipate that the dogs receiving the control AAV2-CAG-GFP vector or eye treatment only will reach end-stage disease and require euthanasia at the usual 10 to 11 months of age. For dogs receiving the CSF injections of direct gene therapy vector or TPP1-producing stem cells, survival could be prolonged indefinitely depending on how effective the treatment is. These dogs will be euthanized either when they reach end-stage disease or when they reach 5 years of age, whichever is sooner in order to analyze the treated tissues.

4. Scientific Description of Experimental Design

In language a scientific colleague can understand, provide a step-by-step, general description of the animal experiments you will perform including experimental groups and timing of procedures and manipulations. For complicated experimental designs, including a flow chart, diagram, or table in the Attachments section is recommended to help the ACUC understand what is proposed. DO NOT describe details of the procedures here as such details are requested later in the form.

We are conducting studies to develop treatments for a fatal neurodegenerative disease called CLN2. Our studies are being conducted using a naturally occurring Dachshund model of CLN2. The disease results from a mutation in the TPP1 gene, and dogs that are homozygous for this mutation develop a progressive neurodegenerative disorder characterized by progressive atrophy and loss of function of the central nervous system and the retina. Affected dogs and people suffer from progressive impairment of neurological functions and from progressive vision loss.

Animals
CLN2 Dachshunds: Signs in the dachshund first become apparent at approximately 5 months of age and progress to a stage where the dogs need to be euthanized at 10 to 11 months of age. Dogs that are

heterozygous for the TPP1 mutation are perfectly healthy as are the homozygous wildtype dogs. Dogs for the research are generated by breeding heterozygous or homozygous affected males to heterozygous females. Puppies are genotyped from a cheek swab at about the age of weaning or earlier (at the time they receive identifying microchip implants). All of the homozygous affected dogs are used for the treatment studies and/or breeding. Some of the heterozygous dogs are used temporarily for breeding, and then after being spayed or neutered, are eventually adopted out. Most of the homozygous normal dogs are adopted out shortly after weaning and after being spayed or neutered. A few homozygous normal dogs are kept temporarily as controls for behavior or vision studies, and then after this control data is collected, they are spayed or neutered and adopted out.

Dogs with other neurologic diseases: Part of our research involves identifying mutations in dogs that result in inherited neurodegenerative diseases. Privately-owned dogs carrying these mutations are sometimes offered as donations to the University or for purchase from non-commercial breeders (primarily show-dog breeders). If these dogs carry a mutation of value to our research, we will obtain the dogs after proper health screening, vaccinations, and quarantine, and the dogs will be incorporated into our research colony, following filing and approval of an amendment. We will breed the dogs and conduct the same therapeutic studies as those described for the Dachshund CLN2 disease. We anticipate that any donated dogs could come from any breed or mixed breed dogs. We have identified mutations of interest in Cane Corsos, Australian Cattle Dogs, Australian Shepherds, Chihuahuas, English Setters, Chinese Cresteds, American Bulldogs, Tibetan Terriers, Golden Retrievers, and several mixed-breed dogs.

Treatment approaches: We are using direct gene therapy and ex vivo gene therapy (stem cell based) approaches to treat the dogs in order to slow or stop the disease progression. Prior to treatment and regularly following treatment, we look at several biomarkers of disease in order to assess treatment efficacy. Each AAV may offer different distribution depending on the target tissues, and therefore we are currently considering 3 different AAVs for CSF delivery including AAV2, AAV9, and AAVrh10. All of these have been used in previous dog studies for other diseases and are therefore under consideration as a therapy for CLN2 disease.

The five TPP1 delivery methods we will use are as follows: (1) Injection of AAV-CAG-TPP1 into the cerebrospinal fluid surrounding the central nervous system; (2) Injection of AAV-CAG-TPP1 into the vitreous of the eye; (3) Injection of autologous bone marrow derived mesenchymal stem cells (MSCs) into the vitreous of the eye after the cells have been transduced with AAV2-CAG-TPP1; (4) Injection of autologous bone marrow derived MSCs into the CSF after the cells have been transduced with AAV2-CAG-TPP1; and (5) periodic injection of recombinant TPP1 enzyme into the vitreous of the eye or into the CSF.
For all of the gene therapy approaches, some dogs are treated using an AAV-CAG-GFP vector in place of the AAV-CAG-TPP1 vector so that we can visualize which cells are expressing the fluorescent transgene. These dogs are used as controls for the therapeutic efficacy trials. The CSF delivery approaches are to treat the brain and spinal cord to prevent CNS degeneration. The injections into the vitreous are to treat the retina to prevent retinal degeneration.
Each dog may receive eye only treatment, CNS only treatment, or a combined eye and CNS treatment. For approaches #1-4, each dog will receive injections at ages between 3 and 6 months. For direct gene therapy approaches #1 and #2, dogs will receive a single injection each for the eye and/or brain. For the stem cell based approaches #3 and #4, dogs may receive 2 injections in each eye and 2 injections into the CSF. For the dogs receiving intravitreal injections of recombinant TPP1 (approach #5), injections will begin between 3 and 5 months of age and be repeated every 2-6 weeks. Each dog will be given an injection of TPP1 in one eye and vehicle (sterile saline) in the other eye (total volume 0.1 mL per eye per injection).

Follow-up measures of treatment efficacy: The dogs are monitored with a panel of neurological and visual disease markers using behavioral testing, neurological and ophthalmological exams, electrophysiological testing, in vivo retinal imaging, and MRI imaging of the CNS. Periodically blood, urine, and CSF samples are

collected for analysis. Eventually all of the treated dogs are humanely euthanized and their tissues are collected for evaluation. We anticipate that the dogs receiving the control AAV-CAG-GFP vector and dogs receiving only eye treatment will reach end-stage disease and require euthanasia at 10 to 11 months of age as is typical for untreated CLN2 affected dogs. For dogs receiving the CSF treatment, survival could be prolonged indefinitely depending on how effective the treatment is. These dogs will be euthanized either when they reach end-stage disease or when they reach 5 years of age, whichever is sooner.

Direct gene therapy:
The gene therapy approach involves injection of a TPP1 gene construct (AAV2-CAG-TPP1, AAV9-CAG-TPP1 or AAVrh10-CAG-TPP1) into the ventricles, lumbar spine, and/or cisterna magna of pre-symptomatic dogs at 3 to 6 months of age. In previous studies we have shown that administration of this construct results in long-term expression of TPP1 in the brain and its release into the cerebrospinal fluid (CSF). The CSF flows to most regions of the brain. Via this flow, we expect the therapeutic normal replacement protein to reach many cells in the brain and retard or prevent their degeneration. We have already shown in studies in which recombinant TPP1 protein is infused into the CSF that we are able to get such widespread distribution of the enzyme. To minimize immune reactions to the TPP1 protein, dogs will be treated with immunosuppressants starting just prior to treatment and continuing for varying periods of time (length of time to be determined empirically).
We will also test direct gene therapy in the eye by injecting the TPP1 gene construct (AAV2-CAG-TPP1) into the vitreous at 3 to 5 months of age. The efficacy of the vector in preventing retinal degeneration will be evaluated by assessing retinal function with electrophysiological techniques and retinal structure using imaging techniques. AAV2 has been shown as the most promising option for intravitreal delivery, but dosing and age of treatment still requires optimization.

Ex vivo gene therapy with autologous mesenchymal stem cells:
We have previously shown that bone marrow-derived mesenchymal stem cells (MSCs) from dogs homozygous for the TPP1 mutation can be genetically modified to overexpress the TPP1 enzyme. A sample of bone marrow is obtained from a forelimb of the dog between 2 and 3 months of age, and the cells are expanded in culture, transduced with the TPP1 expression vector, and then implanted into the vitreous or CSF of the same dog. We have demonstrated therapeutic benefit when the cells are injected into the vitreous, but will conduct studies to determine if they can also be used in the CSF to prevent neurologic signs of disease. Control experiments will be performed by injecting the MSCs transduced to overexpress GFP instead of TPP1. Some dogs will receive both the intravitreal and CSF injections of TPP1-expressing MSCs to determine if combined treatment can prevent all disease signs and preserve useful vision.

Techniques for delivery of therapeutics

CSF Administration via the lateral ventricles or via cisternal puncture: In order for the TPP1 enzyme to reach the central nervous system in significant quantities, the treatment must bypass the blood-brain barrier and therefore requires injection directly into the CSF. This may be done through a surgical procedure into the lateral ventricals or non-surgically via the cisterna magna or lumbar vertebral area. Either procedure will be performed under general anesthesia by a board certified veterinary neurologist. While cisternal delivery is preferable since it is less invasive, it is possible that we will not be able to achieve adequately widespread delivery of the therapeutic throughout the brain with this approach. In this event, intraventricular surgical delivery will be employed.

Intravitreal administration: In order for the TPP1 enzyme to reach the retina in significant quantities, the treatment must bypass the blood-retinal barrier and therefore requires injection directly into the vitreous of the eye using a small gauge needle while the dog is under general anesthesia. Based on previous studies, we expect significant transfer of the enzyme from the vitreous to the retina. The injection will be performed by a

board certified veterinary ophthalmologist experienced with the procedure.

Follow-up Assessments: The following measures will be used to determine whether therapy is capable of preventing or slowing disease progression and to monitor for any complications associated with therapeutic intervention.

Behavioral testing
a. Reversal learning using a T-maze: The reversal learning task is repeated monthly and uses a t-maze apparatus and food reward motivation to measure cognitive abilities in dogs.
b. Visual function testing: Visual function will be tested monthly by assessing the ability of dogs to distinguish between two images that are displayed on adjacent iPad screens using standard food reward-based training methods.

Neurologic Examinations:
For treated and control dogs in the studies, complete physical examinations will be performed on a weekly basis. Body growth will be monitored weekly by body weight measurements. Beginning at 4 months of age, a neurologic examination will be performed weekly until euthanasia. These exams are completed by a licensed veterinarian.

Visual system assessment:
a. Ophthalmic exam: All dogs in the study will undergo routine clinical ophthalmologic exams on at least a monthly basis. These exams are performed in the same manner as is done for clinical patients.
b. Pupillography: A standard protocol will be used to video record and take measurements of the pupillary light reflex (PLR). Recordings will be performed under general anesthesia every 2 months beginning at 4 months of age and continuing until the dogs reach end-stage disease.
c. Electrophysiology: Measurement of electroretinograms (ERGs) and visual evoked potentials (VEPs) will also be performed monthly on all groups of the Dachshunds used in the study under sedation.
d. In vivo imaging: Following the ERG recording session, general anesthesia will be induced and retinal imaging will be performed.

Cardiac Assessments: Noninvasive cardiac assessments using echocardiography and electrocardiography may be performed under sedation in Dachshunds enrolled in these studies every month starting at 6 months of age. Cardiac abnormalities have been previously documented in these dogs and may offer a biomarker for future therapeutic studies.

MRI Imaging: We will perform magnetic resonance imaging (1.5 T or 3.0 T) on each dog once every 4-8 weeks to enable 3-D visualization of the brain. NCL affected dogs develop brain atrophy during the disease progression. We have previously established the volume of the brain and the ventricles in normal dogs and in untreated dogs with CLN2. Therefore, monitoring volume over time in treated dogs will provide us with another biomarker by which to evaluate therapeutic strategies.

Collection of Blood/CSF/Urine/Tears: Blood, urine, tear fluid, and cerebrospinal fluid will be collected periodically while the dogs are alive. For dogs in the therapeutic studies, blood and CSF samples will be collected first at approximately 3.5 months of age and then monthly therefter until they are 24 months of age. If the dogs exceed 24 months of age, the frequency of CSF sampling will be reduced to once every 6 months until they reach 5 years. For analysis of progesterone levels in breeding females, blood will be collected daily once the dogs show signs of heat and analyzed to determine the best days for breeding. Blood may be collected from dogs not currently on study for use by MU collaborators. The blood collections will not exceed the currently approved sampling volumes and frequencies.

Euthanasia: Dogs will be euthanized and necropsied in room D119 of the VMDL which is setup and dedicated for this purpose. The dogs will be sedated in the Comparative Neurology Lab (in most cases, euthanasia is

performed following other procedures for which the dog will have already been sedated). The dog will be placed in a carrier and transported to the VMDL (neighboring building) by the veterinary technician and veterinary neurology research intern where it will be euthanized and necropsy performed immediately thereafter.

4. Justify

1. Justify Use of Animals in your Research

Justify the use of animals for your experimental goals. **DO NOT** describe details of the experimental design or justify animal numbers here.

The purpose of this research is to determine whether therapeutic agents delivered to the brain or eyes of subjects suffering from the fatal neurodegenerative disease neuronal ceroid lipofuscinopsis (NCL) will be effective in preventing or slowing the disease. Before these therapies can be tested in humans, their efficacy and safety need to be evaluated in animals. We have Dachshunds that have a naturally-occurring mutation in the same gene (CLN2) that causes a childhood form of NCL in people. These dogs develop neurodegenerative signs and pathology very similar to the corresponding human NCL. Therefore, these Dachshunds are an ideal model in which to assess treatment efficacy and safety. The translational value of the CLN2 dog model has been demonstrated by the FDA approval of Brineura (www.brineura.com/); successful preclinical trials in our dogs provided the necessary support to conduct clinical trials, which were similarly successful and this therapeutic is now greatly improving quality of life in children with CLN2 disease.
If we are able to obtain donated dogs with other hereditary neurodegenerative diseases that also occur in people, these dogs could be used for the same types of preclincal therapeutic studies to develop effective treatments for these other similar disorders.

2. Justify Animal Species

Justify the choice of species for your study.

To model human NCL for testing therapeutic approaches, an animal model is required. The only known species to naturally develop CLN2 as a result of TPP1 mutations are mice and dogs. We discovered the naturally occurring CLN2 in the miniature Dachshunds, obtained dogs that were heterozygous for the TPP1 mutation, and established a research colony by breeding these dogs. The disease phenotype in mice does not mimic the human disease phenotype nearly as closely as does the Dachshund. In addition, with respect to evaluating therapeutic efficacy and complications, the dog is a much better model because the size and complexity of the central nervous system of the dog is much closer to humans than is that of the mouse. In planning our studies, we decided to maintain the mutation in the miniature Dachshund breed because the disease phenotype was well characterized by us in this breed and these dogs are easy to work with in terms of size and temperament.
Different forms of NCL and related lysosomal storage diseases occur naturally in other dog breeds. If dogs carrying the disease-causing mutations are donated to us, we will be able to readily use them to conduct the same type of therapeutic studies we are currently conducting with the CLN2 Dachshunds.

3. Justify Animal Numbers

Justify numbers of animals to be used (attach timeline or flow chart and power analysis, if possible, to describe study groups). This section should include a description of animals used for colony maintenance (breeders and all offspring produced) as well as a description of experimental animal numbers. Total numbers should match the requested numbers in the species section.

- Animal Numbers Justification

- The Logical Determination of “N” in Animal Experimentation
- Non-Statistical Approach for Calculating the Optimum Number of Animals Needed in Research
- Statistics and the Issue of Animal Numbers in Research
- JUSTIFY ANIMAL NUMBERS EXAMPLE

Pre-Existing Dogs in Colony (9 Category B, 10 Category D):
We currently have 5 females in the breeding colony. These dogs will be used for breeding only and will eventually be adopted out. At the time of this renewal, we have 2 puppies and 2 retiring breeders which will be adopted out within the next 1-2 months. We currently have 8 CLN2 affected dachshunds enrolled in treatment studies and 2 carrier dogs from which we are collecting untreated control data (biologic samples, ERG recordings). The 2 carrier dogs will become part of the breeding colony once they reach sexual maturity and then eventually be adopted out.

Breeding (~200 Category B including dogs that will be adopted out after weaning, immunizations, and spay/neuter):
Over the next 3 years we will need an estimated 40-50 Dachshunds for breeding to generate the 90 CLN2 affected dogs required for the therapeutic studies. In addition to the dogs required for the proposed studies, the planned breeding's are estimated to generate approximately 150 dogs that are carrier dogs (which will be re-entered into the breeding colony or adopted out) or homozygous normal dogs (that will be adopted out). The carrier dogs used for breeding will also be adopted out once we no longer need them for breeding.

Dogs required for these studies are generated by breeding carrier females to either carrier (approximately 80% of the breeding's) or homozygous affected males (approximately 20% of the breeding's). On average, each female will produce 3 litters before being adopted out, and each litter averages 3-4 pups. Of those pups, approximately 3 to 6 of them will be affected with CLN2 disease, and the remainder will be carrier or normal pups. In order to generate the 90 affected dogs required for the therapeutic studies, we estimate that we will need at least 25 productive females over the course of the next 3 years (with a target of 10 females in the breeding colony at any one time). In the past, we have found that some of our females are infertile (ie do not get pregnant after several matings or rounds of AI), or produce very small litters (1 or 2 pups only). These females will be adopted out early, without additional breeding's being attempted. In addition, we will keep some male dogs (approximately 5 per year) until they reach sexual maturity in order to collect semen samples; these samples will be used either for immediate artificial insemination if an appropriate female is in hear or frozen for use in future breedings. In order to maintain genetic diversity, we also need to breed normal unrelated males to carrier females in order to generate additional carrier dogs for breeding. For this we use semen donated from privately owned dogs and breed using artificial insemination. We plan to use 4-6 normal dogs for this purpose.

Therapeutic TPP1 Delivery Approaches (90 Category D):
Based on previous studies, we know that we can achieve statistical significance with a small sample size of 3 dogs per study group due to the severe nature of the disease signs in comparison to normal dogs. The following numbers are estimated based on this sample size and the number of study groups needed to determine optimal treatment conditions (dose, route of delivery, treatment age, etc.)

Injection of AAV2-CAG-TPP1 into the vitreous of the eye:
9 affected dogs. The dose optimization phase is wrapping up with the final 3 dogs, but we may be asked to gather additional safety or efficacy data by a potential pharmaceutical partner in the near future. AAV2-CAG-TPP1 in varying doses will be administered via intravitreal injection into one eye of each dog, and a control vector (AAV2-CAG-GFP) will be injected into the other eye. The efficacy of the treatment in preserving retinal structure and function will be evaluated.

Injection of AAV2-CAG-TPP1 or AAVrh10-CAG-TPP1 or AAV9-CAG-TPP1 into the cerebrospinal fluid:

45 affected dogs (3 AAV viruses, 3 delivery route combinations, dose escalation unknown). Distribution after injection into the CSF will be compared using control vectors (AAV2-CAG-GFP, AAVrh10-CAG-GFP, or AAV9-CAG-GFP). 3 different routes of administration may be tested alone or in combination with one another: intraventricular injection, cisterna magna injection, and lumbar intrathecal injection. The virus and route(s) of delivery that show the most promising distribution will be further tested with the therapeutic TPP1 vector, and the ability of treatment to ameliorate disease progression will be evaluated. Once CNS treatment parameters have been optimized, a combination therapy to treat both the brain and eye will be tested in additional dogs.

Injection of autologous TPP1-producing MSCs into the vitreous of the eye and/or into the CSF:
36 affected dogs (4 delivery route combinations, 2 treatment ages, dose escalation unknown). Autologous MSCs transduced with AAV2-CAG-TPP1 will be injected into the vitreous in varying doses (i.e. numbers of MSCs). The other eye of each dog will receive intravitreal injections of MSCs transduced with a control vector (AAV2-CAG-GFP). The efficacy of the treatment in preserving retinal structure and function will be evaluated. A portion of the dogs receiving MSCs in the vitreous will also receive an injection of autologous bone marrow derived mesenchymal stem cells (MSCs) into the CSF via the lateral ventricles or cisternal puncture after the cells have been transduced with the AAV2-CAG-TPP1 or AAV2-CAG-GFP. The efficacy of the treatment in preserving brain structure and neurological function will be evaluated.

> The first sentence-should this be 5 Cat D dogs currently instead of 10?
> — Schlink, Sarah Nicole, VETERINARIAN, Office of Animal Resources Jul 22, 2022 08:17 AM

5. Animal Husbandry

1. Facilities

In which animal facility will animals be housed?

	Facility
1	[redacted]

2. Housing Outside of Facility

Will animals be housed anywhere other than a designated animal housing facility for more than 12 hours (e.g., a laboratory)?

● Yes ○ No

A. Outside Facilities

Give the location(s) (building and room number) and explain why animals must be housed outside a designated animal housing facility. See the Policy on Housing in Non-designated Areas.

	Outside Facility	Room number or area	Explanation:
1	[redacted]	[redacted]	Some dogs may be housed for short periods in the [redacted] if overnight monitoring is required due to health reasons or for reasons related to medication administration or execution of the study protocols. The lab has standard holding cages of the type used in veterinary clinics for this purpose. This is an ACUC-approved housing area and is periodically inspected by the ACUC. The need for such housing will be infrequent, and when required the dogs will be monitored continuously by lab staff and lab veterinarians will be on call. In cases of severe health emergencies, the dogs will be housed in the Veterinary Medical Hospital intensive care unit. We do not anticipate that this will occur based on our experience with the treatments to date, but we have this contingency if needed.

3. Transportation Between Animal Housing/Use Facilities

Will animals be transported with a private vehicle between animal housing/use facilities?

● Yes ○ No

A. Description of Transportation

Describe how animals will be moved, including caging/transport carriers used (covered microisolator cages, dog/cat carrier, etc.), type of transport used (i.e. personal car or van), and projected transport time. (Note: Animals should be transported in a temperature controlled environment.)

Most transportation of dachshunds will be with a standard dog carrier between LAR where the dogs are housed and the [redacted] where procedures will be performed; both areas are in the [redacted]. Any transport between facilities will be done by OAR staff in University vehicles. In emergency situation, we may have to transport dogs using vehicles of lab staff. In these cases, dogs will be transported in standard dog carriers borrowed from the OAR facility where the dogs are housed. The borrowed carriers will be returned to OAR after transportation is complete. All vehicles used will be temperature controlled. Transportation between facilities should not take more than 15 minutes.

4. Non-Standard Husbandry

A. Does this protocol contain any Prolonged Physical Restraint?

See: ACUC Physical Restraint policy

○ Yes ● No

B. Does this protocol contain any Food/Fluid Regulation?

See: ACUC Food and Fluid Restriction policy

○ Yes
○ No
● Overnight only

C. Does this protocol contain Multiple Survival Surgical Procedures?

See: ACUC Multiple Survival Surgical Procedures policy

○ Yes ● No

D. Does this protocol contain any of the following Non Standard Husbandry?

- ☐ Single housing of social species
- ☐ Wire-bottom cages
- ☐ Special diet/water
- ☐ Extended time to weaning
- ☐ Extended time between cage changes
- ☐ Alternative light cycles
- ☐ Out of range temperatures
- ☐ Cage-size exceptions
- ☑ Other

i. Explain other

We provide supplemental care for the dogs that greatly enhances their welfare and quality of life. We hire our own staff to interact with each dog for at least 20 minutes per day, 7 days per week. Staff play with and socialize the dogs and teach them basic commands. All staff that interact with the dogs are trained and closely supervised by [redacted] a registered veterinary technician with many years of experience in managing research dog colonies.

i. Explain non-standard husbandry and list the length of time the animal will undergo non-standard husbandry.

We provide the supplemental care described above for all of our dogs throughout the time that they are part of our colony; this enhances their welfare and provides a smoother adoption transition for those dogs that are eventually adopted out.

6. Description of Non-Surgical Procedures

1. Sample Collection

Will samples, such as blood or tissues, be collected from live animals? (Include sampling for genotyping.)

● Yes ○ No

A. Sample Type

Type of sample(s):

Cheek swab and blood samples will be collected from young dogs for genotyping.
Blood, urine, tear fluid, and cerebrospinal fluid will be collected periodically while the dogs are alive.
Bone marrow aspirates will also be collected from some dogs while they are alive.
Semen will be collected from dogs to use for breeding via artificial insemination.
Blood and vaginal cytology samples may be collected from prospective breeder females to check on stage of estrus.
A skin sample may be collected to establish fibroblast culture for investigation of metabolic pathways.

B. Sample Volume

Volume of sample(s):

Average of 1-2 ml of blood per draw.
Approximately 2 to 3 ml of urine will be collected per sampling time via free catch if possible.
Tear fluid volume averages 30-80 uL per collection.
For most collections, up to 2 ml of cerebrospinal fluid may be withdrawn at one time; for dogs that will be euthanized immediately following the collection procedure, up to 3 ml of CSF may be taken.
Approximately 0.5 ml of bone marrow aspirate will be collected.
For semen, will collect one ejaculate per dog (varies in volume).
For vaginal cytology, collection of sample on a cotton swab.
For skin sample, a 4-6mm punch will be collected.

C. Sampling Frequency and Duration

Frequency of collection and for how long:

For animals on study, blood and urine will be collected as frequently as every month. For dogs in the treatment studies that are greater than 24 months of age, sampling frequency will be reduced to every 6 months. Collection of blood and urine from animals not on study is variable, but would be infrequent.
Tear fluid collections are performed once per day for 4 consecutive days per month beginning at 4 months of age.
For dogs in the therapeutic studies, CSF samples will be collected first at approximately 10-12 weeks of age and then monthly to every other month, thereafter.
Additional blood and CSF samples may need to be collected for diagnostic purposes if dogs exhibit adverse reactions to any of the treatments or show signs of meningitis. If myelosuppression occurs, blood will be collected weekly to monitor. The necessity of such diagnostic sampling will be determined by veterinary staff participating in the research.
Bone marrow may be collected up to 4 times per animal with at least 1 month between collections.
Semen is collected once before a dog is first used for breeding to assess fertility and then for each breeding, which is performed by a combination of natural breeding and AI with fresh semen. An additional 3-5 samples may be collected to freeze for use in future breedings; suitable frequency is determined by a collaborating theriogenologist.
Blood for progesterone and vaginal cytology is collected/performed each time a bitch is monitored for heat, approximately every other day through proestrus and breeding.
Skin samples may be collected twice per dog with at least 2 months between sample collections.

D. Sampling Method

Method of collection:

Cheek swab collection is performed by inserting a cotton swab into the mouth and rubbing back and forth on the inside of each cheek for 30 seconds per side. Blood is collected via jugular draw into a syringe. Urine will be collected by free catch if possible. If we are unable to obtain samples by free catch, urine will be collected via cystocentesis. Tear fluid is collected by inserting a 3mm x 4mm section of ophthalmic sponge or a schirmer tear strip under the lower eyelid for 60 seconds to absorb the fluid before removing the sponge.

For the CSF collections, the dog will anesthetized and placed in right lateral recumbency. The hair will be clipped just rostral to the occipital protuberance and caudally just past the dorsal spinous process of C2. The site will be aseptically scrubbed using a dilute chlorohexidine solution. The head will be positioned parallel to the table and ventroflexed 120 degrees to facilitate access to the cistern. Guided by pre-defined landmarks, a 22 gauge 1.5 inch spinal needle will be used to percutaneously puncture the dorsal atlanto-occipital ligament and into the subarachnoid space. Approximately 1 to 2 ml up to 3 ml of CSF will be removed using gravity flow. A portion of the CSF (0.5 ml) will be analyzed by an in-house laboratory for total nucleated cell count, red blood cell count, protein concentration and cellular differential. The rest of the CSF will be stored in a -80 Celsius biofreezer for biomarker analysis.

Bone marrow will be aspirated from the humorous bone of Dachshunds under anesthesia using sterile

technique. The hair will be clipped and shaved over the the site of a skin incision made to expose the proximal end of the humorous. The skin will be surgically prepped. Wearing sterile gloves, the surgeon will palpate the proximal portion of the greater tubercle and make a small incision over this structure using a #15 surgical blade. An 11 guage Jamshidi needle will then be inserted into the humorus and the bone marrow sample aspirated into the needle. The needle is then withdrawn. After sample collection the skin is closed with 3-0 non-absorbable suture material. The suture site is monitored daily and the suture is removed 7 to days after wound closure.

Semen will be collected via manual manipulation.

To collect samples for vaginal cytology, a cotton swab is introduced into the vagina or vestibule of the bitch through a sterile tubular vaginal speculum. The mucosa is then gently contacted with the swab and removed, rolled onto a glass slide stained using Diff Quik, and the smear evaluated.

Skin sample is collected via punch biopsy with the dog under sedation. The skin will be sutured and gabapentin used as needed for pain control for approximately 3 days.

2. Induced or Spontaneous Neoplasia

Will induced or spontaneous neoplasia occur in live animals?

○ Yes ● No

3. Non-Surgical Procedures

1	Dog acquisition and breeding	Dog acquisition and breeding Dachshund colony: CLN2 is inherited as an autosomal recessive trait. For our studies, we require homozygous affected dogs, heterozygotes (phenotypically normal), and homozygous normal dogs. At approximately 2 weeks of age, the dogs receive an identifying microchip implant which is injected subcutaneously with an 18 gauge needle by OAR staff. Shortly thereafter, the genotypes of the dogs are determined using a DNA sequencing-based assay performed on DNA obtained from cheek swabs or blood samples drawn from the jugular vein. To generate the required dogs, we will perform primarily carrier to carrier crosses which will produce homozygous normal dogs, heterozygous carriers, and homozygous affected dogs in a 1:2:1 ratio. We are also able to use some of our treated homozygous affected males for breeding (both AI and natural), which has increased our ratio in those litters to 0:2:2. Affected dogs will be used for research protocols and some of the heterozygous dogs and treated affected males will be used for breeding. Heterozygous and normal dogs that will not be needed for these studies will be offered to other researchers upon request or will be spayed or neutered and put up for adoption. In addition, female breeders will be spayed and put up for adoption either after they have delivered 3 to 4 litters, deliver very small litters, or if they fail to produce a litter by 2 years of age. To date, all of the dogs we have put up for adoption have been quickly adopted. The adoption program is coordinated by [redacted] in cooperation with Homes for Animal Heroes. Some breedings will be performed periodically using normal females obtained from outside sources to maintain genetic diversity in the research colony, or from semen collected from privately owned dogs. All of the carriers used for breeding will come from our research colony and all of the normal females will be purchased from approved suppliers through the Office of Animal Resources. For semen obtained from outside dogs, we will contact the private owners and request semen donations. All contacts will be done discreetly via phone. Owners who agree to provide semen samples will either be sent kits for semen collection, preservation, and shipping or, if they are within a reasonable distance from Columbia, will be given the option of having either [redacted] or [redacted] visit them and do the semen collections on site. The semen may be used immediately, if a female is in heat, or may be stored frozen until the appropriate female comes into heat. The resulting puppies will be incorporated into our research colony. All breedings will be managed by [redacted] and [redacted]. They will monitor all females in the colony for estrous via evaluation of vaginal cytology, progesterone levels, and vaginoscopy. Vaginsocopy is performed immediately following vaginal cytology. Using a direct light source (ophthalmoscope) with a speculum, the characteristics of the vaginal mucosa (edema, etc) are evaluated to stage the bitch's progression through proestrus/estrus. Sedation is not usually required, especially if the bitch is in heat. At the appropriate stage of estrous [redacted] and [redacted] will mate the females with selected males using a combination of natural breeding and artificial insemination (AI). Semen for AI will	[redacted]	Procedures will be performed in the [redacted] or in the theriogenology clinic rooms at the [redacted].

Procedure	Description of procedure	Building name	Room number or area
	be collected manually from the males, evaluated for quality, and then deposited via a vaginal route into the uteri of the females. Semen will be collected from males with a teaser bitch present. Ideally the teaser should be in estrus, but when that is not possible, a friendly, non-estrus bitch will be used. The bitch will be held by an experienced handler with her rear quarters facing the male. Semen is collected using digital pressure and massage. The collection will be performed with the male placed on a non-slip surface such as a carpet. Semen is collected without allowing the male to mount. Semen is collected into a latex collection cone with an attached tube. AI will be performed by vaginal insemination which involves inserting a rod loaded with semen into the bitch's vagina and advancing it to the cervix opening. Once the rod is in place, the semen is deposited and the rod is withdrawn. In special cases, dogs may need to be bred via surgical artificial insemination. For example, this procedure will be used if a critical breeding is to be done using preserved semen from a male dog that is no longer available. The procedures for surgical AI are described in the section on surgical procedures. Pregnancy will be evaluated (to determine pup number, viability, etc) via palpation, ultrasonography or radiography.		

	Procedure	Description of procedure	Building name	Room number or area
2	Intravitreal injection for delivery of therapeutics	Intravitreal injection for delivery of therapeutics Studies will be conducted to determine whether intravitreal injections of autologous mesenchymal stem cells (MSCs), or AAV gene therapy vectors, or recombinant TPP1 enzyme can prevent retinal degeneration. Some control eyes will be injected with vehicle solutions only. AAV gene therapy vectors will be administered once. MSCs may be administered a second time if a decline in retinal function is observed; at least 4 weeks will be left between injections. For the enyzme replacement studies, 0.1 mL of a sterile solution of TPP1 protein or vehicle will be injected into the vitreous once every 2-6 weeks depending on results of ERG recordings. All injections will be performed by a board-certified veterinary ophthalmologist ([redacted]). The specific protocol for the eye treatments is as follows: Anesthetize the dog and apply 1 drop of topical proparacaine ophthalmic solution and 1 drop of ofloxacin ophthalmic solution (antibiotic drops) to the eye(s) to be treated (allowing 5 minutes between application for optimal absorption). Place a sterile lid speculum on the eye to be injected and clean the ocular surface with a dilute (1 in 50) povidone-iodine solution. For the injection, the dorsolateral bulbar conjunctiva will be grasped with forceps and the globe rotated ventromedially. The globe will be injected with a 28 or 30-gauge 0.5 inch sterile needle and syringe containing 50 to 200 ul of the therapeutic agent 5-7mm posterior to the limbus with the needle directed posterior to the lens into the mid-vitreous. Following the injection, one drop of ofloxacin ophthalmic solution will be applied. A sub-tenon injection of triamcinolone acetonide (2mg per eye) will be performed immediately following the intravitreal injection by the veterinary opthalmologist to control immune-mediated inflammation. The sub-tenon injection will be repeated at 2 weeks and 4 weeks post-intravitreal injection and monthly therefter. If necessary to control inflammation following a gene therapy injection, triamcinolone acetonide may be injected into the vitreous every 4 to 8 weeks using the procedure described above for intravitreal injection. In vivo imaging is performed immediately following injection and then the dog is recovered from anesthesia. Intraocular pressure measurements are performed 30 minutes following the injection and repeated if necessary until pressure returns to normal. Any uveitis observed following injection will be managed with topical anti-inflammatories or steroids at the discretion of the veterinary opthalmologist (prednisolone, oflaxacin, durazol, nevanac). If deemed necessary by the veterinary ophthalmologist, oral prednisone or carprofen may be administered as well.	[redacted]	Procedures will be performed in the [redacted]

	Procedure	Description of procedure	Building name	Room number or area
3	CSF administration of therapeutics Immunosuppression	CSF administration of therapeutics The gene therapy vector or TPP1-producing MSCs will be delivered to the CSF via the lateral ventricles or via the cisterna magna. Delivery to the lateral ventricles will be performed via a surgical procedure as described in the appropriate section of this protocol. Injection of gene therapy vectors or transduced MSCs via the cisterna magna and/or lumbar vertebral area will be performed by a board certified veterinary neurologist [redacted]. The dog will be anesthetized, and the area for the injection site will be shaved and sterilely prepped. A needle will be inserted through the skin into the cisternal space. Entry into the cisternal space will be identified by the flow of clear CSF fluid from the needle hub. A syringe will then be attached to the needle hub and the vector slowly injected over 1-2 minutes. Immunosuppression 2 weeks prior to planned administration of gene therapy vectors to the CSF or vitreous, dogs will be started on an immunosuppression regimen consisting of cyclosporine (25mg BID until reaching a weight of 4kg; then 35mg BID) and leflunomide (2.5mg/kg PO SID). The Leflunomide will be continued until at least 4 weeks post-injection and then will be discontinued after any inflammation has resolved or if no inflammation is observed. These have been well tolerated in our dogs, but dogs will be monitored for adverse clinical signs and for abnormalities in blood cytology and chemistry analyses. If any pronounced abnormalities are noted, Leflunomide administration will be discontinued.	[redacted]	Procedures will be performed in the [redacted]

	Procedure	Description of procedure	Building name	Room number or area
4	Cognitive ability testing using a T-maze	Assessments Cognitive ability testing using a T-maze The reversal learning task employed by our lab uses a t-maze apparatus and food reward motivation to measure cognitive abilities in canines. The task includes a series of pre-test training phases designed to allow the dogs to become accustomed to the maze and to learn to explore the maze in search of food rewards. The final pre-test phase trains the dogs to consistently seek food rewards in a single arm of the t-maze. After the dogs have been trained to this protocol, food rewards are switched to the opposite arm of the t-maze. For example, if a dog has been trained to expect food reward to be present in the right arm of the maze and absent in the left, this orientation is reversed so that food rewards are only found in the left arm of the maze. We measure cognitive ability (specifically cognitive deficits due to neurodegeneration) by the number of incorrect choices the dog makes before learning to consistently seek food rewards in the opposite arm. This reversal is repeated 3 times at each time point. Dogs are run 5 days a week in sessions consisting of 10 trials each (each trial is a single traversal of the t-maze). Pre-test training begins at 2 months of age and the first data collection occurs at 4 months of age. Data is collected monthly thereafter. Each time point usually consists of 10-15 sessions, allowing for a 1-2 week break between monthly data points. Data are analyzed by calculating the average number of incorrect choices necessary to reach criterion for each reversal. Performance from normal and CLN2 affected, age-matched dogs is compared to look for differences in cognitive abilities caused by neurodegeneration. Application of the t-maze procedure has been published: Sanders DN, Kanazono S, Coates JR, Johnson GS, Johnson GC, Narfstrom K, O'Brien DP, Katz ML: Cognitive decline in a dog model for an inherited neurodegenerative disease using t-maze performance. Journal of Veterinary Behavior: Clinical Applications and Research. 5(3): 154, 2010. It has also been presented as an invited talk at an international meeting on dog behavior.	[redacted]	[redacted]; a dedicated room for this purpose

	Procedure	Description of procedure	Building name	Room number or area
5	Functional vision testing: Visual Tracking Ability	Functional vision testing: Since vision depends upon both the retina and the visual centers in the brain, a functional test is necessary to thoroughly evaluate treatment efficacy with regard to preserving vision. Therefore, the dogs receiving the eye treatment will be trained to distinguish between images displayed on 2 adjacent iPads using food reward and clicker training. The "correct" choice will be the iPad with a black and white stripe grating and the opposite iPad will display a solid grey screen. The dog will be trained to indicate its choice by touching its nose to the correct iPad screen and will then receive a small food reward for each correct choice. Training will require daily 15-30 minute sessions for 6-8 weeks beginning when the dog is 2.5 to 3 months old. After training, testing will be repeated monthly and require daily 15 minute sessions for 1-2 weeks. Visual Tracking Ability In a fully awake dog, cotton balls are dropped from above to the front left and right of the animal, and the dogs are monitored for whether they can visually track the cotton balls.	[redacted]	Procedures will be performed in the [redacted]

	Procedure	Description of procedure	Building name	Room number or area
6	Physical and Neurological Exam Ophthalmic Examinations	Physical and Neurological Exam For treated and control dogs in gene therapy studies, complete physical and neurologic examinations will be performed on a weekly basis. Body growth will be monitored weekly by body weight measurements. Signs of neurologic dysfunction will be assessed subjectively by the clinical neurologic examination [Lorenz MD, Kornegay JN. Neurologic history and examination. In: Lorenz MD, Coates JR, Kent M. Handbook of Veterinary Neurology 5th ed. 2010, Saunders - Elsevier Science; St. Louis, MO pp.3-44]. Components of the neurologic examination include observation (mentation, posture, gait); cranial nerve evaluation; postural reaction testing (conscious proprioceptive positioning, hopping, wheelbarrow, tactile place and extensor postural thrust); spinal reflexes (myotatic and flexor withdrawal); and nociception (superficial and deep). Gait evaluation will be assessed as normal, ataxic and paretic (ambulatory, nonambulatory). Postural reactions will be assessed as normal, decreased or absent. Spinal reflexes will be assessed as normal, decreased or absent. Nociception will be assessed as normal, decreased or absent. Dogs will also be evaluated for abnormal movement or seizure activity. The neurologic examination involves observation of the dog's attention to its environment, postural reactions to assess for limb strength, limb reflexes, reflexes and reactions to assess cranial nerve function, and palpation of the dog for signs of pain. Ophthalmic Examinations All dogs in the study will undergo weekly routine clinical ophthalmologic exams. These exams are done on an awake dog in the same manner as is done for clinical patients. A veterinary ophthalmologist will use a slit lamp to look for abnormalities in the lens and cornea and will then examine the back of the eye including the retina with an ophthalmoscope. Prior to examination of the back of the eye, the pupil will be dilated by applying a drop of sterile 1% tropicamide to the surface of the cornea.	[redacted]	Procedures will be performed in the [redacted]

	Procedure	Description of procedure	Building name	Room number or area
7	Retinal imaging Electroretinography Sensory and/or motor nerve conduction velocity (NCV) measurements	In vivo retinal imaging: The retinas of the dogs will also be imaged with scanning laser ophthalmoscopy (SLO) and optical computed tomography (OCT) using a Spectralis instrument. Imaging with the Sepctralis instrument will be performed immediately following each ERG session with the dog under general anesthesia. Electroretinography: Retinal function will be evaluated in the dogs in therapeutic studies through recording of electroretinograms (ERGs), and visual cortex response will be evaluated through recording of visually evoked potentials (VEPs). ERGs/VEPs will be recorded from the CLN2 dachshunds on a monthly basis beginning at 4 months of age. Dogs will be sedated with dexmedetomidine (20-30 mcg/kg) IM, ketamine (up to 5mg/kg) IM, and/or midazolam (0.25 - 0.5 mg/kg). Vital signs (temperature, respiration, heart rate and pulse quality) will be monitored during sedation. Prior to recording, a contact lens electrode is placed on the surface of the cornea using methylceullulose as a lubricant, and reference and ground needle electrodes are inserted subcutaneously near the base of the ear and on the top of the head over the visual cortex. The eyes are exposed to full field light stimuli and the responses are recorded with a handheld mini-ganzefeld system (HMsERG). The responses are then analyzed with a computer. For VEPs, the contact lens electrode is disconnected and the VEP recordings is taken from the needle electrode that was previously inserted over the visual cortex. Given the muscle relaxant properties and depressant effects of midazolam, using it in combination with dexmedetomidine may result in hypoventilation and/or hypoxemia. To monitor oxygen saturation during sedation, a battery-powered pulse-oximeter (so as not to interfere with the ERG) will be used throughout the procedure. If the SpO2 falls consistently below 92-93%, the procedure will be aborted. If this occurs, the dexmedetomidine will be reversed with atipamezole IM at half the volume of dexmedetomidine used and flumazenil will be administered at 0.01mg/kg IV to reverse midazolam. A mechanical ventilator will be available at all times in case the patient remains hypoxemic or continues to hypoventilate. Other electrodiagnostic procedures such as sensory and/or motor nerve conduction velocity (NCV) measurements will also be performed under the same sedation period if necessary. For these procedures, the sciatic nerve will be stimulated using Teflon coated stainless steel or platinum wire electrodes placed percutaneously in the vicinity of the nerve. The evoked response will be recorded with identical electrodes placed in the innervated muscle or in proximity to the nerve.	[redacted]	Procedures will be performed in the [redacted]

	Procedure	Description of procedure	Building name	Room number or area
8	Pupillography	Pupillography Pupillary light reflex (PLR) recordings will be performed on CLN2 dachshunds every 2 months beginning at 4 months of age and continuing until the dogs are 10 to 12 months old. If lifespan is extended by the treatment, PLRs will be repeated every 4 months after 12 months of age. Dogs will be placed under general anesthesia and dark-adapted for at least 20 minutes prior to recording. Following anesthetic induction, eyes will be moistened using artificial tears, and body temperature will be controlled using a water circulating – heating pad. PLR recordings will be performed using a custom system in which the dog will be aligned with respect to a light stimulus and pupil imaging instrument such that light stimuli will be delivered to the center of the pupil. Each session will be done using a protocol lasting 30 minutes. A session will consist of capturing an video of the pupil in the fully dark-adapted dog with an infrared-sensitive digital camera. The eye will then be presented with visible light stimuli of varying intensity, duration, and color, while the pupil size is continuously monitored with the camera. All digital images of the pupil will be stored along with stimulus conditions in a computer. The highest light intensities that will be used will be the minimum that are required to achieve maximal constriction of the pupil. These intensities are relatively low (orders of magnitude lower than normal daylight), and so pose no risk of inducing light damage to the eye. For the procedures listed above (In vivo retinal imaging, ERGs, and PLRs) the eye will remain open for long periods of time, and therefore the surface of the cornea will be kept moist by administration of 2% methylcellulose or frequent flushing with saline eye solution. In addition, the eye must remain positioned so that the center of the pupil is aligned with the imaging or stimulus device. To maintain eye centration, a small stay suture is placed in the bulbar conjunctiva in the central axis approximately 5 mm superior to the limbus. The end of the suture thread is clamped with a hemostat which then can be used to put tension on the globe to keep it centered.	[redacted]	Procedures will be performed in the [redacted]

	Procedure	Description of procedure	Building name	Room number or area
9	Cardiac Assessments In vivo Imaging of the Brain Pre-surgical stereotaxy Magnetic resonance imaging:	Cardiac Assessments Non-invasive cardiac structure and function assessments may be performed on the Dachshunds enrolled in these studies once every month staring at 6 months of age. If needed, dogs will be sedated for these procedures. Echocardiography: Standard echocardiographic examination will be performed on all dogs. With the dogs laying on a soft pad in right lateral recumbency, a transducer is placed on the chest and emits ultrasonic sound waves through the skin and other body tissues to image the heart tissue walls and valves. The echocardiogram is a noninvasive painless procedure for evaluating the heart structure and function. Electrocardiography: ECG monitoring involves placing small metal electrodes attached to adherent hypoallergenic patches onto the chest and limbs of the dogs. Wires from the electrodes are connected to a recorder that monitors and stores the electrical activity of the heart. ECG recording will be performed for approximately 5 minutes with the dogs laying on a soft pad in right lateral recumbency. Electrocardiography is a noninvasive painless procedure for evaluating the electrical activity of the heart. In vivo Imaging of the Brain CT imaging: Two weeks prior to intraventricular injection, a head CT scan (without contrast) will be performed at the VMTH; imaging will be used for 3-D printing of fiducial array in preparation for surgery. Pre-surgical stereotaxy: The dog will be positioned in sternal recumbency for computed tomography using a helical scanner (Picker PQ 6000) or 1.5 T MR unit located at the VMTH. Following anesthesia induction, the subjects will be fitted with an acrylic bite block. The bite block has plastic fiducial markers adjacent to the muzzle. With the bite block in place, the subject will be placed in dorsal recumbency for imaging in a 3.0 Tesla MRI. A high-resolution T1-weighted 3D pulse sequence is acquired (TR = 22 ms, TE = 9.50 ms, flip angle = 308, FOV = 170 mm, 0.7 _ 0.7 _ 0.7 mm voxels, transaxial slices, 4 averages). Acquisition time for the series is between 35 and 50 minutes. Magnetic resonance imaging: The brains of treated and control dogs will be examined with MRI under general anesthesia (handled by the MU VMTH anesthesia service) at approximately one to two month intervals starting at 3 months of age. Magnetic resonance imaging will be performed with a 1.5-Tesla magnet (MU-VMTH). Pulse sequences will be selected to obtain T1, T2, proton density and flair-weighted sequences in 3 planes. The images will be evaluated by veterinary neurologists and radiologists.	[redacted]	Procedures will be performed at the [redacted] or in the dedicated surgery suite in the [redacted]

	Procedure	Description of procedure	Building name	Room number or area
10	Blood collection from normal dogs at MU outside NCL the colony	We have been approved to collect blood from normal dogs at the University of Missouri outside of the NCL colony. We will collect up to 20 ml per dog for as many dogs as the respective lab approves as a one-time collection. Blood will be collected from dogs free from any studies which may interfere with blood parameters needed for our research. In coordination with the PI of the study and associated lab personnel, we will arrange for collection of blood to not exceed approved quantities per body weight and to minimize undue stress to the animals. Blood will be collected by a member of the research lab who owns the dogs or by [redacted] in coordination with the lab.	[redacted]	TBD based on colony location

7. Substances Used in Animals

1. Substances Used in Animals

List the substances you will give the animals here (including vehicles given to controls, hazards, radiation, etc.):

	Substance	Amount/ Dose/Volume	Route	Frequency/ Duration	Hazard	Pharmaceutical Grade
1	AAV2-CAG-TPP1 or AAV2-CAG-GFP gene therapy vector	100 uL / 1 X 10^9 vg to 1x10^12 vg	Intravitreal		Yes	No
2	AAV2-CAG-TPP1 or AAVrh10-CAG-TPP1 or AAV9-CAG-TPP1 gene therapy vectors	1 - 2 mL / 1.5 - 7.0 X 10^13 vg	CSF; intraventricular or cisternal puncture		Yes	No
3	AAV2-CAG-GFP or AAVrh10-CAG-GFP or AAV9-CAG-GFP gene therapy vectors	1 - 2 mL / 1.5 - 7.0 X 10^13 vg	CSF; intraventricular or cisternal puncture		Yes	No
4	MSC-AAV2-CAG-TPP1 or MSC-AAV2-CAG-GFP gene therapy cells	150 to 250 uL / 0.5 - 8.0 x 10^6 MSCs	Intravitreal		Yes	No
5	MSC-AAV2-CAG-TPP1 or MSC-AAV2-CAG-GFP gene therapy cells	1 mL / 5.0 - 20.0 x 10^6 MSCs	CSF; intraventricular or cisternal puncture		Yes	No

2. Non-Pharmaceutical Grade Substances

For those substances that are marked “no” as pharmaceutical grade, list a justification in the space below. Also, include instructions for how they will be mixed to maintain sterility and adjust pH.

Vectors are obtained from Signagen Lab and while not graded for human use, they are rAAV purified via advanced 2xCsCl ultra-centrifugation and graded for in vivo animal use.

3. Substances Used in Animals Personal Protective Equipment (PPE)

PPE is needed to safely handle most materials in the laboratory. In general, a minimum of gloves and lab coat should be used. Other substances would require more PPE such as eye protection, respiratory protection, fume hood, etc. Please notify laboratory members if there are any special precautions that need to be taken when working with the above substances.

Describe the PPE required to handle these substances. You may group substances (e.g., "All substances" or "non-hazardous substances") if all or some use the same PPE. Please list any substances needing alternative or additional PPE separately. You do not have to include additional PPE needed for work with hazards as that will be described in the Hazards section, however, you may include here as well if you wish.

	Substance	Gloves	Eye Protection	Lab Coat	Face Mask	Fume hood	Biosafety cabinet	Double-Gloves	Other	Other PPE
1	All substances	☑	☐	☑	☐	☐	☑	☐	☐	

Hazardous Agent

If you marked "yes" under Hazard, please complete the "Hazardous Materials" Section that follows.

8. Hazardous Materials

1. Will you use any Biological Hazards?

● Yes ○ No

A. Biological Hazard

List all biological hazards that will be used in live animal work.

	Agent or type of hazard	Donor species	Receiving species	Dose	Route/Volume of Admin.	Frequency of Admin.	Other
1	AAV2-CAG-TPP1 or AAV2-CAG-GFP gene therapy vector	dog	dog	Up to 1x10^12 vg	Intravitreal 100 uL	once	
2	AAV2-CAG-TPP1 or AAVrh10-CAG-TPP1 or AAV9-CAG-TPP1 gene therapy vectors	dog	dog	up to 7 X 10^13 vg	CSF; intraventricular or cisternal puncture 1-2 mL	once	
3	AAV2-CAG-GFP or AAVrh10-CAG-GFP or AAV9-CAG-GFP gene therapy vectors	dog	dog	up to 7 X 10^13 vg	CSF; intraventricular or cisternal puncture 1mL	once	
4	MSC-AAV2-CAG-TPP1 or MSC-AAV2-CAG-GFP gene therapy cells	dog	dog	Up to 8 x 10^6 MSCs	Intravitreal 150 to 250 uL	twice	
5	MSC-AAV2-CAG-TPP1 or MSC-AAV2-CAG-GFP gene therapy cells	dog	dog	Up to 20 x 10^6 MSCs	CSF; intraventricular or cisternal puncture 1 - 2 mL	twice	

B. IBC Protocol Number (if applicable for recombinant DNA or biological materials)

List your IBC Approval Number or attach your current IBC application. (Include attachments in the attached files section.)

07-24

- ☐ Unsubmitted
- ☐ Submitted
- ☑ Approved

i. Expiration date

Expiration Date for IBC#07-24 is 10/24/2022

> Expiration Date for IBC#07-24 is 10/24/2022
>
> — [redacted], Environmental Health & Safety Aug 2, 2022 08:52 AM

C. Biological Hazard - Anticipated Effect(s)

List any anticipated effect(s) of biological hazards on animal.

Dogs undergoing gene therapy will receive gene constructs either directly or via injected transgenic autologous MSCs that will direct synthesis of the protein that is missing in the disease or "reporter" proteins that are fluorescent but have no detectable biological effect. The vectors will be packaged in

coat proteins derived from adeno-associated viruses (AAV2). The vectors will contain no viral genes and will not be capable of replication. Immune-mediated inflammation may occur in response to the AAV or to the produced TPP1 protein. Immunosuppressives will be used to control these reactions and the dogs will be monitored closely for signs necessitating further intervention.

D. Biological Hazard - Housing/Procedure Sites

Where do you anticipate housing/working with animals receiving hazardous or potentially hazardous biological agents? Coordinate with the facility manager then list building and room numbers below.

	Agent	Receiving species	Building	Room or Area	Housing	Procedure
1	All biological hazards	dog	[redacted]	[redacted]	☑	☑
2	All biological hazards	Dog	[redacted]	[redacted]	☑	☐

E. Biological Hazard - Animal Identification

Explain how animals treated with a biological hazard will be identified (ex. cage card, ear tag, etc.)

- ☑ Cage Card
- ☑ Chip
- ☐ Door Sign
- ☐ Other

F. Hazardous Agents or By-Products /Presence

The biological hazard or by-products may be present in which of the following?

- ☐ None
- ☐ Feces/Urine/Bedding
- ☐ Saliva
- ☐ Blood
- ☐ Aerosols
- ☐ Animal bite/scratch
- ☑ Animal carcasses/tissues
- ☐ Surgical site wound or sore
- ☐ Other

G. Biological Hazard - Personal Protection Equipment (PPE) and Engineering Controls

PPE to be worn when handling biological hazards. LIDR ABSL-3 includes protective suit, shoe covers, double gloves, full-face PAPR.

	Biological Hazard	Gloves	Eye Protection	Lab Coat	Double-Gloves	Face Mask	Biosafety cabinet	LIDR ABSL-3	Other	Other PPE
1	AAV2-CAG-GFP or AAVrh10-CAG-GFP or AAV9-CAG-GFP gene therapy vectors	☑	☐	☑	☐	☐	☑	☐	☐	

H. Additional Information

List additional information, i.e., special precautions for pregnant women, immunocompromised individuals, special handling, or storage, etc.

2. Will you use any Chemical Hazards?

○ Yes ● No

3. Will you use any Radiation Hazards?

○ Yes ● No

9. Anesthetic Procedures, Pain Control, Other Clinical Drugs

1. Anesthetics, Preanesthetics & Tranquilizers

Will any anesthetics, preanesthetics, or tranquilizers be used?

● Yes ○ No

2. Preanesthetic Agent(s)

List preanesthetic agents here

Under Frequency of Admin, you could say "as needed for anesthesia" or something similar.

— [redacted], VETERINARIAN, Office of Animal Resources Jul 22, 2022 08:18 AM

	Species	Agent	Dose/Volume	Route	Frequency of Admin.
1	Dog	Glycopyrolate	0.005 mg/kg 0.01 mg/kg	IM,IV	As needed for anesthesia
2	Dog	Dexmedetomidine	0.005 mg/kg 0.01 mg/kg	IV or IM	As needed for anesthesia
3	Dog	Buprenorphine	0.01 mg/kg	IV or IM	As needed for anesthesia
4	Dog	Nalbuphine	0.25 mg/kg	IM	As needed for anesthesia
5	Dog	ketamine	5 mg/kg	IM	As needed for anesthesia
6	Dog	Midazolam	0.1-0.5 mg/kg	IV or IM	As needed for anesthesia

3. Anesthetic Agent(s)

List anesthetic agents here. **Do not list isoflurane here, it will be listed later in the form.**

> Under Frequency of Admin, you could say "as needed for anesthesia" or something similar.
> —[redacted], VETERINARIAN, Office of Animal Resources Jul 22, 2022 08:19 AM

	Species	Agent	Controlled Substance	Dose/Volume	Route	Frequency of Admin.
1	Dog	Propofol		6 mg/kg	IV	As needed for anesthesia

4. Isoflurane Use

A. Will you use isoflurane?

● Yes ○ No

B. What species?

dog

C. How will it be administered?

- ☐ Vaporizer with nose cone
- ☑ Vaporizer with endotracheal tube
- ☐ Induction box connected to vaporizer
- ☐ Open drop method (bell jar, etc.)
- ☐ Other

5. Non-Pharmaceutical Grade Anesthesia

If non-pharmaceutical grade anesthesia must be used, strong scientific justification must be provided. In addition, describe how the solution will be made and stored. Also, describe how pH, osmolarity, and sterility will be kept in acceptable physiological ranges. Please see ACUC policies for Anesthesia and Non-pharmaceutical Anesthesia and ACUC Guidelines for pentobarbital, avertin, inactin, and urethane/chloralose.

6. Monitoring and Life Support

Monitoring and life support systems to be utilized to ensure adequate depth of analgesia or anesthesia and to prevent overdose:

Monitoring of dogs under sedation: Temperature, pulse and respiratory rate are monitored every 5 minutes during periods of heavy sedation.

Anesthesia for basic surgical procedures (Therapeutic delivery, Surgical AI), and non-surgical procedures (MRI, CSF Collection, CSF injections, PLR recording, etc):
The dogs will be maintained in a plane of anesthesia monitoring for ventral eye position, loss of pedal and palpebral reflexes. Heart rate and respiration will be monitored and recorded. Monitoring will consist of continuous electrocardiography, mean systolic blood pressure, end tidal carbon dioxide, temperature, and continuous pulse oximetry. The dogs will be ventilated mechanically to maintain a PaCO2 of 38-45 mm Hg. Crystalloid fluids (LRS) will be administered at 10 ml/kg/hour during anesthesia. Eye lubricating ointment will be applied for procedures not involving eye measurements. For eye procedures, methylcellulose or saline flush will be applied so not to interfere with data collection.

CT and MRI Studies at the Veterinary Medical Hospital: For MRI or CT performed at the College Veterinary

Medical Teaching Hospital (VMTH), the anesthesia will be performed by hospital anesthesiologists using the standard procedures they use on clinical patients. The clinical anesthesia staff will be responsible for all monitoring, life support and post-anesthesia recovery when these procedures are performed at the VMTH.

7. Post Anesthetic Recovery

Complete description of post-anesthetic recovery monitoring and care:

Basic Recovery from Anesthesia:
The dogs will be recovered from anesthesia (in the anesthesia prep area of the [redacted]) and the endotracheal tube removed when the return of pharyngeal reflexes is observed. The dogs will be directly observed several hours by the Veterinary Neurology Research Intern and veterinary technician ([redacted]). Hourly, serial neurologic examinations will be performed by assessing mentation, body position, mobility and cranial nerve evaluation for at least the first 2 hours following recovery. Rectal temperature, pulse and respiration will be monitored hourly until temp > 100 and then repeated q 24 hours until sutures are removed. Fluid therapy using lactated Ringer's solution will be administered at a maintenance rate of 60 ml/kg/day for 1-2 hours following each procedure if needed. If rectal temperature falls below 100 F a thermal heating blanket will be placed beneath the dog. If the dogs show evidence of dysphoria (anxiousness, pacing, non-sensical barking, etc.) or pain (anxiousness, panting, tachycardia, etc.) buprenorphine or butorphanol and/or dexmedetomidine at the doses outlined above will be used as needed to assist with smoother recovery. If any further complications (e.g. seizures, fever, progressive neurologic signs, etc.) arise from the surgery, appropriate standard of care therapy will be administered at the direction of a veterinarian.

Gene Therapy ICV Injections: In addition to the routine described in "basic recovery from anesthesia", the dogs will be directly observed for the first 12 hours after the craniotomy by the Co-I ([redacted]), veterinary neurology research intern, and veterinary technicians [redacted]. If any further complications (e.g. seizures, fever, progressive neurologic signs, etc.) arise from the surgery, appropriate standard of care therapy will be administered at the direction of [redacted].
These dogs will be recovered and monitored in [redacted] using the above stated protocol. Acepromazine may be administered if the dog is anxious during recovery. Buprenorphine (0.015 mg/kg SQ q 4-8 h for the first 24 h) or Morphine 0.5 mg/kg IM q 4-6 h and/ or dexmedetomidine (20-30 mcg/kg IM) will be administered in the immediate post operative period and tramadol (1-4 mg/kg q 6-8 hrs) or gabapentin (10 mg/kg PO q 8 hrs) will be given as needed for pain for 24-48 after surgery.

Recovery of dogs under sedation: Sedatives will be reversed as appropriate, and pulse and respiratory rates will be monitored every 5-10 minutes until recovery.

> Remove [redacted] since not listed elsewhere in protocol.
>
> —[redacted], VETERINARIAN, Office of Animal Resources Jul 22, 2022 08:19 AM

8. Pharmaceutical Analgesia

● Yes ○ No

9. Pharmaceutical Analgesia

	Species	Agent/Non-pharm.	Dose/Volume	Route	Frequency of Admin.
1	Dog	buprenorphine	0.01-0.03 mg/kg	IV, IM, SQ	q 4-12 hrs
2	Dog	Morphine	0.5 mg/kg	IM	q 4-6 hours
3	Dog	Gabapentin	10 mg/kg	PO	q 8 hours
4	Dog	Meloxicam	0.1-0.2 mg/kg	PO, SC, IV	q 24 hours
5	Dog	Butorphanol	0.2 mg/kg	IM, SQ	q 4-6 hours
6	Dog	Tramadol	1-4 mg/kg	PO	q 6-12 hours
7	Dog	Carprofen	2.2 mg/kg or 4.4 mg/kg	PO	q 12 hours or q 24 hours

10. Non-pharmacologic control of pain

○ Yes ● No

11. Paralytic Agents

○ Yes ● No

12. Antibiotics and Other Agents

(Include any emergency drugs, fluids, etc. here)

● Yes ○ No

13. Antibiotics and Other Agents

List other agents such as antibiotics and other emergency drugs

Please move Meloxicam, Butorphanol, Tramadol, Carprofen and Gabapentin to the Pharmaceutical Analgesia table.

—[redacted], VETERINARIAN, Office of Animal Resources Jul 22, 2022 08:21 AM

	Species	Agent	Dose/Volume	Route	Frequency of Admin.
1	Dog	Phenobarbital	2-4 mg/kg	PO, IV, IM	q 12 hrs
2	Dog	Diazepam/Valium	0.5-1 mg/kg	IV	PRN for seizures
3	Dog	Acepromazine	0.01-0.2 mg/kg	IV, SQ	once
4	Dog	cefazolin	20-22 mg/kg	IV	q 90 minutes intraop / 6 hrs
5	Dog	Prednisone	0.25-1 mg/kg	PO	q 12-24 hrs for 2 weeks
6	Dog	Cyclosporine	25 mg until weight reaches 4kg; then 35 mg	PO	q 12 hrs
7	Dog	Atropine	0.02 mg/kg	IV, SQ, IM	PRN for intraop bradycardia
8	Dog	Ephedrine	0.1 - 0.3 mg/kg	IV	once for Intraop hypotension
9	Dog	Atipamezole	half to full volume of dexmedetomidine given	IM	once for dexmed reversal
10	Dog	Tropicamide	1 drop	corneal surface	once/PRN
11	Dog	Methylcellulose	1 drop	corneal surface	once for ERG
12	Dog	Proparacaine	1 drop	corneal surface	once/PRN
13	Dog	ofloxacin	1 drop	corneal surface	SID to QID
14	Dog	prednisolone	1 drop	corneal surface	SID to QID
15	Dog	durezol (Difluprednate)	1 drop	corneal surface	SID to QID
16	Dog	Nevanac (nepafenac)	1 drop	corneal surface	SID to QID
17	Dog	Lidocaine	2.5 mg/kg	SQ	once for bone marrow aspiration
18	Dog	Levetiracetam	2-4 mg/kg	IV, PO	q 8h for seizures
19	Dog	Leflunomide	2-4 mg/kg	PO	q 24h
20	Dog	Dexamethasone	0.1-0.2 mg/kg	IV	Once for intravitreal injection/q24 hrs
21	Dog	triamcinolone	2 mg	sub-tenon injection	every 2-4 weeks

	Species	Agent	Dose/Volume	Route	Frequency of Admin.
22	Dog	flumazenil	0.01 mg/kg	IV	once for midazolam reversal

10. Description of Surgical Procedures

1. Surgical Procedures

Will there be any surgical procedures?

● Yes ○ No

2. Surgery: Pre-surgical Prep

Describe pre-surgical preparation of the animals. Include information about fluid/food restriction, skin, and instrument prep.

Prior to all surgical procedures listed below, the dogs will undergo complete physical and neurologic examinations. Food will be withheld the evening prior to and the morning of the planned surgery. All surgical instruments will be autoclaved.

Intracerebroventricular (ICV) Injections for Delivery of Therapeutics:
The day prior to surgery, the dogs will be started on prednisone (1 mg/kg SID PO for 3-4 days; then 0.5 mg/kg for 3-4 days) to control inflammation. Prior to surgery, urine will be collected by free catch for urinalysis. Blood will be collected for routine CBC and serum biochemistry panel. Buccal mucosal bleeding time will be performed to assess clotting status. The buccal mucosal bleeding times require a special blade instrument by which to lacerate the buccal mucosa and document the time it takes for a blood clot to develop. The dogs will be prepared for aseptic surgery and bathed the evening prior to planned surgery.

Preoperative MRI examination and CSF collection will be performed the day prior at the VHC to determine the catheter target location and the dog will be recovered.

Fiducial Array Placements using bite block: A molding of the mouth will be made to create a bite-block for placement of markers to obtain coordinates for the stereotaxy. A fiducial array will be used for locating the brain ventricles in conjunction with injecting gene therapy vectors into the ventricles. The fiducial array will be temporarily rigidly fixed to the skull of the subject prior to MRI. The procedure is considered a minor surgery as only a single skin incision is required and the cranial vault is not penetrated. The skin is shaved and surgically prepped with 3x chlorohexidine scrub alternating with alcohol wipes. A 1cm skin incision is made on 0.5cm lateral of midline over the bregma landmark. A 3.5mm drill bit with a depth guard of 4mm creates a partial thickness skull hole that does not penetrate the inner calvarial cortex. A Teflon based screw with a profile 8mm above the skull is placed within the created hole. A second hole 2.7mm wide is placed in similar fashion and a second Teflon screw placed. The skin incision is temporarily covered with a bio-occlusive sterile dressing. For imaging, the fiducial array is inserted and secured in the main screw. Imaging is performed as previous. The fiducial array is removed and stored until surgery. A simple interrupted 3-0 nylon suture is placed in the skin to close the defect. On the subsequent day the surgery is performed.

Surgeries will be performed on 3 to 6 month old dogs in the surgical suite of the [redacted] located in the [redacted]. After induction of anesthesia on the day of surgery, the dog will be positioned in sternal recumbency and head secured to head holding apparatus to facilitate positioning. The hair will be clipped from just rostral to the orbital region and caudally just past the dorsal spinous process of C2. A heating pad will be placed beneath the dog. The site will be

aseptically scrubbed 3 times using a dilute chlorohexidine scrub alternating with alcohol wipes. The surgical sight will be 4 quadrant draped. The dog will be positioned into the mouth bit block and the head will be stabilized.

Artificial insemination of dogs:
The female dogs will have complete physical examinations and be prepped for aseptic surgery (see description of surgery for ICV injections). After induction of anesthesia, the dog will be repositioned in dorsal recumbency. The hair on the ventral abdomen will be clipped from the xyphoid to the pubis. A heating pad will be placed beneath the dog. The surgical site will be aseptically scrubbed 3 times using a dilute chlorohexidine scrub alternating with alcohol wipes. The surgical sight will be 4 quadrant draped.

Bone Marrow Collection (not technically a surgical procedure, but included here for information purposes):
The hair is clipped and shaved over the site of a skin incision to made to expose the proximal end of the humorus. The skin is surgically prepped, using the 3X scrub described above. A subcutaneous injection of Lidocaine is given at the site where the bone marrow aspiration needle will be inserted into the hind limb.

3. Surgical Procedures

List surgical procedures (include incision location and size, tissue(s) manipulated, and closure methods and materials).

> Add descriptions of the actual surgeries here and remove the other info about prep and recovery- those are described elsewhere.
>
> —[redacted], VETERINARIAN, Office of Animal Resources Jul 22, 2022 08:28 AM

1	Surgical ICV Injection	Craniotomy and Intraventricular Injection Procedures: The right or left side or both sides of the cranium will be exposed. The incision will begin using a straight midline incision extending from the nasion caudally just past the occipital protuberance. The subcutaneous tissues are separated at midline. Sterile drapes are attached to each side of the incision using Michele clips. The temporalis muscle and periosteum are reflected laterally on both sides of the cranium. A pneumatic drill and burr will be used to create a defect in the cranium over the parietal bone approximately 2 cm by 3 cm. Using the drill and rongeurs, the hole is enlarged to accommodate an ultrasound probe. The procedure will be repeated on the opposite side. Size of lateral craniectomy has been modified to fit the ultrasound probe. Hemorrhage from boney sinuses will be controlled with bone wax. Hemorrhage from soft tissues will be controlled using bipolar cautery. The intraventricular injections will be performed via ultrasound guided or stereotaxy techniques. Ultrasound guided injection: A pneumatic drill and burr will be used to create a defect in the cranium over the parietal bone approximately 2 cm by 3 cm. Using the drill and rongeurs, the hole is enlarged to accommodate an ultrasound probe. The procedure will be repeated on the opposite side. Size of lateral craniectomy has been modified to fit the ultrasound probe. Hemorrhage from boney sinuses will be controlled with bone wax. Hemorrhage from soft tissues will be controlled using bipolar cautery. Sonography is performed by a veterinary radiologist. Real-time B-mode sonography of the brain is performed using a 7.5-MHz intraoperative ultrasound probe. The ultrasound probe is covered using sterile sleeve specifically designed for probe in use. Sagittal and transverse images of the lateral ventricle are attained. The meninges exposed for the lateral craniectomy are incised at the needle entry site. A 1.5 inch 22 guage spinal needle is inserted into the brain guided by ultrasound imaging. Specifically, the needle will be inserted into the middle suprasylvian gyrus and directed medioventrally into the ventricle. When sonography reveals the needle tip in the right lateral ventricle a 1 ml syringe is attached to the hub of the biopsy needle and up to 1.0 ml of the recombinant AAV solution will be injected into the lateral ventricle. The volume of the injected solution is considered a small amount and adverse effects with this volume is not anticipated. To detect any changes, such as hemorrhage, the brain will be monitored by ultrasound for approximately 30 seconds after removal of the needle. Stereotaxic guided injection procedure: The system is a magnetic resonance (MR) imaging based frameless stereotactic surgical system. The device name is “Brain sight Veterinary Neuronavigation system”. This is the first and only system of its kind made specifically for veterinary use. The requestor is an investigator in establishing the accuracy and precision of the device from previous work at Washington State University. The advantages of the proposed protocol over previous ultrasound guidance are multiple. First, the MR software of this device will allow for a superior topographic	Survival	[redacted]	Surgical suite of the [redacted]

Procedure	Description of procedure	Survival or Terminal	Building Name	Room Number or area
	evaluation of the subject's brains as well as the potential for volumetric studies. The precision and accuracy of the system is considered the highest of currently available minimally invasive techniques. The morbidity associated with the procedure will likely be less because of a smaller craniotomy defect and the ability of preplanned needle entry and trajectory. Following anesthesia induction, the subject is refitted with the bite block. The patient will be placed in dorsal recumbency and a small area over the parietal bone is clipped and prepared for surgical approach with a betadine scrub in an aseptic manner. The subject's head is secured to the surgical table via a C-clamp head and skull screws. The fiducial markers are co-registered with the 3-D MRI software and video capture device. A tracking pen is then co-registered with the software to act as a reference in the coordinate system. The tracking pen is connected to the C-clamp by an articulated arm. The tracking pen identifies an area on the scalp over the parietal bone with the correct entry and trajectory for penetration into the lateral ventricle. The articulated arm is locked into place. A small skin (1.0 cm diameter) skin incision at the site of contact between the pen and scalp is made. The tracking pen is replaced with a 0.5cm craniotome for skull removal. The underlying dura is then pierced with a dural hook. The craniotome is replaced with an automated syringe. The automated syringe is set to insert into a predetermined depth as predetermined by the MRI software. Once at the set depth, 0.1 cc of negative pressure is placed on the syringe. If fluid suggestive of cerebrospinal fluid is aspirated, the syringe with the compound is injected into the ventricle. Following injection, the syringe is left in place for 5 minutes prior to removal. The head clamp and bite block is removed. Bilateral Injection Procedure: Depending upon the distribution of the AAV after injection one side, both ventricles may need to be injected using the procedures already described. The same approach will be performed by extending the dissection of the temporalis muscle on the opposite. The stereotaxic apparatus will be positioned to the coordinates and the injection of the AAV repeated on the opposite side. Closure – The meningotomy and craniectomy will not be repaired. The temporalis muscle fascia will be apposed using 2-0 PDS in a simple interrupted pattern. The subcutaneous tissue will be apposed in 2 layers using 3-0 PDS in a simple continuous pattern. The skin will be apposed with 3-0 nylon in a simple interrupted pattern.			

	Procedure	Description of procedure	Survival or Terminal	Building Name	Room Number or area
2	Artificial insemination of dogs:	Artificial insemination of dogs: Description of surgical procedure: A 5 to 8 cm midline abdominal incision will be made and the uterus exteriorized using sterile technique. Using an intravenous 18 gauge catheter inserted through the uterine wall, semen collected prior to surgery will be injected into the uterus. The abdomen will be closed by standard two-layer closure using 3-0 PDS in a simple interrupted pattern for the abdominal wall and 3-0 nylon in a simple interrupted pattern for the skin.	Survival	[redacted]	[redacted]
3	Bone Marrow Collection (not technically a surgical procedure, but included here for information purposes):	Bone Marrow Collection (not technically a surgical procedure, but included here for information purposes): Wearing sterile gloves, the proximal portion of the greater tubercle is palpated and an incision over this structure is made using a #15 surgical blade. An 11 guage 10 cm Jamshidi needle is introduced through the incision and pushed into contact with the lateral portion of the greater tubercle in the fossa where the lateral glenhumeral ligament attaches. The needle is passed into the bone using a drilling motion aiming the needle at an angle to pass down the medullary cavity. When the needle has penetrated partially through the cortex, the sylet is removed and the needle is passed into the medullary cavity. The needle is then moved back and forth slightly and then withdrawn. The sample is retrieved by placing the stylet into the needle and pushing the biopsy into sterile culture medium. The skin is then closed with 3-0 non-absorbable suture material. The suture site will be monitored daily and the suture will be removed in 7 to 10 days.	Survival	[redacted]	[redacted]

4. Surgery: Post-operative Care

Describe post-operative care (include **both short and long-term care**; monitoring, surgical wound care including suture removal, and list drugs and doses anticipated to be used).

Surgical ICV Injection
Short- term care: The dogs will be recovered from anesthesia and the endotracheal tube removed once pharyngeal reflexes are observed. The dogs will be directly observed for the first 12 hours after the craniotomy and injection by the Co-I, veterinary research intern, and veterinary technicians. Hourly, serial neurologic examinations will be performed by assessing mentation, body position, mobility and cranial nerve evaluation. Rectal temperature, pulse and respiration will be monitored hourly and fluid therapy using lactated Ringer's solution (60 ml/kg/day) will be administered for 12 hours following each procedure. The craniotomy site will be monitored for swelling and cool-packed every 4 hours until 12 hours after surgery. If rectal temperature falls below 100 F a thermal heating blanket will be placed beneath the dog. Dogs will be recovered overnight in room E201 before returning to animal housing in Vet Med the following day.

Acepromazine may be administered if the dog is anxious during recovery. Buprenorphine (0.015 mg/kg SQ q 4-8 h for the first 24 h) or Morphine 0.5 mg/kg IM q 4-6 h and/ or dexmedetomidine (20-30 mcg/kg IM) will be administered in the immediate post operative period and tramadol (1-4 mg/kg q 6-8 hrs) or gabapentin (10 mg/kg q 8 hrs PO) will be given as needed for pain for 24-48 hours after surgery.

Long- term care: The surgical site will be monitored daily for redness, swelling and drainage. Sutures will be removed in 7 days. The neurologic examination will be repeated every 24 hours for 1 week and then weekly until 4 weeks post surgery. Dogs may be administered a 2 week course of prednisone following surgery to address inflammation. Dogs will be administered cyclosporine and/or leflunomide for the duration of the study until euthanasia to minimize potential immune reactions to the TPP1 protein which could be recognized a “foreign” by the immune system of TPP1-null dogs. The dogs will be monitored for improvement or regression of their neurologic status. Dogs will be kept and neurologic exams repeated weekly until either their neurologic signs indicate euthanasia is necessary (using the criteria described elsewhere in this protocol), or until they reach 5 years of age. Any treated dogs reaching 5 years of age will be euthanized and the tissues collected for biochemical and histopathological analyses.

Artificial insemination of dogs
The dogs will be recovered from anesthesia and the endotracheal tube removed once pharyngeal reflexes are observed. Rectal temperature, pulse and respiration will be monitored at least hourly until full recovery. If rectal temperature falls below 100 F a thermal heating blanket will be placed beneath the dog. If the dogs show evidence of discomfort, additional buprenorphine will be administered. Miloxicam 0.2 mg/kg initially then 0.1 mg/kg daily will be used as needed for the treatment of post-operative pain. The incision site will be monitored daily for evidence of pain, redness or swelling. The sutures will be removed 7 to 10 days after surgery.

Bone marrow aspiration
The same principles and procedures will be used for post-operative care, excluding the use of analgesics, as for the artificial insemination procedure. If pain or tenderness is observed after the procedure, carprofen or buprenorphine will be administered for 24-48 hours following the procedure.

5. Surgery: Special Needs

Special needs of the animals following surgery:

N/A

6. Surgery: Length of Time Alive

Length of time animals will be kept alive following surgery:

From 4 months to full life span or when clinical signs reach endpoint (refractory seizure control; unable to eat/swallow without difficulty; become nonambulatory).

11. Potential Pain or Physical Stress

Potential Pain and/or Distress

Note: Animal Welfare Act regulations define a painful procedure as "any procedure that would reasonably be expected to cause more than slight or momentary pain ... in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures." Procedures reasonably expected to cause pain in the absence of anesthetics or pain relieving drugs should be considered to have the potential to cause pain even with the use of such drugs.

1. Potential Side-Effects and Adverse Health Effects

Describe any potential side-effects or anticipated adverse health effects of all procedures listed in the preceding sections: animal husbandry, description of non-surgical procedures, anesthetic procedures, and surgical procedures.

Dogs with CLN2 disease experience progressive neurodegeneration leading to presentation of neurological signs at approximately 6 to 7 months of age. Signs include progressive cognitive decline, visual impairment, ataxia, and myoclonic seizures (at end stage).
Minimally-invasive tests (i.e. collection of blood samples or any non-surgical procedure requiring sedation or anesthesia) are associated with minor discomforts and minimal risks of causing consequential harm. Blood collection procedures or placing an intravenous catheter may cause a small amount of swelling or discoloration at the puncture site, but this usually resolves within 24 hours. Endotracheal intubation may cause tracheal irritation during the postanesthetic period. General anesthesia is associated with risks for development of complications, which might be minor (i.e., slight decrease in blood pressure or some depression of breathing) or major (i.e., paralysis, cardiac arrest or death). Dogs will be monitored regularly throughout general anesthesia by a veterinary technician and assistance will be provided by the veterinary neurology research intern as needed to maintain vital signs within a healthy range.
The collection of CSF via cisternal tap or vector delivery to the spinal cord is associated with minimal risks because the procedure involves placing a needle near the spinal cord. These collection procedures are routinely performed in the hospital and will be performed using standard procedures described in this protocol. The CSF collection procedure will be performed by a veterinarian with necessary training from a veterinary neurologist ([redacted]) to minimize the associated risks. Vector delivery to the spinal cord will be performed by a veterinary neurologist ([redacted]) who is experienced with the procedure. Preliminary experiments have already been conducted with the gene therapy protocols and the dogs have been followed for up to 9 months post-treatment. In the dogs treated with our current protocols, no adverse health effects have been observed, so none are expected for these treatments. Histopathological analyses will be performed after the dogs are euthanized. These analyses will indicate whether any subclinical adverse effects occurred.
There are no associated risks with brain imaging using MRI other than previously described risks associated with anesthesia. Metallic objects will be
avoided when using MRI. All individuals involved with the study have undergone viewing of the MRI safety video.

Post operative complications of intracranial surgery include those involving damage or injury to the brain and other systemic abnormalities. Iatrogenic injury to the brain often results in intracranial signs that are present immediately on recovery from anesthesia or evolve within 48 to 72 hours. Intracranial hemorrhage, increasing cerebral edema, increasing intracranial pressure and ischemia due to cerebrovascular disease are most often the causes of neurological deterioration after surgery. Injection of the solution will be performed over a one minute procedure to lessen complication of increased intracranial pressure. Moreover, the volume of injected solution is considered small in amount and we do not anticipate any adverse effects based on this injection volume. The dogs may have temporary hemi-neglect which may last for several days post surgery. Hemi-inattention or hemineglecsyndrome refers to a phenomenon in which a patient with a structural forebrain lesion ignores input from the contralateral half of its environment. Since stimuli are interpreted primarily in the cerebral hemisphere contralateral to the stimulus side, the side that the patient ignores is contralateral to the side of the lesion. These patients may eat from only ½ of the food bowl, turn the opposite direction when called by name on the ignored side, and ignore or have difficulty localizing nociceptive (skin pinch) stimuli when applied contralateral to the side of the brain lesion. The dog will be humanely euthanized if it becomes comatose, develops decerebrate posturing or evidence of severe meningitis.
Infectious complications are possible but rare. Seizures are possible. Seizures occurring in the immediate postoperative period are treated as in dogs not undergoing surgery. Intravenous diazepam bolus doses are given acutely if seizures occur. If recurrent seizure activity ensues, a constant infusion of diazepam or propofol may be needed for seizure control. Maintenance anticonvulsants listed elsewhere in this protocol are initiated if seizures continue to occur. Incision complications include swelling, drainage, seroma formation and infection. The skin incision will be monitored closely. Meningitis may occur secondary to injection of the recombinant AAV vector. Immunosuppression with cyclosporine will minimize this complication, and prednisone will be used to address meningitis if it occurs. Of the non-neurological complications of intracranial surgery, pneumonia is the most common. The most common factor to contribute to pneumonia

is aspiration of food or other digestive material. Withholding food the evening prior to surgery minimizes this risk. Megaesophagus also may be an associated risk factor for the development of pneumonia in dogs after intracranial surgery. Fever is usually the first sign of pneumonia. Thoracic radiography will assist with further evidence of aspiration pneumonia. Long term immunosuppression can increase the risks of infections and cancer. Animals will be carefully monitored for adverse effects, and animals will be treated with antimicrobials/antinflammatories as needed, or euthanized, based onconsultations with OAR Veterinary Staff.

Intravitreal injections carry the risk of causing uveitis. Injected animals will be carefully monitored by a veterinary ophthalmologist and uveitis will be treated with appropriate topical and oral medications.

2. Assurance of Limited Discomfort and Pain

Describe how it is assured that discomfort and pain are limited to that which is unavoidable for the conduct of this experimentation.

Appropriate analgesics, sedatives, and anesthetics will be used for all procedures that could cause more than momentary discomfort or pain. The only procedure that we anticipate could cause momentary pain are needle sticks required for blood draws in awake dogs or for injection of medications. The dogs tolerate these quite well with minimal exhibition of pain or discomfort, especially after they get used to the procedures. To limit pain and discomfort in affected animals, they are euthanized before the disease signs become severe enough that they would be judged to entail significant discomfort or pain. Untreated affected animals, if allowed to die naturally, will live to approximately 12 months of age. Based on our prior experience with affected dogs, we have elected to euthanize at around the age of 10.5 months. At this stage the dogs exhibit significant visual impairment, moderate ataxia with occasional falling, and the first appearance of seizures may occur. At this stage, the dogs begin to have difficulty eating on their own and it is often necessary to supplement with hand-feeding. Behaviorally the dogs still appear to enjoy interaction with their caretakers.

3. Pain and Distress Form

Is there a Pain and Distress form associated with this protocol?

See: Painful or Distressful Procedures

○ Yes ● No

12. Disposition of Animals

1. Animal Disposition

Check all that apply

- ☑ Adoption (See MU adoption policy)
- ☐ Market
- ☑ Euthanasia
- ☐ Transfer to different project, PI, or another institution
- ☐ Returns to breeding colony, herd, source, owner, or wildlife site
- ☐ Other

2. Euthanasia

Euthanasia Statement

As noted in the Guide, “Euthanizing animals is psychologically difficult for some animal care, veterinary, and research personnel, particularly if they perform euthanasia repetitively or are emotionally attached to the animals being euthanized (Arluke 1990; NRC 2008; Rollin 1986; Wolfle 1985). When delegating euthanasia responsibilities, supervisors should be sensitive to this issue.”

A. Method of Euthanasia

Select the method of euthanasia

- ☐ Inhalant agent
- ☐ Physical Method without Anesthesia
- ☐ Physical Method with Anesthesia

Unselect this one

— [redacted], VETERINARIAN, Office of Animal Resources Jul 22, 2022 10:25 AM

- ☑ Noninhalent Pharmaceutical Agent

Select this one

— [redacted], VETERINARIAN, Office of Animal Resources Jul 22, 2022 10:25 AM

B. Euthanasia Descriptions

	Species	Agent/Method	Dose/Volume	Route
1	Dog	pentobarbital	390 mg/ml; 1 ml/4.5 kg	IV

C. Additional Explanation of Euthanasia Procedures

Include any additional explanation of euthanasia procedures here.

To confirm death, the dogs will be monitored with a stethoscope by a veterinarian after drug administration to confirm that heartbeat and respiration have stopped.

D. Scientific Justification for Use

- ☑ AVMA Approved Method
- ☐ Not AVMA Approved Method

E. Secondary (Physical) Means of Assuring Euthanasia

- ☑ Bilateral pneumothorax
- ☐ Cervical dislocation
- ☐ Decapitation
- ☐ Exsanguination
- ☐ Removal of vital organs
- ☐ Other

13. Project Information

1.

Associate	Role	Responsibilities	Animal Care & Use	OHSP Training	P&D Training	Survival Surgery
Whiting, Rebecca Elizabeth Hannah [redacted]	Principal Investigator Authorized to order animals Editor		☑ Sep 12, 2024	☑ Sep 12, 2024	⊖	⊖
[redacted]	Co-Investigator Editor	Surgery Euthanasia	☑ Aug 24, 2024	☑ Jun 15, 2022	⊖	☑ Aug 24, 2024
[redacted]	Co-Investigator Editor		☑ May 9, 2022	☑ Nov 18, 2022	⊖	☑ May 28, 2003
[redacted]	Key Personnel		☑ Oct 19, 2021	☑ May 17, 2022	⊖	⊖
[redacted]	Key Personnel	Surgery	☑ Mar 30, 2023	☑ Oct 19, 2022	⊖	☑ Nov 26, 2002
[redacted]	Key Personnel	Euthanasia	☑ Mar 4, 2024	☑ Sep 19, 2022	⊖	☑ Jan 18, 2012
[redacted]	Key Personnel		☑ Aug 3, 2022	☑ Aug 3, 2022	⊖	⊖
[redacted]	Key Personnel		☑ Apr 8, 2024	☑ Jun 1, 2022	☑ Apr 9, 2024	☑ Apr 8, 2024
[redacted]	Key Personnel		☑ Sep 4, 2024	☑ Jun 1, 2022	⊖	☑ Sep 4, 2024
[redacted]	Key Personnel	Surgery Euthanasia	☑ Jul 12, 2022	☑ Jul 12, 2022	⊖	⊖
[redacted]	Key Personnel		☑ Oct 13, 2021	☑ May 22, 2022	⊖	⊖
[redacted]	Key Personnel		☑ Jan 23, 2022	☑ Feb 26, 2022	⊖	☑ Jan 23, 2022
[redacted]	Key Personnel		☑ Sep 5, 2024	☑ Oct 25, 2022	⊖	⊖
[redacted]	Key Personnel		☑ Apr 26, 2022	☑ May 11, 2022	⊖	☑ Apr 19, 2024
[redacted]	Key Personnel		⊖	⊖	⊖	☑ Feb 21, 2020
[redacted]	Key Personnel		☑ Nov 21, 2023	☑ Nov 27, 2023	⊖	☑ Nov 21, 2023

Associate	Role	Responsibilities	Animal Care & Use	OHSP Training	P&D Training	Survival Surgery
[redacted]	Key Personnel		⊖	⊖	⊖	⊖
[redacted]	Key Personnel		☑ Sep 7, 2023	☑ Feb 2, 2022	⊖	⊖
VHC, Clinical Staff VM&S acuc+clinicalstaff@missouri.edu	Key Personnel		⊖	⊖	⊖	⊖
VHC, Residents acuc+VHC@missouri.edu	Key Personnel		⊖	⊖	⊖	⊖
[redacted]	Key Personnel	Surgery	☑ Jun 28, 2022	⊖	⊖	⊖
[redacted]	Key Personnel	Surgery	⊖	⊖	⊖	☑ Dec 11, 2019
[redacted]	Key Personnel		☑ May 17, 2022	☑ May 17, 2022	⊖	⊖
[redacted]	Protocol Creator Editor		☑ Oct 16, 2023	☑ May 5, 2023	⊖	☑ Oct 16, 2023

Please note under Responsibilities who may perform surgery and euthanasia

—[redacted], VETERINARIAN, Office of Animal Resources Jul 22, 2022 10:26 AM

2. Training and Qualifications

Provide a description of the training and qualifications for each individual listed above under Protocol Associates. Provide adequate detail to allow the ACUC to determine if the individual has adequate training and experience with the species and procedures to perform their role proficiently. If they do not have prior training or experience, how will this be obtained?

Please complete the Employment Status for all personnel

—[redacted], VETERINARIAN, Office of Animal Resources Jul 22, 2022 10:26 AM

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
1	[redacted]	[redacted] is a board certified veterinary oncologist in the College of Veterinary Medicine. In the course of this clinical practice he routinely performs bone marrow aspirations on canine patients. He will perform this procedure on our research dogs as a means of obtaining bone marrow for culturing mesenchymal stem cells.	Perform bone marrow aspirations on dogs to obtain mesenchymal stem cells	[redacted] is a board certified veterinary oncologist in the College of Veterinary Medicine. In the course of this clinical practice he routinely performs bone marrow aspirations on canine patients. He will perform this procedure on our research dogs as a means of obtaining bone marrow for culturing mesenchymal stem cells.	Full-time employee
2	[redacted]	[redacted] is a Registered Veterinary Technician with 20 years experience in a clinical setting and 10 years experience in a research setting working primarily with dogs and cats. She has extensive experience in canine surgery, anesthesia, electrophysiology and behavior. During her 10 years in the research laboratories, she has been responsible for dog colony management, including health and care oversight. She hires and supervises part-time student workers who walk and socialize our research dogs and has assisted with numerous litter deliveries.	Oversee dog colony; supervise student workers; monitor care and health of dogs; assist with all surgical procedures, monitor vital signs during sedation/anesthesia; perform electroretinograms; perform behavior studies; collect blood samples; assist with reproductive procedures.	[redacted] is a Registered Veterinary Technician with 20 years experience in a clinical setting and 10 years experience in a research setting working primarily with dogs and cats. She has extensive experience in canine surgery, anesthesia, electrophysiology and behavior. During her 10 years in the research laboratories, she has been responsible for dog colony management, including health and care oversight. She hires and supervises part-time student workers who walk and socialize our research dogs and has assisted with numerous litter deliveries.	Full-time employee

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
3	[redacted]	[redacted] Associate Professor is a faculty member at the University of Missouri, College of Veterinary Medicine and is a board certified veterinary neurologist of the American College of Veterinary Internal Medicine. She co-directs the Comparative Neurology Program at MU. She has extensive experience in neurology and neurosurgery and has characterized a number of neurodegenerative disorders. She will train the veterinary neurology research intern in CSF collection and neurologic exam. [redacted] will perform the injections or surgery necessary for gene therapy vector delivery to CSF. She has experience with all of these procedures.	Perform surgeries and fluid collections; supervise other veterinary staff; euthanasia; Monitor overall and neurological health of the dogs; collect blood and CSF samples, perform surgeries or procedures to inject gene therapy vectors; perform MRI and other imaging.	[redacted] Associate Professor is a faculty member at the University of Missouri, College of Veterinary Medicine and is a board certified veterinary neurologist of the American College of Veterinary Internal Medicine. She co-directs the Comparative Neurology Program at MU. She has extensive experience in neurology and neurosurgery and has characterized a number of neurodegenerative disorders. She will train the veterinary neurology research intern in CSF collection and neurologic exam. [redacted] will perform the injections or surgery necessary for gene therapy vector delivery to CSF. She has experience with all of these procedures.	Full-time employee
4	[redacted]	A veterinary medicine student with significant experience working with dogs in our colony. She has been trained in the proper methods of dog socialization, medication administration, testing cognitive behavior, and to assist with procedures by [redacted]	dog socialization, medication administration as directed by veterinarians, behavioral studies, monitoring during estrous and pregnancy, assist with other procedures as needed	They have been trained in the proper methods of dog socialization, medication administration, testing cognitive behavior, and to assist with procedures by [redacted]	Grad student/ Professional student
5	[redacted]	[redacted] is a board-certified veterinary pathologist who has over 25 years experience performing necropsies on dogs and other animals.	Perform or assist with necropsies.	[redacted] is a board-certified veterinary pathologist who has over 25 years experience performing necropsies on dogs and other animals.	Full-time employee

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
6	[redacted]	[redacted] has over 30 years of experience directing and conducting animal experiments using rats, mice, primates, and dogs. He has direct experience with electrophysiology, surgery, euthanasia, blood sampling, behavioral studies, and tissue collections. His experience is reflected in many of his 115 scientific publications.	Contribute to study design; approve specific aspects of protocol procedures, necropsy/ assist with tissue acquisitions.	[redacted] has over 30 years of experience directing and conducting animal experiments using rats, mice, primates, and dogs. He has direct experience with electrophysiology, surgery, euthanasia, blood sampling, behavioral studies, and tissue collections. His experience is reflected in many of his 115 scientific publications.	Full-time employee
7	[redacted]	[redacted] has 5 years experience working with our dog colony.	Culture and prepare autologous mesenchymal stem cells for injection into dogs, perform ERGs and in vivo retinal imaging; assist with necropsy; handle and process biologic samples from dogs (blood, CSF, urine, tears, tissues collected during necropsy); culture fibroblasts from skin biopsy	[redacted] has several years experience with each procedure and has been trained in all procedures by [redacted] and [redacted]. Any in vivo data collection is overseen by lab veterinary staff.	Full-time employee and Postdoc fellow/ Resident
8	[redacted]	[redacted] will not be working directly with the animals, but she will be processing samples collected from the dogs. She has extensive experience working with canine biologic samples and tissues and cell culture, and she has received all necessary safety training.	Culture and prepare autologous mesenchymal stem cells for injection into dogs; handle and process biologic samples from dogs (blood, CSF, urine, tears, tissues collected during necropsy).	[redacted] will not be working directly with the animals, but she will be processing samples collected from the dogs. She has extensive experience working with canine biologic samples and tissues and cell culture, and she has received all necessary safety training.	Full-time employee

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
9	[redacted]	[redacted] is a board-certified veterinary ophthalmologist who has extensive experience in intraocular injections, ophthalmic examinations, and treatment of eye pathology for dogs and other animals.	Perform intravitreal injections, sub-tenon injections, and ophthalmic exams. Manage ocular health.	[redacted] is a veterinary ophthalmologist who will work on a contract basis as needed	Part-time employee and Courtesy appointment/ Adjunct
10	VHC, Clinical Staff VM&S	anesthesia, MRI, cardiac evaluation	anesthesia, MRI, cardiac evaluation	anesthesia, MRI, cardiac evaluation	Full-time employee
11	VHC, Residents	anesthesia, MRI, cardiac evaluation	anesthesia, MRI, cardiac evaluation	anesthesia, MRI, cardiac evaluation	Full-time employee and Postdoc fellow/ Resident

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
12	[redacted]	[redacted] performs all of these duties routinely in the Veterinary Medical Teaching Hospital for outside clients. She is a licensed veterinarian, completed a three year residency in clinical reproduction, and obtained board certification as a theriogenologist in 2006. She has extensive experience with the required procedures.	Oversee dog reproduction; provide pre- and post-delivery care of mothers and puppies; collect and preserve semen; perform AI; perform or assist with surgical AI and caesarean section deliveries when required. She is responsible for working with [redacted] on the breeding management of the dogs. This entails monitoring bitches by behavior testing, vaginal cytology, vaginoscopy and blood collection for serum progesterone concentrations. Additionally, she performs semen collection, semen evaluation, semen freezing, artificial insemination (vaginally or surgically), supervised natural matings, pregnancy examinations by palpation, ultrasonography and radiography and occasional assistance with dystocias.	[redacted] performs all of these duties routinely in the Veterinary Medical Teaching Hospital for outside clients. She is a licensed veterinarian, completed a three year residency in clinical reproduction, and obtained board certification as a theriogenologist in 2006. She has extensive experience with the required procedures.	Full-time employee

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
13	[redacted]	[redacted] performs all of these duties routinely in the clinics of the VMTH. He is a licensed veterinarian and board certified as a theriogenologist. He has performed such duties and procedures for almost 30 years in service and research programs of at least 3 universities and a colony of guide dogs (Guiding Eyes for the Blind, Patterson, New York).	[redacted] is responsible for the breeding management of the dogs. This entails monitoring bitches by behavior testing, vaginal cytology, vaginoscopy and blood collection for serum progesterone concentrations, semen collection, semen evaluation, semen freezing, artificial insemination (Vaginally or surgically), supervised natural matings, pregnancy examinations by palpation, ultrasonography and radiography and occasional assistance with dystocias.	[redacted] performs all of these duties routinely in the clinics of the VMTH. He is a licensed veterinarian and board certified as a theriogenologist. He has performed such duties and procedures for almost 30 years in service and research programs of at least 3 universities and a colony of guide dogs (Guiding Eyes for the Blind, Patterson, New York).	Full-time employee
14	Whiting, Rebecca Elizabeth Hannah	Rebecca E.H. Whiting, PhD: Dr. Whiting has been conducting research on the effects of hereditary neuronal ceroid lipofuscinosis (NCL) in the Dachshund model for over 10 years, and she has worked closely with the team of veterinary specialists and basic researchers involved in these projects for the majority of that time.	Experimental design and protocol development, perform vision assessments on dogs including ERG, in vivo retinal imaging, pupillography, and funtional vision testing, oversee trainees assisting with procedures.	Rebecca E.H. Whiting, PhD: Dr. Whiting has been conducting research on the effects of hereditary neuronal ceroid lipofuscinosis (NCL) in the Dachshund model for over 10 years. She has worked with and been trained by veterinary ophthalmologists and has developed expertise in assessing visual function in dogs using electroretinography, quantitative pupillary light reflex measurement, behavioral testing, and retinal imaging.	Full-time employee

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
15	[redacted]	[redacted] is a licensed veterinarian and a veterinary research intern in the University of Missouri, College of Veterinary Medicine Comparative Neurology Program. She will work closely with the other veterinarians involved in the project to manage the health of the dogs and to assist with treatments and neurological assessments. As a veterinarian, she has training and experience with all the procedures she will perform or assist with, and [redacted] will provide any additional training needed for procedures such as CSF collection or surgery.	Provide veterinary oversight of all procedures requiring sedation and/or anesthesia of the dogs. Collect CSF, blood, and skin biopsy samples. Perform complete neurologic, ophthalmic, and physical exams of dogs. Provide veterinary care for the dogs.	[redacted] is a licensed veterinarian and a veterinary research intern in the University of Missouri, College of Veterinary Medicine Comparative Neurology Program. She will work closely with the other veterinarians involved in the project to manage the health of the dogs and to assist with treatments and neurological assessments. As a veterinarian, she has training and experience with all the procedures she will perform or assist with, and [redacted] will provide any additional training needed for procedures such as CSF collection or surgery.	Full-time employee and Postdoc fellow/ Resident
16	[redacted]	[redacted] is an undergraduate student with an interest in veterinary medicine and has significant experience working with dogs.	dog socialization, medication administration as directed by veterinarians, behavioral studies, monitoring during estrous and pregnancy, assist with other procedures as needed	[redacted] has been trained in the proper methods of dog socialization, medication administration, testing cognitive behavior, pregnancy monitoring, and to assist with procedures by [redacted]	Part-time employee and Undergraduate student
17	[redacted]	[redacted] is an undergraduate student with an interest in veterinary medicine and has significant experience working with dogs.	dog socialization, medication administration as directed by veterinarians, behavioral studies, monitoring during estrous and pregnancy, assist with other procedures as needed	[redacted] has been trained in the proper methods of dog socialization, medication administration, testing cognitive behavior, pregnancy monitoring, and to assist with procedures by [redacted]	Part-time employee and Undergraduate student

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
18	[redacted]	[redacted] was previously employed by our lab as an undergraduate research assistant. He worked extensively with our dogs and was trained in the proper methods of dog socialization, medication administration, testing cognitive behavior, and to assist with procedures by [redacted]	[redacted] will rejoin the lab as a [redacted]. He will primarily work to analyze biologic samples from the dogs, but may observe or assist with other procedures as required. He will be overseen by either the veterinary technician or veterinarian at all times for any dog procedures.	[redacted] will be trained by [redacted] in proper handling of biologic samples. He has 2 years of experience and has been trained in the proper methods to assist with dog procedures by [redacted]	Grad student/ Professional student
19	[redacted]	An undergraduate student with an interest in veterinary medicine and has significant experience working with dogs.	dog socialization, medication administration as directed by veterinarians, behavioral studies, monitoring during estrous and pregnancy, assist with other procedures as needed	The student has been trained in the proper methods of dog socialization, medication administration, testing cognitive behavior, and to assist with procedures by [redacted]	Part-time employee and Undergraduate student
20	[redacted]	An undergraduate student with an interest in veterinary medicine and has significant experience working with dogs.	dog socialization, medication administration as directed by veterinarians, behavioral studies, monitoring during estrous and pregnancy, assist with other procedures as needed	The student has been trained in the proper methods of dog socialization, medication administration, testing cognitive behavior, and to assist with procedures by [redacted]	Part-time employee and Undergraduate student
21	[redacted]	An undergraduate student with an interest in veterinary medicine and has significant experience working with dogs.	dog socialization, medication administration as directed by veterinarians, behavioral studies, monitoring during estrous and pregnancy, assist with other procedures as needed	The student has been trained in the proper methods of dog socialization, medication administration, testing cognitive behavior, and to assist with procedures by [redacted]	Part-time employee and Undergraduate student

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
22	[redacted]	An undergraduate student with an interest in veterinary medicine and has significant experience working with dogs.	dog socialization, medication administration as directed by veterinarians, behavioral studies, monitoring during estrous and pregnancy, assist with other procedures as needed	The student has been trained in the proper methods of dog socialization, medication administration, testing cognitive behavior, and to assist with procedures by [redacted]	Part-time employee and Undergraduate student
23	[redacted]	An undergraduate student with an interest in veterinary medicine and has significant experience working with dogs.	dog socialization, medication administration as directed by veterinarians, behavioral studies, monitoring during estrous and pregnancy, assist with other procedures as needed	The student has been trained in the proper methods of dog socialization, medication administration, testing cognitive behavior, and to assist with procedures by [redacted]	Part-time employee and Undergraduate student

Training Requirements

Note: The ACUC required Basic Training can be found at: https://research.missouri.edu/acqa/. This training must be updated every three years in order to receive protocol approval.

Note: It is the Principal Investigator's responsibility to ensure that all persons listed in Protocol Associates above participate in the MU Occupational Health and Safety Program. See Section 7:020 MU Business Policy and Procedures Manual for details. For enrollment procedures visit the OHSP website.

3. Funding Source

What is the funding source for this project? (Note: If funded internally or by a non-peer-reviewing agency, a peer review of scientific merit may be required.)

- [x] PHS (NIH, CDC, FDA, NSF, NASA)
- [ ] DoD
- [ ] VA
- [ ] AHA
- [ ] USDA
- [ ] Foundation/Industry
- [x] Internal
  - **i.** Specify funding source

    MU School of Medicine (TRIUMPH grant)
- [x] Other
  - **i.** Specify funding source:

National Institutes of Health, National Eye Institute

14. Refinements or Literature Search

Attach relevant files in the attached files section.

1. Painful Procedures

Any procedure that may potentially cause more than momentary or slight pain or distress requires a literature search for animal alternatives.

Are you performing any procedures that may potentially cause more than momentary or slight pain or distress?

● Yes ○ No

2. USDA Covered Species

Does this protocol utilize animals covered by the Animal Welfare Act or assigned to Category E? (AWA covered species include all warm blooded animals except birds, rats of the genus Rattus, and mice of the genus Mus, bred for use in research, horses not used for research purposes, and other farm animals.)

● Yes, includes USDA covered species or Category E ○ No

3. Includes USDA covered species or Category E

Search for Animal Alternatives

In the literature search and in the written narrative, replacement by non-animal systems, reduction in numbers of animals and refinement of experimental methods (the three R's) must be addressed.

Provide at least two sources of information: one of these sources must be a scientific literature database; documented expert consultation may be used as one source of information.

If you are in the School of Medicine and need assistance with this item, please contact [redacted]. Others can contact the Zalk Veterinary Medical Library, at MU CVM VetMed Library for help.

See also:

https://www.nal.usda.gov/awic/sample-searches
https://library.missouri.edu
Literature Search Help

A. Source 1: Literature Database

Complete the information below:

	Date of Search	Name of Database	Years Covered by Search	Keywords and Search Strategy
1	7/19/22	PubMed	all years	see narrative

B. Source 2: Literature Database

For the second source you may use a literature database search or an expert consultation (see following question).

	Date of Search	Name of Database	Years Covered by Search	Keywords and Search Strategy
1	7/19/2022	Web of Science	All Years	See narrative

C. Source 2: Expert Consultation (alternative)

For the second source you may use a literature database search or an expert consultation. Documented expert consultation may be used as one source of information.

No Sources...

D. Animal Alternatives Narrative

Based on the information from the sources above, provide a written narrative of alternatives to procedures that may potentially cause more than momentary or slight pain or distress. The narrative should be such that the ACUC can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough.

If a possible alternative was identified or is known, but will not be employed, discuss why.

The goal of our collective studies is to determine whether administration of gene therapy based therapeutics can prevent the progression of disease symptoms in the CLN2 form of Neuronal Ceroid-Lipofuscinosis for which the TPP1 mutant dog is a naturally-occurring model. To determine if a suitable alternative for this goal exists, searches were performed of the Web of Science (all years) and PubMed (all years) databases on July 19, 2022 using the search terms indicated below.

PubMed
Neuronal Ceroid Lipofuscinosis AND animals AND alternatives: 21
Neuronal Ceroid Lipofuscinosis AND in vitro: 120
Neuronal Ceroid Lipofuscinosis AND computer simulation: 5
Neuronal Ceroid Lipofuscinosis AND animals: 971

Web of Science
Neuronal Ceroid Lipofuscinosis AND animals AND alternatives: 12
Neuronal Ceroid Lipofuscinosis AND in vitro: 89
Neuronal Ceroid Lipofuscinosis AND computer simulation:1
Neuronal Ceroid Lipofuscinosis AND animals: 764

Replacement: Of the papers identified as containing both the indicated search words and neuronal ceroid lipofuscinosis, we were familiar with most because of our years of research in this field. Examining these papers and at least the abstracts of those published since our last similar search in 2019, we did not identify any suitable alternative to the dog model. Most instances of in vitro work offer preliminary information that then needs to be validated in an animal model. The computer simulation papers refer to work surrounding the genetic basis of the disease and are not relevant to therapeutic studies. Mouse models may offer preliminary results regarding a therapeutic but cannot predict potential results in humans as well as can the proposed dog studies. Ultimately, even promising mouse studies must be validated in a large animal model before being translated for human use. Some porcine models for various forms of NCL are under development through the use of genetic modification, but these are in the early stages of determining the similarity of the resulting phenotype to that observed in children with the disease. In addition, they are not validated for their ability to successfully predict

outcomes of therapies in children. Companies with the resources to develop the gene therapy vectors for human applications have requested that we conduct the preclinical studies in dogs because of their doubts about how predictive cell culture and mouse studies are for human applications and because of FDA requirements.

Reduce: The minimum number of animals to reach statistical significance will be utilized in this study. Based on previous studies, we are able to achieve statistical significance with a small sample size of 3 dogs per group due to the severe nature of deficits associated with this disease in comparison with normal animals. In most cases, if an approach lacks promise after even 1 or 2 animals we will look for ways to improve the approach before treating any additional animals in order to optimize animal use. Whenever possible, we stagger study enrollment so that preliminary data can be obtained from a single dog before repeating the same therapeutic parameters in even a single additional dog. In this way, we can refine our approach as often as possible and not waste any opportunity for improvement.

Refinement: The techniques described in this protocol are designed to elicit the least possible pain or distress to the dogs. Our primary goal is always to develop therapeutic approaches that will readily translate for use in people, and as such, we do not perform procedures or surgeries that would not be suitable for use in people. Surgeries will be performed by highly trained and experienced veterinary neurologists using anesthetics and analgesics that have been established as the most effective and safe by veterinary practitioners. Dogs will be closely monitored by veterinary specialists (neurology and ophthalmology) and by experienced veterinary technicians for any signs of pain or distress; if such signs are seen the neurologists and other veterinary staff will be consulted as needed. The neurologists involved in the study keep up with the current literature and attend scientific meetings where pain and distress management techniques for dogs are presented. Any applicable relevant information garnered from these sources will be applied in these studies to make certain that the most effective techniques for ensuring the best quality of life for the dogs are utilized.

15. Investigator Assurances

1. ABSL-2 Assurance

I will provide training to the husbandry/veterinary staff at least 48 hours prior to exposing animals to a biohazard regarding (but not limited to): the health hazards and symptoms of the biohazard(s) being used; husbandry related research specific SOP's (e.g. handling live exposed animals and contaminated cages); and animal/carcass disposition.

- ● Yes, I will meet the requirements of this statement.
- ○ No, I will not meet the requirements of this statement.
- ○ Not Applicable

2. Investigator Assurances

- ☑ 1. The information provided herein is accurate to the best of my knowledge.
- ☑ 2. Procedures involving vertebrate animals will be performed only by trained or experienced personnel, or under the direct supervision of trained or experienced persons.
- ☑ 3. Any change in the care and use of vertebrate animals involved in this protocol, will be promptly forwarded to the MU ACUC for review; such changes will not be implemented until the committee's approval is obtained.
- ☑ 4. The number of animals proposed is the minimum necessary to conduct valid experimentation.
- ☑ 5. I assure that I am not unnecessarily duplicating previous experiments.
- ☑ 6. I have considered alternative methods to using animals.

- [x] 7. I understand that animal housing must be coordinated with the facility veterinarian and/or facility manager and that approval of this protocol does not guarantee space to house animals.

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