Van Andel Research Institute
Animal Study Protocol

Leave Blank	
Protocol #:	**03-09-015**
Approval Date:	**September 29, 2003**
Expiration Date:	**September 29, 2006**

PLEASE TYPE

A. ADMINISTRATIVE DATA

Laboratory: Laboratory of Cancer Genetics

Principal Investigator: Bin Tean Teh, M.D., Ph.D.

Mailing Address: Van Andel Research Institute, 333 Bostwick NE, Grand Rapids, MI-49503

Telephone: 1-616-234-5296 Fax: 1-616-234-5297 Email: bin.teh@vai.org

Protocol Title: *Functional Analysis of BHD Using Knockout Strategy*

Initial Submission ☒ Renewal ☐ or Modification ☐

List the names of all individuals authorized to conduct procedures involving animals under this protocol and identify key personnel (e.g., co-investigator(s)), providing their laboratory, telephone, fax, and email:

Name	**Dept/Affiliation**	**Phone**	**Fax**	**Email**
Bin Tean Teh	*Lab of Cancer Genetics*	*616.234.5296*	*616.234.5297*	*bin.teh@vai.org*
Jindong Chen	*Lab of Cancer Genetics*	*616.234.5578*	*616.234.5579*	*jindong.chen@vai.org*
Sok Kean Khoo	*Lab of Cancer Genetics*	*616.234.5536*	*616.234.5537*	*sok-kean.khoo@vai.org*
Bart Williams	*Cell Signaling and Carcinogenesis*	*616.234.5308*	*616.234.5309*	*bart.williams@vai.org*

☒ Internally supported research

☒ Funding source: *National Cancer Center*

Title of grant application: *Functional analysis of the Birt-Hogg Dube gene (BHD), a novel kidney cancer gene, in renal cell carcinoma (RCC), and in kidney tumorigenesis and development*

Submission deadline or dates of funding: *7/1/03 – 6/30/04*
(with a one-year renewal available)

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B. ANIMAL REQUIREMENTS

1. Species: *Mus musculus*
2. Strain or subspecies: C57BL/6J, TG or KO from a non-vendor
3. Approximate age, weight, or size: embryo to adult
4. Sex: Both male and female
5. Source (name of outside vendor or supplier):
   (NOTE: Animal order will not be processed without an approved protocol number on the order.)
   Vivarium breeding stock, Charles River, and Jackson Labs.
6. Average daily census: 235
7. Primary housing location(s):
   (NOTE: Facility manager must certify below that facility has the resource capability to support the study. If animals will be housed in a lab or anywhere else outside the central facility for more than 12 hours, provide building and room number.)
   Vivarium SPF barrier facility
8. Location(s) where manipulation will be conducted:
   (NOTE: Animals cannot be removed from the animal facility and then returned. Animals cannot be taken to the 4th floor laboratory area and kept overnight without the Vivarium Director's approval.)
   Vivarium
9. Number of animals to be used:
   Year 1: 897 Year 2: 692 Year 3: 0
   Total for the duration of entire study: 1589

C. TRANSPORTATION

Transportation of animals must conform to all institutional guidelines/policies and federal regulations. If animals will be transported on public roads or out of state, describe efforts to comply with USDA regulations. If animals will be transported between facilities, describe the methods and containment to be utilized. If animals will be transported within a facility, include the route and elevator(s) to be utilized.

It may be necessary, on a few occasions, to transfer live animals to the 4th or 5th floor for experimental purposes. These animals will be transported from the Vivarium in cages with filter tops on carts. The cages will be carried through the cagewash area, passed through the hall door and placed on a cart that has been placed outside the facility in the corridor. The mice will be transported to the 4th or 5th floor in the freight elevator. These mice will not be returned to the facility and will be euthanized within 12 hours of leaving the facility. The cages will be returned to the dirty cagewash area for cleaning and autoclaving.

D. STUDY OBJECTIVES

Briefly explain in language **understandable to a layperson** 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.

Birt-Hogg-Dubésyndrome (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. 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 knockouting BHD gene in mouse or specific mouse tissues such as kidney, intestine, lung will increase our knowledge of the BHD gene and its effects on tumor formation in kidney and other organs, and other biological processes. These studies will provide insight into 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.

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

In aim #1, we will create *BHD*-deficient (conventional knockout) mice, study their phenotype and the expected kidney tumors.

In aim #2, we will create kidney-specific, intestine-specific, and lung-specific BHD-deficient (knockout) mice, to confirm the corresponding tumor formation.

The 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.

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E. RATIONALE FOR ANIMAL USE
(Use additional sheets if necessary.)

1. Explain your rationale for animal use.
   (NOTE: The rationale should include reasons why non-animal models cannot be used.)

   No in vitro model can replicate the complex processes of tumorigenesis in living animals. This tumor model provides an excellent model system for deriving information that is directly applicable to our understanding of tumor formation in all animals, including man.

2. Justify the appropriateness of the species selected.
   (NOTE: The species selected should be the lowest possible on the phylogenetic scale.)

   The use of non-mammals has significant limitations in research. Although many different non-mammalian species (such as frogs, squid, zebrafish, and birds) can model a specific component of a system, there is not one single non-mammalian species that models a complete mammalian system accurately and reliably. In addition, there are many mammalian diseases for which models in non-mammals do not exist.

   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 & care. In addition, this widespread use has led to the 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 on 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 the best small animal model for human disease.

3. Justify the number of animals to be used.
   (NOTE: The number of animals should be the minimum number required to obtain statistically valid results.)

The mouse strains needed for these experiments:

The CMV-Cre transgenic mouse will be obtained from the Repository.

The ERTM-Cre transgenic mouse will be obtained from the Repository.

The Ksp-Cre knock-in mouse will be obtained from the Repository.

The Sglt2-Cre transgenic mouse will be obtained from the Repository.

The Villin-Cre transgenic mouse will be obtained from the Repository.

The C57BL/6 mice will be obtained from the Vivarium breeding supply.

The FLPeR mice will be obtained from the Repository.

The BHD-flox (KOH(R4R3-BHD)046BT4A3) conditional knockout mice will be obtained by crossing BHD knockout chimeric mice to C57BL/6 mice.

Experiment #1:

We will first cross the BHD-flox strain with the CMV-Cre strain. This experiment will create *BHD* knockout mice to allow for the phenotypic observation of both homozygous and heterozygous *BHD* knockouts. We expect the BHD-deficient mice will develop BHD disease similar to human's. Since homozygous deletion of BHD will lead to embryonic lethal, we will do the following conditional knockout experiments in related organs/tissues.

Experiment #2:

Since mating BHD-flox with CMV-cre strain will result in homologous embryonic lethal, we will cross BHD-flox mice with ERTM-Cre strain, which is responsive to the application of tamoxifen, a synthetic estrogen. Unlike CMV-Cre strain, ERTM-Cre mice will not express Cre until expose to tamoxifen. This will allow us to test and control freely systemic response to BHD elimination at different life stage. However, this experiment is not suitable for production of heterozygous BHD mice like Experiment #1. So both experiment #1 and experiment #2 are both necessary.

Experiment #3:

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 feature of BHD syndrome. So it is logical to try to further assess the role of BHD in kidneys.

Experiment #4:

In this experiment, we will further cross the BHD-flox mice with Sglt2-cre mice, which express Cre in another type of kidney cells. Since it is not clear that kidney cancer come from which type of kidney cells, it is necessary to use different kidney Cre-expressing strains.

Experiment #5:

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 intestine.

Experiment #6:

Since the BHD-construct contains neomycin antibiotic gene that may lead to unknown background, we will cross the BHD-flox chimeric mice to FLPeR mice to remove the neomycin gene for reducing some unnecessary background or noise.

Number Breakdown for Experiment #1:
This experiment involves crossing the BHD-Flox strain to CMV-Cre to generate conventional BHD knockout mice. To obtain the mice for phenotype assessment, 684 mice will be used. The number are listed below (refer to the attached mouse breeding calculator spreadsheet.

Postnatal evaluation:
- Day 1 after birth ..... 10 null mice; 10 heterozygous mice; 10 wild type mice
- Day 7 after birth ..... 10 null mice; 10 heterozygous mice; 10 wild type mice
- Day 21 after birth ..... 10 null mice; 10 heterozygous mice; 10 wild type mice

Pre-pubertal evaluation
- (8 weeks old) ..... 10 null mice; 10 heterozygous mice; 10 wild type mice

Mature adult evaluation:
- 16 weeks old..... 10 null mice; 10 heterozygous mice; 10 wild type mice
- 24 weeks old..... 10 null mice; 10 heterozygous mice; 10 wild type mice
- 52 weeks old..... 10 null mice; 10 heterozygous mice; 10 wild type mice

Aging mice
- (78 weeks old) ..... 10 null mice; 10 heterozygous mice; 10 wild type mice

Histological and pathological examinations:
- ..... 10 null mice; 10 heterozygous mice; 10 wild type mice

Establishment of fibroblast cell lines:
- ..... 10 null mice; 10 heterozygous mice; 10 wild type mice

Prenatal period of evaluation for embryos:
- Prenatal 10 days post coitum .... 10 heterozygous mice
- Prenatal 12 days post coitum .... 10 heterozygous mice

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Summary of Number Breakdown: Total Experiment Animals Needed:

100 homozygous mice, 120 heterozygous mice, and 100 wild-type mice for a total of 320 experimental mice.

Breeding Step#1- breeding to get KOH Heterozygous & KOH wild-type:

Twelve KOH BHD-flox/+ mice will be bred twice to 12 Cre tg/tg mice.

These will be set up as paired mating over time.

Given an approximate little of 10 pups, that should produce 240 offspring in the F1 generation.

Of these 240:

½ of the total, or roughly 120 pups, will be double hets (KOH BHD flox/+; Cre tg/+).

½ of the total, or roughly 120 pups, will be wildtype/het (KOH BHD +/+; Cre tg/+)

Total mice used and produced in breeding Step #1: 264

Breeding Step #2-Breeding to Get KOH Homozygous:

20 KOH BHD flox/+; Cre tg/+ mice (from Breeding Step #1) will be bred twice to 20 KOH flox/flox mice (this does not count the 20 mice used in pared mating, they have been counted in Step #1). These will be set up as paired mating over time.

Given an approximate little of 10 pups, that should produce 400 offspring in the F2 generation.

Of these 400:

¼ of the total, or roughly 100 pups, will be KOH homo; Cre het (KOH BHD flox/flox; Cre tg/+).

Total mice used and produced in breeding Step #2: 420

Subtotals:

Breeding Step 1: 264

Breeding Step 2: 420

evaluation for embryos *20*

Total for Experiment #1: 704

Number Breakdown for Experiment #2:

This experiment involves crossing the mouse strain BHD-flox (KOH(R4R3-BHD)046BT4A3) to ERTM-Cre to generate a tamoxifen-sensitive conditional knockout.

To generate the mice for this experiment, 342 mice are needed. The numbers are given below; in additional, please refer to the attached mouse breeding calculator apreadsheet.

Neonates 15 null mice; 15 heterozygous mice; 15 wild type mice

Mature (24 weeks) 15 null mice; 15 heterozygous mice; 15 wild type mice

Histological and pathological examinations:

15 null mice; 15 heterozygous mice; 15 wild type mice

Summary of Number Breakdown:

45 null (homozygous) mice (from Step #2 below), 45 heterozygous mice (from Step #1 below), and 45 wild-type mice (from Step #1 below) for a total of 135 experimental mice.

Breeding Step #1-Breeding to Get KOH BHD Hetrozygous & KOH Wild type:

Si x KOH BHD flox/+ mice will be bred twice to six Cre tg/tg mice.

These will be set up as paired mating over time.

Given an approximate little of 10 pups, that should produce 120 offspring in the F1 generation.

Of these 120:

½ of the total, or roughly 60 pups, will be double hets (KOH BHD flox/+; Cre tg/+).

½ of the total, or roughly 60 pups, will be wildtype/het (KOH BHD +/+; Cre tg/+)

Total mice used and produced in Breding Step #1: 132

Breeding Step#2-Breeding to Get KOH Homozygous:

10 KOH flox/+; Cre tg/+ mice (from Breeding Step #1) will be bred twice to 10 KOH flox/flox mice (this does not count the 10 mice used in pared mating, they have been counted in Step #1). These will be set up as paired mating over time.

Given an approximate little of 10 pups, that should produce 200 offspring in the F2 generation.

Of these 200:

¼ of the total, or roughly 50 pups, will be KOH homo; Cre het (KOH BHD flox/flox; Cre tg/+).

Total mice used and produced in Breeding Step #2: 210

Subtotals:

Breeding Step1	132
Breeding Step 2	210
Total:	342.

Number Breakdown for Experiment #3-5:

The same number as experiment #1 except the Cre strain are different.

Number Breakdown for Experiment #6:

This experiment involves crossing the mouse strain BHD-flox mice to FLPeR-Frt strain to generate a non-neomycine BHD-flox mice.

To generate the mice for this experiment, 72 mice are needed. The numbers are given below.

Summary of Number Breakdown:

This experiment will produce about 30 double heterozygous (BHD-flox/+, Frt tg/+).

Breeding Step-Breeding to Get BHD-flox hetrozygous & FLPeR-Frt heterozygous mice:

Three BHD flox/+ mice will be bred twice to three FLReP-frt tg/tg mice.

These will be set up as paired mating over time.

Given an approximate little of 10 pups, that should produce 60 offspring in the F1 generation.

Of these 60:

½ of the total, or roughly 30 pups, will be double hets (KOH BHD flox/+; Frt tg/+).

½ of the total, or roughly 30 pups, will be wildtype/het (KOH BHD +/+; Frt tg/+)

So totally 66 mice (3+3+60) are needed in this experiment.

DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES
(Use additional sheets if necessary)

Briefly explain the experimental design and specify all animal procedures. This description should allow the IACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study.

This protocol includes 5 experiments.

Experiment #1-5:

This experiment will create *BHD* knockout mice to allow characterization of both homozygous and heterozygous *BHD* knockouts. Dr. Pam Swiatek, Special Program Investigator of Laboratory of Germline Modification will be generating the *BHD* knockout mice under her protocol number 01-03-004 (entitled "Gene Targeting/Sperm Cryopreservation Service"). The knockout will be evaluated at nine intervals during five principal phases of life: 1) embryogenesis, 2) neonatal period, 3) peripubertal growth, 4) mature adulthood, and 5) aging. At each interval 10 –/– and 10 +/– mice will be evaluated, with 10 +/+ mice as controls. 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.

Specifically address the following:

1. **Experimental injections or inoculations**
   (substances, e.g., infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedules)

   For experiment #1, human kidney tumor-derived cell lines will be injected subcutaneously into the flank of the mouse. The injectate will be at a concentration of 100,000 cells per 100 microliters suspended in a PBS solution and administered one time, in a volume of 0.2 ml.

2. **Blood withdrawals**
   (volume, frequency, withdrawal sites, and methodology)

   t applicable.

3. **Surgical procedures**
   (provide details of survival and non-survival surgical procedures in Section G.)

Not applicable.

4. **Radiation**
   (dosage and schedule)

   Not applicable.

5. **Methods of restraint**
   (e.g., restraint chairs, collars, vests, harnesses, slings, etc.)
   Include how animals are restrained for routine procedures like blood withdrawals. Prolonged restraint must be justified with appropriate oversight to ensure it is minimally distressing. Describe any sedation, acclimation, or training to be utilized.

   Not applicable.

6. **Animal identification methods**
   (e.g., ear tags, tattoos, collar, cage card, implant, etc.)

   Cage cards and ear notches.

7. **Other procedures**
   (e.g., survival studies, tail biopsies, etc.)

   Not applicable.

8. **Resultant effects, if any, that the animals are expected to experience**
   (e.g., pain or distress, ascites production, etc.)

   Animals will be observed daily. Weight will be monitored weekly during normal observation periods. Animals will be monitored for weight loss, lethargy, loss of appetite, and euthanized when necessary.

9. **Other potential stressors**
   (e.g., food or water deprivation, noxious stimuli, environmental stress)
   In addition, specify procedures to monitor and minimize distress. If a study is USDA Classification E, indicate any non-pharmaceutical methods to minimize pain and distress.

   While every effort will be made to avoid causing pain/distress to mice, some mice may experience adverse effects associated with tumor growth. Tumor burden is usually associated with lethargy, shortness of breath (lung metastasis), and weight loss. A loss of > 20% body weight is an indicator of euthanization. In all cases, it is essential that mice are observed on a regular basis, and mice are euthanized when it is necessary.

10. **Experimental endpoint criteria**
    (e.g., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral

abnormalities, clinical symptomatology, or signs of toxicity)
Experimental endpoint criteria must be specified when the administration of tumor cells, biologics, infectious agents, radiation, or toxic chemicals are expected to cause significant symptomatology or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified.

The normal endpoint for the subcutaneous tumor model is the growth of the tumor to the limit of 1 cm^3. In the case where an animal does not appear to develop tumors and fails to exhibit any of the symptoms of distress (itemized below), the animal will be sacrificed at one year from the experimental inoculation.

A loss of > 20% body weight is indicative of euthanasia at any time during the experiment. When mice appear distressed, e.g., lethargy, ulcerations, loss of appetite for more than one day they will be euthanized. In particular, for the knockout experiment, animals may potentially exhibit some skin lesions. This is not considered an endpoint of the experiment, unless they appear to cause distress to the animal.

11. **Veterinary care**
(indicate desired plan of action in case of animal illness, e.g., initiate treatment, call investigator prior to initiating treatment, euthanize)

Daily veterinary care will be provided to all animals by the Vivarium staff. The Vivarium staff will consult with the attending veterinarian Dr. Joan Koelzer (616) 437-6415 or the alternate attending veterinarian Dr. Diane Egedy (616) 827-2950 when necessary. In the case animals are found sick or dead the PI will be notified via email and phone. PI will be notified with symptomology, disposition and animal identifier. In the event the PI cannot be reached, associates in the PI's lab will be contacted. In the event PI and his/her associates cannot be contacted any sick mice will be treated at the discretion of the Vivarium staff or attending veterinarian. Any animals found dead will be placed in a -20 refrigerator.

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F. SURGERY

If proposed, complete the following:
(NOTE: Use additional sheets if necessary.)

Surgery will not be performed in conjunction with this protocol.

1. Identify and describe the surgical procedure(s) to be performed. Include preoperative procedures (e.g., fasting, analgesic loading), and monitoring and supportive care during surgery. Include the aseptic methods to be utilized.

2. Who will perform surgery and what are their qualifications and/or experience?

3. Where will surgery be performed and postoperative care provided (building and rooms)?

4. If survival surgery, describe postoperative care required, frequency of observation, and identify the responsible individual(s). Include detection and management of postoperative complications during work hours, after hours, weekends, and holidays.

5. If non-survival surgery, describe how humane euthanasia is enacted and how death is determined.

6. Are paralytic agents used during surgery? If yes, please describe how ventilation will be maintained and how pain will be assessed.

7. Has major survival surgery been performed on any animal prior to being placed on this study? [Major survival surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation).]
   If yes, please explain:

8. Will more than one major survival surgery be performed on an animal while on this study?
   If yes, please justify:

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G. PAIN OR DISTRESS CATEGORY:

Check the appropriate category and indicate the approximate number of animals in each.
NOTE: The sum of all three categories should equal the total cited in both Section B, question #9 (total number of animals used for the duration of the entire study) and Section E, question #3 (provide a justification for the total number of animals used in this protocol).

		Number of Animals
☒	Category 1 – Minimal, Transient, or No Pain or Distress.	1589
☐	Category 2 – Pain or Distress Relieved by Appropriate Measures	
☐	Category 3 – Unrelieved Pain or Distress***	

******NOTE: If animals are indicated in Category Three, a written scientific justification is required to explain why the appropriate use of anesthetics, analgesics, sedatives, or tranquilizers during and/or following painful or distressful procedures are contraindicated in this study.***

H. ANESTHESIA, ANALGESIA, TRANQUILIZATION, OTHER AGENTS

For animals indicated in Section H, category 2, specify the anesthetics, analgesics, sedatives, or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route, and schedule of administration.

Not applicable.

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I. METHOD OF EUTHANASIA OR DISPOSITION OF ANIMALS AT END OF STUDY

Indicate the proposed method of euthanasia. If a chemical agent is used specify the dosage and route of administration. If the method(s) of euthanasia include those **not** recommended by the AVMA Panel Report on Euthanasia (e.g., decapitation or cervical dislocation without anesthesia), provide scientific justification why such methods must be used. Indicate the method of carcass disposal if not described in Section K below.

The mice will be euthanized by inhalation of 100% CO_2 until respiration has ceased.

J. HAZARDOUS AGENTS

Use of hazardous agents requires the approval of the institutional Biosafety Office/Committee. Attach documentation of approval for the use of recombinant DNA or potential human pathogens.

Hazardous Agent	Yes	No	Agent	Biosafety Approval Date	Tracking Number
Radionuclides	☐	☒			
Biological agents	☒	☐	Human cell lines		
Hazardous chemicals or drugs	☐	☒			
Recombinant DNA	☒	☐			

Additional safety considerations:

1. Study Conducted at Animal Biosafety Level: 1 ☐ 2 ☒ 3 ☐ 4 ☐

2. Practices and procedures required for the safe handling and disposal of contaminated animals and material associated with this study to include methods for the removal of radioactive waste and, if applicable, the monitoring of radioactivity:

   Universal precautions, including lab coats, gloves and eye protection will be worn while working with the mice, particularly while administering the human cell line injections.

   An MSDS for the ATCC cell lines is attached.

3. Other:

   Not applicable.

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K. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS

(e.g., cell lines, antiserum, etc.)

1. Specify Material:

   Human kidney tumor-derived cell lines obtained from the American Type Culture Collection (ATCC).

2. Source:

   Material Sterile or Attenuated: Yes ☒ No ☐

   If derived from rodents, has the material been MAP/RAP/HAP tested*?*
   (MAP - Mouse Antibody Production;
   RAP - Rat Antibody Production;
   HAP - Hamster Antibody Production)

   NOTE: If Yes, attach copy of results: Yes ☒ No ☐

   All cell lines will be MAP tested prior to use and results submitted to Bryn Eagleson, Laboratory animal Resource Director, and Kaye Johnson, IACUC Administrative Assistant.

3. I certify that the MAP/RAP/HAP-tested materials to be used have not been passed through rodent species outside of the animal facility in question and/or the material is derived from the original MAP/RAP/HAP-tested sample. To the best of my knowledge the material remains uncontaminated with rodent pathogens.

   Initials of Principal Investigator: Bin Tean Teh, M.D., Ph.D.

L. TRANSGENIC AND KNOCKOUT ANIMALS

Describe any phenotypic consequences of the genetic manipulations to the animals. Describe any special care or monitoring that the animals will require.

The phenotype for Experiment #2 is unknown. The animals will be monitored for signs of the experimental endpoint criteria (as cited in Section F). Any animals displaying such criteria will be euthanized.

M. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY

List any special housing, equipment, animal care (e.g., special caging, water, feed, or waste disposal, environmental enhancement, etc.).

Not applicable.

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N. PRINCIPAL INVESTIGATOR CERTIFICATIONS

1. I certify that I have attended the institutionally required investigator training course.

   Year of course attendance: **2001** Location: **VARI**
2. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.
3. I certify that all individuals working on this proposal who are at risk are participating in the Institution's Occupational Health and Safety Program.
4. I certify that the individuals listed in Section A are authorized to conduct procedures involving animals under this proposal, have attended the institutionally required investigator training course, and received training in the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); and procedures for reporting animal welfare concerns.
5. For all Category 3 Proposals *(see Section H)*:
   I certify that I have reviewed the pertinent scientific literature and the sources and/or databases noted below, and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not.
6. I certify that I will obtain approval from the IACUC before initiating any significant changes in this study.
7. I certify that I will notify the IACUC regarding any unexpected study results that impact the animals. Any unanticipated pain or distress, morbidity, or mortality will be reported to the attending Veterinarian and the IACUC.
8. I certify that I am familiar with and will comply with all pertinent institutional, state, and federal rules and policies.

Principal Investigator:

Name: Bin Tean Teh, M.D., Ph.D. Signature: Date: 9/10/2003

O. CONCURRENCES

Supervisory concurrence as applicable:

Name: Signature: Date:

Safety Office/Committee Certification of Review and Concurrence:
(Required of all studies utilizing hazardous agents.)

Name: Signature: Date:

Facility manager/Veterinarian certification of resource capability in the indicated facility to support the proposed study:

Facility:

Name: Signature: Date:

Facility:

Name: Signature: Date:

Comments:

Attending Veterinarian certification of review and consultation on proper use of anesthetics and pain relieving medications for any painful procedures:

Name: Signature: Date:

P. FINAL APPROVAL

Certification of review and approval by the Institutional Animal Care and Use Committee:

Name: Signature: Date:

List any attachments here:

- The IRB approval letter.
- MAP tests of the cell lines

  Please note that the cell lines were submitted for MAP tests citing the ATCC number. Therefore, when reviewing the MAP tests, please be aware that the following cell lines were cited under different numbers:

  - *SW-839 was cited as HTB-49*
  - *Caki-2 was cited as HTB-47*
  - *786-O was cited as CRL-1932*
  - *769-P was cited as CRL-1933*
  - *A-704 was cited as HTB-45*
  - *A-498 was cited as HTB-44*
  - *ACHN was cited as CRL-1611*
  - *SW-156 was cited as CRL-2175*
  - *Caki-1 was cited as HTB-46*

- MSDS from the ATCC for the cell lines
- rDNA approval letter (pending receipt)
- Knockout mice calculator