Protocol Information

Reference Number: 100290
Protocol Number: 09-09-022
Title: Functional analysis of BHD Using Conditional Knockout Strategy
Category: Standard
Protocol Type: Amendment

PI: Teh, Bin
Department:
Location:
Author: Huang, Dan
Status: Approved
Emergency Phone: 616.234.5578
Submittal Date: 12/17/2010 1:50 PM

Approval Date: 12/21/2010 12:00 AM
Effective Date: 12/21/2010 12:00 AM
Renewal Date: 9/14/2011 12:00 AM
Next Review Date: 9/14/2011 12:00 AM
Stock Protocol: No
Expiration Date: 9/14/2012 12:00 AM
Inactive Date:

Amendment to Protocol Form

1 Amendment to Protocol

Are you amending this protocol**only**to update the associates?

a ☐ Yes

b ☑ No

2 [1b] Amendment Reason

Briefly indicate the reason for this amendment. This should focus on**why**the protocol is being amended.

Our preliminary data have suggested that mTOR signaling pathway and PPARa signaling pathway may be implicated in the pathogenesis of kidney tumor in BHD knockout mice. Therefore, we are amending this protocol to investigate the effect of pharmacological inhibition of mTOR and PPARa signaling on the formation of kidney cysts and tumors. We will use 2 small molecule inhibitors to inhibit mTOR and PPARa signaling pathways. The 2 inhibitors are rapamycin (mTOR inhibitor) and MK-886 (PPARa inhibitor). To better monitor the formation of kidney cyst and tumor, we will also add imaging procedures (ultrasound and CT) which will quantify the size of kidney cyst and tumor. Imaging will be performed before, during and after treatment to examine drug effect.

3 [1b] Amendment Summary

Briefly outline**what**is changed in the amendment.

We are amending this protocol to add: (1) imaging procedures to monitor kidney cyst and

tumor formation in BHD knockout mice, including high frequency ultrasound and CT methods; (2) drug treatment to interfere the formation of kidney cyst and kidney tumors.

The imaging procedures will be done in collaboration with Dr. Anthony Chang. Before we image large number of animals, we will take a few mice (10-20) to perform a pilot study to figure out the best imaging procedures and choose between ultrasound and CT method to image large number of animals.

For the drug intervention to prevent or delay the formation of kidney cysts and kidney tumors in BHD knockout mice, we will use 2 drugs. One is rapamycin (an mTOR inhibitor), the other is MK-886 (PPARa inhibitor). Our preliminary data have suggested that mTOR signaling pathway and PPARa signaling pathway may be implicated in the pathogenesis of kidney tumor in BHD knockout mice.Imaging will be performed before, during and after treatment to examine drug effect. The imaging procedures can quantify the size of kidney cyst and tumor.

Two experimental groups are added in the Experimental design section to reflect these changes.

Imaging procedures and anesthesia have been added in Mouse prodedures section.

Important Things to Note

1 Important Note Regarding the Overall Protocol:

- Save your work often.
-
- Make sure to choose your species on the first screen. If you do not, you will not see all of the questions. Once you select the species you will see the icon with a heart symbol on your protocol outline menu on the right of your screen.
-
- In order to save a protocol for the first time, you must enter the name of the PI.
-
- The protocol number is generated only after approval. This field cannot be filled in.
-
- Attach documents to the protocol using the add link/file icon found at the beginning of each section.
-
- An E-signature from the PI is required for IACUC review.

-

Administrative Information:

1 Reference Number

Reference Number
100290

2 Protocol Number

Protocol Number
- 09-09-022 -

3 Title

Title
Functional analysis of BHD Using Conditional Knockout Strategy

4 Continuation of Study

Is this a continuing study from a previously approved protocol that is due to expire?

a ☑ Yes

b ☐ No

5 [4a] Parent Protocol

If this protocol is replacing an expiring protocol please list the parent protocol number.
06-10-028

6 Protocol Category

Protocol Category
Standard

7 Protocol Type

Protocol Type
Amendment

8 Important Note Regarding Roles on a Protocol:

Only individuals identified as the protocol creator, author, PI or Co-PI can access the protocol

prior to approval and are authorized to make amendments and renewal changes.

Therefore, it is best that different individuals be identified in these roles so that the protocol can move forward in the event that one party is not available to log into the system.

A typical scenario might be as follows:

-
- Created by : Administrative Assistant
-
- Author: Postdoctoral Fellow
-
- PI: Principal Investigator
-

9 Created By

Created By

Teh, Bin	(616) 234-5296	Bin.Teh@vai.org

10 Author

Author

Huang, Dan	(616) 234-5683	dan.huang@vai.org

11 Principal Investigator

Principal Investigator

Teh, Bin	(616) 234-5296	Bin.Teh@vai.org

12 Protocol Associates

Protocol Associates

Noyes, Sabrina

Co-PI ☐ **Key Associate** ☑ **Authorized To Order Animals** ☐

Responsibilities: Administrative support - submit, renew, amend protocols.

Comments:

Standard Procedures: Standard Procedure | Type | Species

Ooi, Aikseng

Co-PI ☐ **Key Associate** ☑ **Authorized To Order Animals** ☑

Responsibilities: Set up matings, monitor animals, perform euthanasia, and necropsies.

Comments:

Standard Procedures:

Standard Procedure	Type	Species
Breeding	Breeding	Mus musculus
Post-mortem-Necropsy	Post-mortem Procedures	Mus musculus

Schumaker, Tina

Co-PI ☐ **Key Associate** ☑ **Authorized To Order Animals** ☐

Responsibilities: Daily observation of general health and watch for criteria for euthanasia; drug treatment; intraperitoneal injections

Comments:

Standard Procedures:

Standard Procedure	Type	Species
Breeding	Breeding	Mus musculus
Identification Method-Cage Cards	Animal Identification	Mus musculus
Identification Method-Ear Punches	Animal Identification	Mus musculus
Inoculations-Intraperitoneal	Inoculations	Mus musculus
Oral Dosing-Gavage	Inoculations	Mus musculus

Williams, Bart

Co-PI ☐ **Key Associate** ☑ **Authorized To Order Animals** ☐

Responsibilities: Provide advice on mating procedures and experimental design.

Comments:

Standard Procedures:

Standard Procedure	Type	Species

Chang, Anthony

Co-PI ☐ **Key Associate** ☑ **Authorized To Order Animals** ☐

Responsibilities: Imaging

Comments:

Standard Procedures:

Standard Procedure	Type	Species
Imaging-Ultrasound	Imaging	Mus musculus
Imaging-X-Ray	Imaging	Mus musculus

Huang, Dan

Co-PI ☐ **Key Associate** ☑ **Authorized To Order Animals** ☑

Responsibilities: Set up matings, monitor animals, drug treatment, perform euthanasia, necropsies, assist in imaging; intraperitoneal injections

Comments:

Standard Procedures:

Standard Procedure	Type	Species
Breeding	Breeding	Mus musculus
Post-mortem-Necropsy	Post-mortem Procedures	Mus musculus
Imaging-Ultrasound	Imaging	Mus musculus
Imaging-X-Ray	Imaging	Mus musculus
Inoculations-Intraperitoneal	Inoculations	Mus musculus
Oral Dosing-Gavage	Inoculations	Mus musculus

13 Accounts

Accounts

10301A-4603
10301A-4611
10301A-4613

14 Emergency Phone

Emergency Phone

616.234.5578

15 Internal Funding Information

Is this Protocol funded (either partially or entirely) through internal source(s)?

a ☑ Yes

b ☐ No

16 External Funding Information

Is this protocol funded (either partially or entirely) through external source(s)?

a ☑ Yes

b ☐ No

17 [16a] External Funding Table

Please fill out the External Funding Table.

External Funding Agency	Grant PI	Grant Title	Project Period
Pfizer Global Pharmaceuticals	Bin Teh	Reversal of Sunitinib Resistance in RCC by target	09/01/2010 - 08/31/2011

Rationale For Animal Use

1 Limitations of Mathematical and/or Computer Models

The following statements outline the limitations of mathematical and/or computer models in research. Please read the statements and indicate if they are applicable to your protocol.

Mathematical, computer, and physical models, which have a long tradition of use in the physical sciences and engineering, can complement animal experimentation. The use of computers as research tools in biomedicine has dramatically increased as more biological processes are understood quantitatively and described in mathematical relationships. Although the use of computers alone cannot produce new biological information, they enable scientists to analyze vast amounts of data and test ideas. For example, computer simulations have extended scientists' ability to use three-dimensional visual images to relate structure and biochemical function of molecules. More recently, high performance computing has made it possible to observe phenomena that previously could only be inferred. The development of new imaging technologies, such as ultrasound or nuclear magnetic resonance spectroscopy, has provided a spectrum of noninvasive tools that permits visualization of soft tissues and organs in the intact organism without causing pain or distress. Reference: The Johns Hopkins Center for Alternatives to Animal Testing (CAAT)

Mathematical models and computer simulations rely heavily on predictions based on complete knowledge of a system. Since the interactions involved in complex biological systems and pathways are still largely unknown, mathematical and computer models are extremely limited in their ability represent a complex living system, thus necessitating the use of animal models in biomedical research.

Is this statement applicable to this protocol?

a ☑ Yes

b ☐ No

2 Limitations of Culture Systems

The following statement outlines the limitations of cell, tissue and organ culture systems in research. Please read the statement and indicate if it is applicable to your protocol.

The culture of cells, tissues, and organs of animal and human origin in an environment outside the body, collectively known as in vitro systems, has reached a high level of sophistication and allows scientists to study the effects of substances on cellular events in isolation from other biological phenomena. Such methods often provide reliable data that may be difficult or impossible to obtain in whole animals. The fact that the above tests are conducted in isolated systems, independent of other complex biological systems, creates limitations in their interpretation. In the end, the validity of such tests must be verified by testing in appropriate mammalian model systems and possibly in later human clinical trials. Reference: The Johns Hopkins Center for Alternatives to Animal Testing (CAAT)

Is this statement applicable to this protocol?

a ☑ Yes

b ☐ No

3 Statement of Justification for Animal Use

The following statements outline the justification of the use of mice in research. Please read the statements and indicate if they are applicable to your protocol.

Microorganisms (e.g., yeasts, bacteria), invertebrates, and lower vertebrates are used to provide simple, manageable systems to gain insight into fundamental processes that are relevant to understanding the nature of human diseases and disorders. In particular, the wide array of marine and freshwater invertebrates represent a great potential for biomedical research. These lower organisms are excellent models for the study of certain basic life processes because they permit manipulation and reduce complexity that can obscure understanding of a basic biological process. Although the fundamental knowledge obtained using these diverse species is generally applicable to humans, interspecies transfer of information must be approached with caution and requires validation in higher animals. Reference: The Johns Hopkins Center for Alternatives to Animal Testing (CAAT)

Mice have significant advantages over other mammals as research subjects. Due to their short generation time and prolific breeding, investigators are able to perform studies in a cost-effective, time-efficient manner while using minimal numbers of animals. Due to their small size, they can be easily handled and have relatively minimal housing and care needs. Due to their common use as research subjects, a voluminous body of literature is available encompassing their breeding, housing, development and care. In addition, this widespread use has led to their intensive investigation of all aspects of mice in research (behavioral, genetic, biochemical, etc.) and the development of highly useful cell lines, biochemical products and more. Mice bear their young in utero which mimics human gestation and facilitates studies in early embryonic development. Finally, although mice are low on the phylogenetic scale, the structure and function of genes is very similar between mice and humans. For all these reasons and more, mice are a suitable small animal model for biomedical research.

Are these statements applicable to this protocol?

a ☑ Yes

b ☐ No

4 Duplicative Research?

Is this protocol duplicative of previously reported research?

a ☐ Yes

b ☑ No

5 [4b] Search for Duplication.

Reduction

Federal regulations require assurance from the principal investigator that the work proposed in this research does not unnecessarily duplicate previous studies. You must include what databases were searched, which years were searched, the dates that the search covered, and the keywords used.

Please fill out the table below and attach a copy of the search to this protocol using the attach icon at the beginning of this section.

An excellent resource on this subject is the Animal Alternatives and Welfare: A Search Guide located on the Emory University Woodruff Health Sciences Center Web Site (see link at top of question).

Date of Search	Years Searched	Databases Searched	Key Words Searched
7/28/09	All	Pubmed	Bhd, VHL, double knockout

6 [4b] Duplication Statement.

Please provide a short narrative (few sentences) describing the results of the search. The written narrative should include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine that duplicative studies have not been done. If the proposed studies are similar to those done previously, please indicate how the studies differ.

No related publications found.

7 [4b] Replacement and Refinement Search Table.

Federal regulations require a written description of the methods and sources used to determine that neither alternative non-animal (replacements) nor less painful (refined) animal model methods with which to perform this research.

You must include what databases were searched, which years were searched, the dates that the search covered, and the keywords used. It is suggested that one of the keywords of the search be alternatives and the other words include the species of the animal and painful procedures to be used. For example, alternatives + mouse + acites.

Please fill out the table below and attach a copy of the search to this protocol.

An excellent resource on this subject is theAnimal Alternative and Welfare: A Search Guidelocated on the Emory University Woodruff Health Sciences Center Web Site.

Date of Search	Years Searched	Databases Searched	Key Words Searched
7/28/09	All	Pubmed	Bhd, Rb, double knockout

8 [4b] Replacement and Refinement Statement.

Please provide a short narrative (few sentences) describing the results of the search. The written narrative should include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine the existence of replacement or refinement methods. If an alternative is found that is useful, but not relevant for the particular project, the principal investigator must justify, in writing, why it cannot be used.
No references were found which indicates that this is an appropriate method.

Mouse Procedures (Mus musculus) #1

1 Study Goals and Objectives

Briefly explain the aim of the study and why the study is important...

- to human or animal health
- the advancement of knowledge or
- the good of society.

The response MUST be in language understandable to a layperson.

Birt-Hogg-Dubé syndrome (BHD) is a hereditary cancer syndrome associated with a wide spectrum of diseases, including kidney tumors, skin tumors, colon tumors and lung diseases. We have mapped the BHD gene in chromosome 17 and recently it was identified as a novel kidney cancer gene. BHD has been considered as a tumor suppressor gene. Tumor suppressor genes are genes that protect a cell from one step on the path to cancer. The goal of this project is to exploit our knowledge of the genetic disease, Birt-Hogg-Dubé (BHD) syndrome, to improve the understanding of tumor suppressor genes and their mechanism in causing tumor formation. By knocking out the BHD gene in mouse or specific mouse tissues such as kidney, intestine, or lung, we will increase our knowledge of the BHD gene and its effects on tumor formation in kidney and other organs. These studies will provide insight into the discovery of drug targets that will hopefully lead to better treatment and care for both patients with BHD mutations and other forms of cancer involving the same cancer-causing pathways.

Since tumorigenesis requires more than one genetic change to progress, knockout of one tumor suppressor gene may not guarantee the formation of tumor. Knockout of two or three tumor suppressor genes may be necessary. The VHL gene is another important kidney cancer -related gene associated with VHL syndrome. VHL mutations have been identified in approximately 70% of kidney cancers. However, knockout of VHL alone in the mouse kidney has not led to the formation of kidney tumors, implying that additional genetic mutations in other kidney cancer-related genes may be required. To address this issue, we decided to develop a double gene knockout mouse model involving the BHD and VHL genes. Another gene retinoblastoma (RB) is also an important tumor suppressor gene that is associated with tumor initiation. This gene was the first tumor suppressor cloned and is a negative regulator of the cell cycle. Since mutations in these genes have been identified in kidney cancers, we know that these genes are associated with kidney cancers. Using the kidney-specific combination knockout strategy, we expect that these mice can produce kidney cancer since the disruption of these genes is only restricted to the kidneys. The goal of this project is to exploit our knowledge of the genetic kidney disease and to improve the understanding of tumor suppressor genes and their mechanism in causing tumor formation. Currently, the VHL -, Bhd-, and Rb knockout mice are available in our repository.

Our specific aims in this research proposal are:
1) to determine the functional role of the BHD gene in kidney cancer,
2) to confirm that the BHD gene is required for the development of kidney, intestine and lung cancers and that inactivation of the gene will induce related tumorigenesis, and

3) to determine whether double or triple knockouts could promote tumorigenesis.

First, we will create a BHD conditional knockout mouse strain, also called a BHD-flox strain. This conditional knockout strain should not manifest any phenotype, since the knockout is not completed until the animal is exposed to Cre. Cre is the abbreviation of Cre recombinase which is used as a tool to modify genes and chromosomes. Cre deletes a segment of DNA (Cre sequence) in an experimental animal and has been used to generate animals with mutations limited to certain cell types. Flox refer to a gene that has been marked by flanking two special Cre sequences. The gene marked with Cre sequences still function normally until it is exposed to Cre recombinase. The floxed gene will be disrupted once it is exposed to Cre recombinase that is supposed to cut the gene following the two special Cre sequences flanking it.

Once we have established the conditional knockout, we will breed this knockout to various tissue-specific Cre strains to create kidney-specific, intestine-specific, and lung-specific BHD-deficient knockout mice, to confirm the corresponding tumor formation.

The following proposed studies will provide important new information concerning the functional biology of the BHD gene in mice. As a consequence, it will lead to better understanding of the gene in humans, and facilitate the development of early identification, intervention and therapeutic strategies for related patients.

2 SSB

SSB

C57BL/6

TG or KO from a non-vendor

3 Strain Sources

Strain Sources

Strain Name	Strain Source
Ksp-Cre knock-in mice	Repository
Villin-Cre transgenic mice	Repository
C57BL/6	Vivarium breeding supply
VHL-flox/flox transgenic mice	Repository
RB-flox/flox transgenic mice	Repository
BHD-/VHL-flox mice	Crossing BHD-flox/flox mice with VHL-flox/flox mice
BHD-flox conditional knockout	Crossing the BHD knockout chimeric mice (Dr. Swiatek's protocol 07-07-020) to C57BL/6 mice
Sglt2-Cre transgenic mice	Generated from Dr. Teh's protocol 06-10-028

4 Standard Procedures

Standard Procedures

NAME: Breeding

ANESTHETIC OPTIONS

ASSOCIATES

Name:	**Ooi, Aikseng**
Name:	**Schumaker, Tina**
Name:	**Huang, Dan**

PARALYTIC AGENTS

ASSOCIATED MEDICATIONS

RESTRAINT METHODS

VITAL SIGNS

LOCATION

NAME: Identification Method-Cage Cards

ANESTHETIC OPTIONS

ASSOCIATES

Name:	**Schumaker, Tina**

PARALYTIC AGENTS

ASSOCIATED MEDICATIONS

RESTRAINT METHODS

VITAL SIGNS

LOCATION

NAME: Identification Method-Ear Punches

ANESTHETIC OPTIONS

ASSOCIATES

Name:	**Schumaker, Tina**

PARALYTIC AGENTS

ASSOCIATED MEDICATIONS

RESTRAINT METHODS

VITAL SIGNS

LOCATION

NAME: Post-mortem-Necropsy

ANESTHETIC OPTIONS

ASSOCIATES

Name:	**Ooi, Aikseng**
Name:	**Huang, Dan**

PARALYTIC AGENTS

ASSOCIATED MEDICATIONS

RESTRAINT METHODS

VITAL SIGNS

LOCATION

NAME: Imaging-Ultrasound

ANESTHETIC OPTIONS

Name:	**Isoflurane**
Dose/Route:	inhale 2.5-3 percent in oxygen
Frequency:	once or twice per month
Duration:	15 min

ASSOCIATES

Name:	**Chang, Anthony**
Name:	**Huang, Dan**

PARALYTIC AGENTS

ASSOCIATED MEDICATIONS

RESTRAINT METHODS

VITAL SIGNS

LOCATION

NAME: Imaging-X-Ray

ANESTHETIC OPTIONS

Name:	**Isoflurane**
Dose/Route:	inhale 2.5-3 percent in oxygen
Frequency:	once or twice per month
Duration:	15 min

ASSOCIATES

Name:	**Chang, Anthony**
Name:	**Huang, Dan**

PARALYTIC AGENTS

ASSOCIATED MEDICATIONS

RESTRAINT METHODS

VITAL SIGNS

LOCATION

NAME: Inoculations-Intraperitoneal

ANESTHETIC OPTIONS

ASSOCIATES

Name:	**Schumaker, Tina**
Name:	**Huang, Dan**

PARALYTIC AGENTS

ASSOCIATED MEDICATIONS

Name:	**Chemical - Rapamycin**
Dose/Route:	100 ul volume using 1 cc syringe and 27 gauge needle
Frequency:	
Duration:	10 months

RESTRAINT METHODS

VITAL SIGNS

LOCATION

NAME: Oral Dosing-Gavage

ANESTHETIC OPTIONS

ASSOCIATES

Name: **Schumaker, Tina**

Name: **Huang, Dan**

PARALYTIC AGENTS

ASSOCIATED MEDICATIONS

RESTRAINT METHODS

VITAL SIGNS

LOCATION

5 Investigator Procedure

Investigator Procedure

6 Authorized Amounts Per Pain/Distress Category

Authorized Amounts Per Pain/Distress Category

Stress Level	Requested	On Order	Received	Available
Category 1	3202	0	511	2691

Strains:

	Requested	On Order	Received	Available
Totals	3202	0	511	2691

7 Euthanasia Methods

Euthanasia Methods

CO2 Asphyxiation

☑ **Primary** ☐ **Secondary**

Comments:

Animal Disposition

1 Animal Disposition

Any animals found dead during husbandry care will be disposed of as stated on the Animal Room Data Sheet posted on each animal room door. In addition, the appropriate personnel, as stated on the Animal Room Data Sheet, will be notified by email or by phone. If carcasses are to be stored for disposal, they will be bagged, stapled, dated, and placed in a -20 ºC freezer designated for carcass storage.

Following euthanasia and post-mortem studies, all disposable animal material will be bagged and stapled.

Animal carcasses are placed in clear plastic bags and stored in a -20 degree Celsius freezer designated for animal carcasses. The storage freezer is clearly labeled with biohazard signs. Every week, carcasses are collected in biohazard bags which are then placed into biohazard corrugated cardboard boxes. These boxes are then picked up by a professional medical waste vendor (Stericycle) for incineration.

Do you require any special animal disposition procedures?

a ☐ Yes

b ☑ No

Experimental Design

1 Experimental Group

In order to effectively fill in the Experimental Design section you must first fill in :

a. The Protocol Associates Section
b. The Standard and Investigator Designed Procedures
c. The Authorized Amounts per Pain/Distress Category

:	Experiment #1
Number of Animals::	718
Species:	Mus musculus
Maximum Stress Level:	Category 1
Justification:	Previously, in Dr. Teh's protocol, 06-10-028, we generated BHD flox-heterozygous mice. We will cross these mice to Ksp-Cre to generate tissue-specific knockout mice. This cross will take place using BHD-Flox mice which have already been crossed to FLPeR-frt mice in order to remove the neomycin cassette.Experiment #1, Breeding Step #1A- Breeding to get BHD Heterozygous & BHD wild-type:Twelve BHD-flox/+ mice (obtained from Experiment #1) will be bred twice to 12 Cre tg/tg mice (obtained from the Repository).These will be set up as paired matings over time.Given an approximate litter of 10 pups, that should produce 240 offspring in the F1 generation.Of these 240: 50%of the total, or roughly 120 pups, will be double heterozygous (BHD flox/+; Cre tg/+).50%of the total, or roughly 120 pups, will be wildtype/heterozygous (BHD +/+; Cre tg/+)Total mice used and produced in Breeding Step #1A = 252Experiment #1, Breeding Step # 1B-Breeding to Get BHD Homozygous:20 BHD flox/+; Cre tg/+ mice (obtained from Breeding Step #2A) will be bred twice to 20 BHD flox/flox; Cre +/+ mice (obtained from Dr. Teh's protocol 06-10-028). These will be set up as paired mating over time.Given an approximate litter of 10 pups, that should produce 400 offspring in the F2 generation.Of these 400: 25% of the total, or roughly 100 pups, will be BHD homozygous; Cre heterozygous (BHD flox/flox; Cre tg/+).25% of the total, or roughly 100 pups, will be double heterozygous (BHD flox/+; Cre tg/+).Total mice used and produced in Breeding Step #1B = 400Totals for Experiment #1:Breeding Step 1A: 252Breeding Step 1B: 40010% increase for death,error, etc. 66Total for Experiment #1: 718
Standard Procedures:	**Breeding** **Identification Method-Cage Cards** **Identification Method-Ear Punches** **Post-mortem-Necropsy** **Breeding** **Identification Method-Cage Cards** **Identification Method-Ear Punches** **Post-mortem-Necropsy** **Breeding** **Identification Method-Cage Cards** **Identification Method-Ear Punches** **Post-mortem-Necropsy** **Breeding**

Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy

Sequence And Timing:

In this experiment, BHD-flox strain will be crossed with Ksp-Cre mice to give rise to kidney-specific BHD knockout mice. Kidney cancer is one of the important features of BHD syndrome, and Ksp-Cre mice expres Cre in the proximal tubular, the distal tubular, and the collecting duct cells of the kidney. So it is logical to try to further assess the role of BHD in kidneys.We will use the mouse tails to extract DNA for genotyping. The PCR technique will be used to amplified the genotype-specific DNA sequences and the genotypes will be figured out by the specific PCR bands.The mice will be bred and then euthanized by CO2 at the various timepoints (3 months old, 6 months old, 9 months old, 12 months old, 15 months old, 18 months old, 21 months old, and 24 months old). If any animal presents criteria for euthanasia as cited in Vivarium SOP #6.031 – Euthanasia of Mice, they will be euthanized at that time. We expect that the heterozygous and homozygous animals may develop tumors and kidney disease. Live-born mice will be checked for kidney malformations as well as for physiological or behavioral defects such as weakness, seizures etc. In addition, anatomical and histological analyses will be performed. The animals will be necropsied after euthanasia. Most of the organs (kidneys, lungs, liver, spleen, intestines will be examined for abnormalities. The tissues will be fixed in 4% paraformaldehyde or frozen in -80C freezer for DNA/RNA/Protein analysis.This experiment does not include any imaging or blood collection.

:	Experiment #2
Number of Animals::	718
Species:	Mus musculus
Maximum Stress Level:	Category 1
Justification:	This experiment involves crossing the BHD-Flox strain to Sglt2-Cre to generate tissue-specific knockout mice. This cross will take place using BHD-Flox mice which have already been crossed to FLPeR-frt mice in order to remove the neomycin cassette (these mice are the result of Dr. Teh's protocol 06-10-028).Experiment #2 uses the same number of animals and the same breeding scheme as Experiment #1.

Standard Procedures:

Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches

Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy

Sequence And Timing:

In this experiment, we will further cross the BHD-flox mice with Sglt2-cre mice, which also express Cre kidney cells, but in the proximal tubular cells only. The human disease RCC (Renal Clear Carcinoma) is believed to derive from the proximal tubular cells of the kidney, but there is currently no compelling evidence of this. Since it is not clear that kidney cancer comes from which type of kidney cells, it is necessary to use different kidney Cre-expressing strains.We will use the mouse tails to extract DNA for genotyping. The PCR technique will be used to amplified the genotype-specific DNA sequences and the genotypes will be figured out by the specific PCR bands.The mice will be bred and then euthanized by CO2 at the various timepoints (3 months old, 6 months old, 9 months old, 12 months old, 15 months old, 18 months old, 21 months old, and 24 months old). If any animal presents criteria for euthanasia as cited in Vivarium SOP #6.031 – Euthanasia of Mice, they will be euthanized at that time. We expect that the heterozygous and homozygous animals may develop tumors and kidney disease. Live-born mice will be checked for kidney malformations as well as for physiological or behavioral defects such as weakness, seizures etc. In addition, anatomical and histological analyses will be performed.The animals will be necropsied after euthanasia. Most of the organs (kidneys, lungs, liver, spleen, intestines will be examined for abnormalities. The tissues will be fixed in 4% paraformaldehyde or frozen in -80C freezer for DNA/RNA/Protein analysis.This experiment does not include any imaging or blood collection.

:	Experiment #3
Number of Animals::	718
Species:	Mus musculus
Maximum Stress Level:	Category 1
Justification:	This experiment involves crossing the BHD-Flox strain to Villin-Cre to generate tissue-specific knockout mice. This cross will take place using BHD-Flox mice which have already been crossed to FLPeR-frt mice in order to remove the neomycin cassette (these mice are the result of Dr. Teh's protocol 06-10-028).Experiment #2 uses the same number of animals and the same breeding scheme as Experiment #1.

Standard Procedures:

Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches

Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy

Sequence And Timing:

In patients with confirmed deletion of BHD in the intestine, there is an increased level of colon cancer. To confirm whether BHD deletion in intestine will cause colon cancer, we will cross BHD-flox strain with Villin-cre mice, which express Cre in the intestine.We will use the mouse tails to extract DNA for genotyping. The PCR technique will be used to amplified the genotype-specific DNA sequences and the genotypes will be figured out by the specific PCR bands.The mice will be bred and then euthanized by CO2 at the various timepoints (3 months old, 6 months old, 9 months old, 12 months old, 15 months old, 18 months old, 21 months old, and 24 months old). If any animal presents criteria for euthanasia as cited in Vivarium SOP #6.031 – Euthanasia of Mice, they will be euthanized at that time. We expect that the heterozygous and homozygous animals may develop tumors and colon cancer or related intestinal diseases. Live-born mice will be checked for intestinal malformations as well as for physiological or behavioral defects such as weakness, seizures etc. In addition, anatomical and histological analyses will be performed.The animals will be necropsied after euthanasia. Most of the organs (kidneys, lungs, liver, spleen, intestines will be examined for abnormalities. The tissues will be fixed in 4% paraformaldehyde or frozen in -80C freezer for DNA/RNA/Protein analysis.This experiment does not include any imaging or blood collection.

:	Experiment #4
Number of Animals::	524
Species:	Mus musculus
Maximum Stress Level:	Category 1
Justification:	In this experiment, we will cross the BHD-flox/flox mice (from Dr. Teh's protocol 06-10-028) with VHL-flox/flox mice (from the Repository) to generate double BHD-/VHL-flox heterozygous mice. Heterozygous BHD-flox/+; VHL-flox/+ mice will be intercrossed to produce double homozygous BHD-flox/flox;VHL-flox/flox mice. BHD-flox/flox;VHL-flox/flox mice will be further bred to CMV-Cre mice (from the Repository) to produce BHD-flox/+;VHL-flox/+;CMV-Cre heterozygous knockout mice.We need to produce around 100 BHD-flox/+; VHL-flox/+;CMV-Cre mice as a result of Experiment #4.Experiment #4, Breeding Step #4A-Breeding the BHD-flox/flox and VHL-flox/flox to produce BHD-flox/+,VHL-flox+ heterozygous:3 BHD-flox/flox mice (male or female, obtained from Dr. Teh's protocol 06-10-028) will be bred to 3 VHL-flox/flox (male or female, obtained from VAI repository), bred once to produce 30 offspring. These will be set up as paired matings over time.Of these 30: 100% of the total, or 30 pups, will be BHD-flox/+;VHL-flox/+ mice. We need 20 BHD-flox/+;VHL-flox/+ mice for experiment#4B. Total mice used and produced in Breeding Step #4A = 36Experiment #4, Breeding Step #B-Intercrossing the BHD-flox/+;VHL-flox/+ to produce BHD-flox/flox;VHL-flox/flox:10 BHD-flox/+,VHL-flox/+ males will be bred to 10 BHD-flox/+;VHL-flox/+ females from Breeding Step #4A, bred twice to produce 200 offspring. These will be set up as paired matings over time.Of these 200:6% of the total, or roughly 12 pups, will be double homozygous (BHD flox/flox; VHL- flox/flox)Total mice used and produced in Breeding Step #4B = 220Experiment #4, Breeding Step #C-Breeding to Get BHD-flox/+;VHL-flox/+;CMV-Cre Heterozygotes:10 double homozygous (BHD flox/flox; VHL- flox/flox) mice from Breeding Step #4B will be bred to 10 CMV-Cre mice from VAI repository, bred twice to produce 200 offspring. These will be set up as paired mating over time.Please note that we suppose all the 10 CMV-Cre mice are heterozygotes. Therefore, the offspring of this Breeding Step #4C, 50% will be BHD-flox/flox;VHL-flox/flox;CMV-Cre mice.Of these 200: 50% of the total, or roughly 100 pups, will be BHD-flox/flox; VHL-flox/flox;CMV-Cre mice.Total mice used and produced in Breeding Step #4C = 220Totals for Experiment #4Breeding Step 4A: 36Breeding Step 4B: 220 Breeding Step 4C: 22010% increase for death,error, etc. 48Total for Experiment #4: 524
Standard Procedures:	**Breeding** **Identification Method-Cage Cards** **Identification Method-Ear Punches** **Post-mortem-Necropsy** **Breeding**

Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy
Breeding
Identification Method-Cage Cards
Identification Method-Ear Punches
Post-mortem-Necropsy

Sequence And Timing:

Previous studies showed that knockout of VHL or BHD alone in mice has not developed kidney tumors (except for kidney cysts), indicating two or more genetic changes may be required for kidney tumorigenesis. In this experiment, we are going to double knockout the kidney cancer-related genes in mice to see whether tumors will form in the kidney. We already have created BHD-flox/flox mice from Dr. Teh's protocol 06-10-028 BT and VHL-flox/flox mice are available in our VAI repository.First, the BHD-flox/flox mice will breed to VHL-flox/flox mice to generate heterozygous BHD-flox/+;VHL-flox/+ mice. The generated BHD-flox/+;VHL-flox/+ mice will subject to intercross to produce homozygous BHD-flox/flox;VHL-flox/flox mice. Then, the homozygous BHD-flox/flox;VHL-flox/flox mice will breed to CMV-Cre

lethal, double homozygous knockout mice are not expected in this experiment.We will use the mouse tails to extract DNA for genotyping. The PCR technique will be used to amplified the genotype-specific DNA sequences and the genotypes will be figured out by the specific PCR bands.The mice will be bred and then euthanized by CO2 at the various time points (3 months old, 6 months old, 9 months old, 12 months old, 15 months old, 18 months old, 21 months old, and 24 months old). If any animal presents criteria for euthanasia as cited in Vivarium SOP #6.031 – Euthanasia of Mice, they will be euthanized at that time. We expect that the heterozygous and homozygous animals may develop kidney tumors and colon cancer or related intestinal diseases.
Live-born mice will be checked for intestinal malformations as well as for physiological or behavioral defects such as weakness, seizures etc. In addition, anatomical and histological analyses will be performed.The animals will be necropsied after euthanasia. Most of the organs (kidneys, lungs, liver, spleen, intestines will be examined for abnormalities. The tissues will be fixed in 4% paraformaldehyde or frozen in -80C freezer for DNA/RNA/Protein analysis.

This experiment does not include any imaging or blood collection.

:	Experiment #5
Number of Animals::	524
Species:	Mus musculus
Maximum Stress Level:	Category 1
Justification:	This experiment involves crossing the mouse strain RB-flox/flox to VHL-flox/flox to generate a RB-flox/flox;VHL-flox/flox mouse strain. This cross will take place using RB-flox/flox mice and VHL-flox/flox mice that are available in our repository. We need to produce around 100 RB-flox/+;VHL-flox/+; CMV-Cre mice as a result of Experiment #8.Experiment #5, Breeding Step # 5A-Breeding the RB-flox/flox and VHL-flox/flox to produce RB-flox/+; VHL-flox/+ heterozygous:3 RB-flox/flox mice (male or female, obtained from VAI repository) will be bred to 3 VHL-flox/flox (male or female, obtained from VAI repository), bred once to produce 30 offspring. These will be set up as paired matings over time.Of these 30: 100% of the total, or 30 pups, will be RB-flox/+;VHL-flox/+ mice. We need 20 RB-flox/+;VHL-flox/+ mice for experiment#5B. Total mice used and produced in Breeding Step #5A = 36Experiment #5, Breeding Step #5B-Intercrossing the RB-flox/+;VHL-flox/+ to prduce RB-flox/flox;VHLflox/flox:10 RB-flox/+;VHL-flox/+ males will be bred to 10 RB-flox/+;VHL-flox/+ females from Breeding Step #5A, bred twice to produce 200 offspring. These will be set up as paired matings over time.Of these 200:6% of the total, or roughly 12 pups, will be double homozygous (RB- flox/flox; VHL- flox/flox)Total mice used and produced in Breeding Step #5B = 220Experiment #5, Breeding Step #5C-Breeding to Get RB-flox/+; VHL-flox/+;CMV-Cre Heterozygotes:10 double double homozygous (RB-flox/flox; VHL- flox/flox) mice from Breeding Step #8B will be bred to 10 CMV-Cre mice from VAI repository, bred twice to produce 200 offspring. These will be set up as paired mating over time.Please note that we suppose all the 10 CMV-Cre mice are heterozygotes. Therefore, the offspring of this Breeding Step #5C, 50% will be RB-flox/flox;VHL-flox/flox;CMV-cre mice.Of these 200: 50% of the total, or roughly 100 pups, will be RB-flox/flox; VHL-flox/flox;CMV-Cre mice.Total mice used and produced in Breeding Step #5C = 220Totals for Experiment #5Breeding Step 5A: 36Breeding Step 5B: 220 Breeding Step 5C: 22010% increase for death,error, etc. 48 Total for Experiment #5: 524
Standard Procedures:	**Identification Method-Cage Cards** **Identification Method-Ear Punches** **Breeding** **Post-mortem-Necropsy** **Identification Method-Cage Cards** **Identification Method-Ear Punches**

Breeding
Post-mortem-Necropsy
Identification Method-Cage Cards
Identification Method-Ear Punches
Breeding
Post-mortem-Necropsy
Identification Method-Cage Cards
Identification Method-Ear Punches
Breeding
Post-mortem-Necropsy
Identification Method-Cage Cards
Identification Method-Ear Punches
Breeding
Post-mortem-Necropsy
Identification Method-Cage Cards
Identification Method-Ear Punches
Breeding
Post-mortem-Necropsy
Identification Method-Cage Cards
Identification Method-Ear Punches
Breeding
Post-mortem-Necropsy
Identification Method-Cage Cards
Identification Method-Ear Punches
Breeding
Post-mortem-Necropsy

Sequence And Timing:

The RB gene is another important tumor suppressor gene. Unpublished data indicated that the RB gene is associated with kidney tumorigenesis. As mentioned in Experiment #4, two or more genetic changes may be required for kidney tumorigenesis. In this experiment, we are going to double knockout the kidney cancer-related genes in mice to see whether tumors will form in the kidney. We already have created RB-flox/flox mice and VHL-flox/flox mice are available in our VAI repository.First, the RB-flox/flox mice will breed to VHL-flox/flox mice to generate heterozygous RB-flox/+;VHL-flox/+ mice. The generated RB-flox/+;VHL-flox/+ mice will subject to intercross to produce homozygous RB-flox/flox;VHL-flox/flox mice. Then the homozygous RB-flox/flox;VHL-flox/flox mice will breed to CMV-Cre mice (available in VAI repository) to give rise to heterozygous RB-+/-;VHL-+/-, CMV-Cre mice. Since homozygous knockout mice are embryonic lethal, double homozygous

	genotype-specific DNA sequences and the genotypes will be figured out by the specific PCR bands.The mice will be bred and then euthanized by CO2 at the various time points (3 months old, 6 months old, 9 months old, 12 months old, 15 months old, 18 months old, 21 months old, and 24 months old). If any animal presents criteria for euthanasia as cited in Vivarium SOP #6.031 – Euthanasia of Mice, they will be euthanized at that time. We expect that the heterozygous and homozygous animals may develop kidney tumors and colon cancer or related intestinal diseases. Live-born mice will be checked for intestinal malformations as well as for physiological or behavioral defects such as weakness, seizures etc. In addition, anatomical and histological analyses will be performed.The animals will be necropsied after euthanasia. Most of the organs (kidneys, lungs, liver, spleen, intestines will be examined for abnormalities. The tissues will be fixed in 4% paraformaldehyde or frozen in -80C freezer for DNA/RNA/Protein analysis.This experiment does not include any imaging or blood collection.
:	Experiment #6 Pilot study
Number of Animals::	20
Species:	Mus musculus
Maximum Stress Level:	Category 1
Justification:	20 homozygous mice produced in Experiment 2 will be used in this study ranging from 2 month of age to 12 month of age. 2 mice from each age group (age 2, 4, 6, 8, 10, and 12 month) will be used in the first round of imaging. Based on results, imaging for certain age group may be repeated once to verify findings. Therefore, we need a total of 20 mice in this pilot study.
Standard Procedures:	**Breeding** **Identification Method-Cage Cards** **Identification Method-Ear Punches** **Post-mortem-Necropsy** **Imaging-Ultrasound** **Imaging-X-Ray**
Sequence And Timing:	Homozygous mice produced in Experiment 2 will be selected based on birth date to meet the age group outlined in previous section.Imaging prodedures will be performed according to vivarium SOPs. Imaging will be performed before, during and after treatment to examine drug effect. The imaging procedures can quantify the size of kidney cyst and tumor.After imaging the mice will be sacrificed by CO2 euthanasia.

:	Experiment #7 Drug treatment
Number of Animals::	50
Species:	Mus musculus
Maximum Stress Level:	Category 1
Justification:	50 homozygous female mice produced in Experiment 2 will be used for this drug study. They will be randomly divided into 5 groups (10 mice each group). 10 mice per group was selected for statistical reasons.
Standard Procedures:	**Breeding** **Identification Method-Cage Cards** **Identification Method-Ear Punches** **Post-mortem-Necropsy** **Imaging-Ultrasound** **Imaging-X-Ray** **Inoculations-Intraperitoneal** **Oral Dosing-Gavage**
Sequence And Timing:	One group will receive vehicle control treatment; the second group will receive 10 mg/kg rapamycin treatment; the third group will receive 50 mg/kg rapamycin treatment; the fourth group will receive 50 mg/kg MK-886 treatment; the fifth group will receive 10 mg/kg rapamycin plus 50 mg/kg MK-886 treatment. Treatment will begin when the mice are at 2 months of age. Rapamycin will be prepared in ethanol at 50 mg/ml as a stock solution and then formulated in a water solution containing 5% PEG-300, 4% ethanol, and 5% Tween 80 (Nogueira de Francishchi J et al., Br J Pharmacol, 1993). The administration of rapamycin will be intraperitoneal injection every other day in a 100 ul volume using a 1cc syringe and 27 gauge needles. MK-886 will be prepared in a water solution of 0.5% carboxymethylcellulose (CMC). The administration of MK-886 will be oral gavage every other day in a 200 ul volume (Biserni A et al., Mol Pharmacol, 2008). We will image the mice 1-2 times per month to monitor the formation of kidney cyst and kidney tumor and drug response. The imaging procedure can quantify the size of kidney cyst and tumor. Imaging will be performed before, during and after drug treatment. Since the BHD tumors typically form at 10-12 month of age, we will start treating the mice at age 2 month and continue treatment for 8-10 months. If the imaging procedure shows promising results, we may terminate the treatment earlier. Monitoring will be through daily observation of all the animals by Vivarium caretaker staff. They will observe the animals' general health and watch for criteria for euthanasia. In addition, we will record bodyweights for all the animals on a weekly basis. If any animal develops any other criteria, other than tumor size limit, for euthanasia as specified in the Vivarium SOP #6.031 - Euthanasia of Mice, we will euthanize the animal. By the end of drug treatment, all of the animals (with tumors or not) will be sacrificed by CO2. Following euthanasia, the primary and secondary tumors

will be analyzed by microarray and flow cytometry. Half of the tumor tissue will be formalin fixed/paraffin embedded and immunohistochemical staining will be performed. The remaining tissue will be frozen in liquid nitrogen.

Potential Stressors

1 Potential Stressors

Will the animals be exposed to potential stressors such as food or water deprivation, noxious stimuli, environmental changes, prolonged restraint, paralytic agents or other stressors?

a ☐ Yes

b ☑ No

Special Considerations for Animal Care

1 Use of Genetically Engineered Mice

Does this protocol require the generation or use of genetically engineered mice?

a ☑ Yes

b ☐ No

2 [1a] Expected Phenotypic Consequences

Are there expected phenotype consequences or any special care and monitoring required for these animals?

a ☑ Yes

b ☐ No

3 [2a] Explain Consequences and Special Care

Please explain.

We expect that these animals may have kidney tumors, colorectal cancer, and lung cysts. If mice present criteria for euthanasia, we will euthanize the animal.

4 [1a] Special Considerations

Does this protocol call for any special considerations such as special housing, equipment, or animal care?

a ☐ Yes

b ☑ No

Agents - Hazardous

1 Hazardous Agents

Hazardous Agents

2 Agents - User Named

Agents - User Named

Agent Name: **MK-886**
Agent Type:
Hazardous ? ☑
Description: PPARa inhibitor from Tocris Bioscience. See attached MSDS.

Agent Name: **Rapamycin**
Agent Type:
Hazardous ? ☑
Description: mTOR inhibitor from LC Laboratories. See attached MSDS.

3 Add User Named Agent to Master List

Would you like your user named agents to be added to the master list?

a ☐ Yes

b ☑ No

4 Pathogen Report

Do you agree to submit a copy (paper or electronic) of each pathogen report to the IACUC office prior to initiating the work?

a ☑ Yes

b ☐ No

5 Human Derived Agents, Tissues or Cell Lines

Will you be using human derived agents, tissues, or cell lines?

a ☐ Yes

b ☑ No

6 Procedure Agent

Procedure Agent

Name	Agent Type	Hazardous ?	Description
Isoflurane	Chemical-Anesthesia	☑	
Chemical - Rapamycin	Chemical	☐	

Veterinary Care

1 Veterinary Care

Please read this information regarding Van Andel Institute's veterinary animal care.

The institution has a contract veterinarian and a backup, on call, veterinarian.

Joan Koelzer, DVM (Contract Veterinarian)
Joan Koelzer, D.V.M., is the contract Veterinarian. The contract Veterinarian assists in the semiannual animal facility inspections using the procedures and recommendations set forth in the Guide. As a voting member of the IACUC, the contract Veterinarian also attends IACUC meetings, reviews all animal protocols, and participates in the semiannual review of the Animal Care and Use Program. If the animal care program does not meet the requirements, she recommends to the IO, the Vivarium Director, and the IACUC Chairperson (Chair) the procedures which must be followed for the program to achieve compliance. The Veterinarian has the authority to suspend any animal protocols that do not comply with the Guide or the Animal Welfare Act. In addition to these duties, the Veterinarian is available for consultation regarding animal health. Dr. Koelzer can be contacted by cell phone or e-mail at joan.koelzer@vai.org.

Diane Egedy, DVM (On-Call veterinarian)
Diane Egedy, D.V.M., is available for emergency on-call care only when the contract Veterinarian is not available. Dr. Egedy is a practicing veterinarian at the Cascade Animal Clinic, located within 10 miles of the Institution.

2 Veterinary Role

The veterinarian's institutional role is to plan, oversee and advise:

- Disease detection and surveillance, prevention, diagnosis, treatment, and resolution
- Handling and restraint
- Anesthetics, analgesics and tranquilizer drugs
- Methods of euthanasia
- Surgical and postsurgical care
- Promotion and monitoring of animal's physical and psychological well-being
- Oversees adequacy of the husbandry program
- Involved in the review and approval of all animal care and use, e.g., via a role on the IACUC
- Training of institutional staff in the care and use of laboratory animals
- Assists in establishment and/or monitoring of occupational health and safety program
- Monitors for zoonotic diseases
- Advises on and monitors biohazard control policies and procedures relevant to the animal care and use program

Do you have a question? Click **here** to post.
Protocol FAQ's - Click **here** for frequently asked questions.

Transportation

1 Procedure Location Summary

Procedure Location Summary

Species	Standard	Location	Pre-Location	Post-Location	Duration

2 Transport to External Location

Will you be transporting animals to an external location?

a ☐ Yes

b ☑ No

3 Following the Transportation SOP?

For animal transportation will you perform the transportation according to the transportation SOP?

a ☑ Yes

b ☐ No

AAALAC Procedures

1 AAALAC Procedures

Please select all procedures that are being used on this protocol.

- Animal Identification
- Breeding
- Post-mortem Procedures

PI Certifications **PI Use Only

1 Required Certifications Checklist

I certify that:

a ☑ I will follow "best practices" for animal care and use in accordance with the mission of the IACUC.

b ☑ I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.

c ☑ there are neither non-animal nor less painful/distressful animal models/methods with which to perform this research.

d ☑ for any Pain and Distress Category 3 procedures, I have found no valid alternatives to procedures described herein which may cause more than momentary pain or distress, whether relieved or not.

e ☑ I have read and I understand all SOPs and IACUC guidelines related to submitting this protocol.

f ☑ the individuals listed as either authors or associates on this protocol are authorized to conduct the procedures involving animals as noted on the Indication of Roles Form.

g ☑ all individuals using this protocol will be properly trained in the care and use, safety, manipulations, humane treatment, and euthanasia methods for the species specified in the protocol.

h ☑ I have attended the institutionally required investigator training and/or refresher training.

i ☑ all associates listed on this protocol have attended all the appropriate training or refresher training.

j ☑ all individuals working on this proposal who are at risk are participating in the Institution's Occupational Health and Safety Program.

k ☑ I will obtain approval from the IACUC before initiating any significant changes in this study.

l ☑ this protocol, once approved, will only be referenced in grant applications in which this protocol is being used.

m ☑ I will notify the IAUCC regarding any unexpected study results that impact the animals and that any unanticipated pain or distress, morbidity or mortality will be reported to the attending veterinarian and the IACUC.

n ☑ I will, to the best of my ability, comply with all pertinent institutional, state, and federal guidelines, rules, policies and recommendations regarding animal care and use.

2 Certification Statements - Check All?

Did you check all the Certification Statements?

a ☑ Yes

b ☐ No