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|---|---|---|---|
| | Major Version Incremented | Nguyen, Tony | 10/13/2020 4:57 PM |
| | Amendment AMEND202001135 closed (Approved) | Nguyen, Tony | 10/13/2020 4:57 PM |

Amendment Approved: AMEND202001135

| | | | |
|---|---|---|---|
| | Opened Amendment | Mao, Qingcheng | 10/12/2020 11:22 AM |

Amendment: AMEND202001135

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|---|---|---|---|
| | Letter Sent | Clark, Emily W. | 9/8/2020 1:59 PM |

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| | Letter Prepared | Clark, Emily W. | 9/8/2020 1:58 PM |

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|---|---|---|---|
| | Approval Period Edited | Clark, Emily W. | 9/8/2020 1:57 PM |
| | Designated Member Review Submitted | Sullivan, Jane | 9/8/2020 12:30 PM |
| | Response Submitted | Mao, Qingcheng | 9/8/2020 11:39 AM |
| | Clarification Requested by IACUC Member | Administrator, System | 9/8/2020 10:57 AM |

Hello,

The IACUC had additional questions regarding this item. Please revise/respond as needed and submit back to our office.

Thank you,
Stephanie

| | | | |
|---|---|---|---|
| | Clarification by Designated Reviewer Requested | Huang, Stephanie W | 9/8/2020 10:57 AM |

Hello,

The IACUC had additional questions regarding this item. Please revise/respond as needed and submit back to our office.

Thank you,
Stephanie

| | | | |
|---|---|---|---|
| | Designated Reviewers Assigned | Huang, Stephanie W | 9/8/2020 10:57 AM |
| | Assigned to Designated Review | Huang, Stephanie W | 9/8/2020 10:54 AM |
| | Agenda Item Removed | Huang, Stephanie W | 9/8/2020 10:49 AM |
| | Private Comment Added | Sullivan, Jane | 9/6/2020 10:22 AM |

I have one question to clear up an inconsistency on genotyping methods, but other than that, no questions or comments

| | | | |
|---|---|---|---|
| | Ancillary Review Submitted | Cashman, Judy L | 9/2/2020 2:07 PM |
| | Ancillary Reviews Managed | Cashman, Judy L | 9/2/2020 2:06 PM |
| | Tags Managed | Cashman, Judy L | 9/2/2020 2:06 PM |
| | OHRs attached | Cashman, Judy L | 9/2/2020 2:06 PM |
| | Meeting Assigned | Clark, Emily W. | 9/1/2020 8:10 AM |
| | Pre-Review Submitted | Clark, Emily W. | 9/1/2020 8:10 AM |
| | Response Submitted | Mao, Qingcheng | 8/31/2020 5:18 PM |

| | | | |
|---|---|---|---|
| | Clarification by Pre-Reviewer Requested | Clark, Emily W. | 8/31/2020 12:49 PM |

Hi Qingcheng, Please respond to the veterinary Reviewer Notes and edit the protocol as requested. Let me know if you need help or have any questions. Thanks! Emily

| | | | |
|---|---|---|---|
| | Vet Consult Submitted | Stocking, Kim | 8/31/2020 11:33 AM |

Do you accept the submission? yes

| | | | |
|---|---|---|---|
| | Private Comment Added | Stocking, Kim | 8/31/2020 11:32 AM |

I had a couple of comments but I don't need to review the response unless there's a vet question or concern.

| | | | |
|---|---|---|---|
| | Vet Consult Sent | Stocking, Kim | 8/31/2020 11:00 AM |

| Activity | Author | Activity Date |
|---|---|---|
| Vet Consult Sent | Clark, Emily W. | 8/27/2020 11:29 AM |
| Response Submitted | Mao, Qingcheng | 8/27/2020 11:07 AM |
| Clarification by Pre-Reviewer Requested | Clark, Emily W. | 8/27/2020 10:30 AM |

Hi Qingcheng, I created a cardiac puncture under isoflurane euthanasia procedure and added it to your experiment. This is based on a Standard procedure that is currently being developed by OAW. Please take a look at the procedure and make sure it matches what you do in the lab (or edit, if needed). Thanks! Emily

| | | |
|---|---|---|
| Response Submitted | Mao, Qingcheng | 8/26/2020 11:24 AM |
| Clarification by Pre-Reviewer Requested | Clark, Emily W. | 8/24/2020 4:18 PM |

Hi Qingcheng, Let's make a few changes before this goes to the vets for their pre-review. Please respond to the Reviewer Notes and edit the protocol as requested. Let me know if you need any help or have any questions. Thanks! Emily

| | | |
|---|---|---|
| Tags Managed | Kunsman, Robyn | 8/24/2020 9:35 AM |
| Coordinator Assigned | Clark, Emily W. | 8/7/2020 7:40 AM |

Assigned to Emily W. Clark

| | | |
|---|---|---|
| Coordinator Assigned | Jimenez, Selesteen | 8/6/2020 3:07 PM |

Assigned to OAW Blue Team

| | | |
|---|---|---|
| Assigned Portfolio ID | Jimenez, Selesteen | 8/6/2020 3:07 PM |
| Submitted | Mao, Qingcheng | 8/6/2020 2:28 PM |
| Protocol Created | Mao, Qingcheng | 7/31/2020 2:23 PM |

# Basic Information

1. *** Select research team:**
   Mao
2. *** Title of protocol:**
   Germ-free rederivation of humanized hPXR/hCYP3A4 and hCYP3A4 mice
3. *** Short title:**
   4035-07: Germ-free hPXR/hCYP3A4 mice
4. *** Summary of research:**
   It has been reported that the expression and activity of mouse hepatic Cyp3a11, the murine ortholog of human CYP3A4, are significantly suppressed in germ-free (GF) mice compared to those in conventional (CV) mice. This suggests that the gut microbiome plays an important role in modulating host metabolism of drugs by altering the expression and activity of hepatic drug-metabolizing enzymes. Human CYP3A4 is the most important cytochrome P450 enzyme that metabolizes ~50% of all drugs. Human CYP3A4 and its murine ortholog Cyp3a11 are target genes of the nuclear receptor PXR. Secondary bile acids, generated through metabolism of primary bile acids by the gut microbes, induce CYP3A4 via PXR. Lithocholic acid (LCA) and deoxycholic acid (DCA), especially LCA, are typical secondary bile acids that are known PXR activators. Animal data to date led us to hypothesize that the gut microbiome can alter the expression (and hence activity) of CYP3A via PXR. However, due to species differences in ligand binding specificities between human and mouse PXR, it is not clear whether the same mechanism operates in humans. Therefore, to test our hypothesis and to demonstrate the role of the gut microbiome in regulating CYP3A4 in humans, we propose animal studies that use a novel germ-free humanized PXR/CYP3A4 mouse model. To achieve this goal, we first need to create germ-free humanized hPXR/hCYP3A4 mice. Humanized hPXR/hCYP3A4 mice express human PXR and human CYP3A4, but are deficient in the mouse Pxr and Cyp3a genes. Humanized hCYP3A4 mice, which will be used as a control, express only human CYP3A4 and are also deficient in the mouse Pxr and Cyp3a genes. These humanized CV mice will be obtained from a collaborator at the University of Pittsburgh. Once GF mice are created, we will compare the expression and activity of human CYP3A4 between GF and CV mice. If our hypothesis is correct, we expect that the expression (and hence activity) of human CYP3A4 in the liver and intestine of GF humanized hPXR/hCYP3A4 mice are significantly lower than those in CV mice. As a control, no differences between GF and CV humanized hCYP3A4 mice (which do not express human PXR) would be expected if the gut microbiome modulates the expression of human CYP3A4 via human PXR.
5. *** Principal investigator:**
   Qingcheng Mao

**6. * What is the intention of the animal protocol?**

Experimental Research

# Experimental Research Protocol Addition

1. * **Will the protocol include breeding?**
   - (●) **Yes**
   - ( ) No

# Protocol Team Members

**1. Identify each additional person involved in the design, conduct, or reporting of the research:**

| Name | Role | Involved in Animal Handling | Authorized To Order Animals | E-mail | Phone |
|---|---|---|---|---|---|
| [redacted] | Graduate Student | yes | yes | [redacted] | |
| GNAC Research Support | Other | yes | no | | |

FERPA
RCW 42.56.070(1)

**2. If veterinary care will be provided by individuals outside of DCM or WaNPRC, provide the name, credentials and contact information below:**

N/A

# Funding Sources

## 1. Identify each organization supplying funding for the protocol:

| | Funding Organization | eGC1 Number(s) |
|---|---|---|
| View | National Institute of General Medical Sciences (NIGMS) | |

# Scientific Aims

## 1. * Scientific aims of the research:

Our hypothesis is that the gut microbiome modulates host metabolism of drugs by altering the expression (hence activity) of human CYP3A4 through the nuclear receptor human PXR.

To test this hypothesis, our specific aims are:

Aim 1: Create two germ-free mouse models, namely, germ-free humanized hPXR/hCYP3A4 mice and germ-free humanized hCYP3A4 mice.

Aim 2: Determine the expression and activity of human CYP3A4 in the liver and intestine of GF and CV humanized hPXR/hCYP3A4 and hCYP3A4 mice.

## 2. * Using language understandable to non-scientists, describe the goals and significance of the protocol to humans, animals and science:

Over the last decade, there has been an exponential rise in research into the gut microbiome that links the gut microbiome to human physiology and diseases; however, the role of the gut microbiome in drug pharmacology remains largely underexplored. Specifically, very little information is available on how the gut microbiome modulates host metabolism of drugs by altering the expression and activity of human drug-metabolizing enzymes. The studies proposed in this project will fill this critical gap in knowledge. In this project, we will focus on investigating the role of the gut microbiome in altering the expression (and hence activity) of the most important drug-metabolizing enzyme in humans, namely the human CYP3A4. To date, evidence regarding alteration of CYP3A expression or activity by the gut microbiome has come almost exclusively from animal studies. However, whether the gut microbiome can modulate host metabolism of drugs by altering the expression of human CYP3A4 is not known. To address this question, we propose to use a novel germ-free humanized CYP3A4 mouse model. These studies will address a critical gap in our understanding of the role of the gut microbiome in modulating host metabolism of drugs, and ultimately drug efficacy and toxicity. Given its vast interindividual variability, the gut microbiome may represent a new source of interindividual variability of drug PK, efficacy or toxicity. Therefore, our studies will have significant implications in precision medicine and will be important for optimizing dosing regimen of drugs with better efficacy and safety.

## 3. * Provide a statement to address the potential harm to the animals on this study (e.g., pain, distress, morbidity, mortality) relative to the benefits to be gained by performing the proposed work:

We do not expect any harm to mice on these studies. Mice will be anesthetized before tissue collection and will be closely monitored. Veterinary staff will be consulted when necessary.

# Experiments

Note: If you will be administering cells, cell lines, sera or other biologicals to rodents, contact the Rodent Health Monitoring Program (RHMP, rhmp@uw.edu). Testing may be required prior to administration to rodents.

**1. * Define the experiments to be used in this protocol:**

| Name | Species | USDA | Count | Count by Pain Category | Procedures | Husbandry Exception Types |
|---|---|---|---|---|---|---|
| Germ-free rederivation of humanized hPXR/hCYP3A4 and hCYP3A4 mice | Mice | no | 111 | B: 48<br>C: 63<br>D: 0<br>E: 0 | ▪ Euthanasia: CO2 Followed by Secondary Methods (>10 days of age) (Standard)<br>▪ Euthanasia: Mao: Exsanguination via Cardiac Puncture, Under Isoflurane Anesthesia, Anesthesia Machine (Team) | Mice - No husbandry or enrichment exceptions. |

**2. Will any single animal undergo more than one survival surgery? (include any animal that underwent surgery prior to use on this protocol)** ○ Yes ● **No**

# Procedure Personnel Assignment

## 1. * Select the team members who will be performing each procedure:

| Procedure | Species | Is USDA Species | Team Members |
|---|---|---|---|
| Euthanasia: CO2 Followed by Secondary Methods (>10 days of age), ver. 2 (Standard) | Mice | no | Xin Chen |
| Euthanasia: Mao: Exsanguination via Cardiac Puncture, Under Isoflurane Anesthesia, Anesthesia Machine, ver. 1 (Team) | Mice | no | Xin Chen |

## 2. Team member training:

First Name Last Name Training

**FERPA**

**RCW 42.56.070(1)**

| Course | Category | Source | Stage | Stage Number | Completion Date | Expiration Date |
|---|---|---|---|---|---|---|
| Animal Use Laws & Regulations | General | Online | Basic Course | Stage 1 | 10/21/2019 | 10/21/2024 |
| Animal Use Medical Screening | General | Online | Basic Course | Stage 1 | 10/28/2019 | 10/31/2022 |
| Orbital Injection, Mouse Anesthetized | Procedure | In Person | Basic Course | Stage 1 | 11/15/2019 | |
| Mouse Hands-On Laboratory | Animal Handling | In Person | Basic Course | Stage 1 | 11/15/2019 | |

No experience data to display

**Qingcheng Mao**

| Course | Category | Source | Stage | Stage Number | Completion Date | Expiration Date |
|---|---|---|---|---|---|---|
| Mouse Hands-On Laboratory | Animal Handling | In Person | Basic Course | Stage 1 | 4/22/2003 | |
| Mouse Online Course: Working with Mice at UW | Animal Handling | Online | Basic Course | Stage 1 | 8/23/2012 | |
| Animal Use Medical Screening | General | Online | Basic Course | Stage 1 | 7/16/2019 | 7/31/2022 |
| Animal Use Laws & Regulations | General | Online | Basic Course | Stage 1 | 3/8/2019 | 3/8/2024 |

No experience data to display

| | | | |
|---|---|---|---|
| **GNAC** | **Research Support** | No training data to display | No experience data to display |

# Animal Details

## 1. * How are animals acquired?

Transferred from an Outside Institution

## 2. Describe the acquisition for:

**a. Transferred from an Outside Institution, provide Institution and Protocol Number (note: DCM must approve transfers from outside institutions):**
University of Pittsburgh

**b. * If animals were transferred, please provide an explanation of all procedures the animal(s) experienced prior to transfer (i.e substance administrations, surgeries, dietary restrictions, etc.):**
Conventional humanized hPXR/hCYP3A4 and conventional humanized hCYP3A4 mice will be provided from a collaborator at the University of Pittsburgh. These mice will not undergo any procedures prior to transfer.

Germ-free mice will be created using these conventional mice at the University of Washington.

## 3. Identification of individual animals (other than cage cards):

**a. Method(s) (e.g., ear punch/tag, tattoo, tagging/banding, radio collar, etc.) (Note: If method is implantation (e.g. PIT tag), create or select an Implant procedure to describe the details. If method is surgical (e.g., satellite tag), create or select Survival Surgery procedure to describe the details):**
ear tag in one ear

**b. Will external identification be replaced if it falls off/out? If yes, describe the plan for replacement:**
Yes, re-tag an ear tag in a different ear

**c. Will external identification be removed as part of the protocol (e.g., radio collars on field animals)? If yes, describe the plan for removal:**
No

## 4. Identify strain/stock for rodents and genetically modified animals:

| | Species | Is USDA Species | Strain | Genetically Modified Strain | Phenotype Description |
|---|---|---|---|---|---|
| View | Mice | no | FVB hCYP3A4 | yes | None |
| View | Mice | no | FVB hPXR/hCYP3A4 | yes | None |

# Animal Number Adjustments

"Animals Identified in Experiments" is the total number of animals per pain category listed in all experiments on this protocol. If more or fewer animals will be used on the protocol (see Help Text for examples), click Update to enter this new number in the corresponding "Adjusted Animal Count" column. **Only input numeric values in this field; 0 is acceptable.**
If no adjustment is required, the values in the "Animals Identified in Experiments" and "Adjusted Animal Count" columns must match. Click Update in each Pain Category row to input the matching value.
For questions about adjusting animal numbers, contact OAW.

## 1. * Click Update to adjust the number of animals to be used or produced for this protocol:

| | Species | USDA Covered Species | Pain Category | Animals Identified in Experiments | Adjusted Animal Count |
|---|---|---|---|---|---|
| View | Mice | no | Pain Category B | 48 | 48 |
| View | Mice | no | Pain Category C | 63 | 63 |
| View | Mice | no | Pain Category D | 0 | 0 |
| View | Mice | no | Pain Category E | 0 | 0 |

## 2. If you adjusted the number of animals for this protocol, explain why:

N/A

## 3. If you will be using animals to train personnel or to practice procedures included in this protocol, describe below:

A small number of mice (5) will be used for personnel training. In addition, an extra number of mice (10) is requested to cover other needs such as experimental loss. These have been requested within the experiment.

## 4. Supporting documents:

| Document Name | Date Modified |
|---|---|
| There are no items to display | |

# Alternatives and Duplication Searches

**Display Procedures that cause pain or distress:** none

1. **Record all searches for any previous research that this protocol might duplicate:**

| | Search Date | Searched Databases | Other |
|---|---|---|---|
| View | 8/5/2020 | Web of Science (searches multiple databases)<br>PubMed/Medline | N/A |

2. **Briefly describe the results of your searches and why you can or cannot incorporate the findings. Or, if a literature search was not performed, describe the methods used to determine that alternatives are not available or feasible:**

   We did not find alternative procedures that do not cause pain or distress in our search. among animal models, mice are most commonly used based on our search. Other rodent models such as rats might be used. However, humanized hPXR/hCYP3A4 and humanized hCYP3A4 rats are not available. WE therefore will use CV and GF humanized hPXR/hCYP3A4 and humanized hCYP3A4 mice for our proposed studies.

3. **Confirm that you have made every effort to ensure that this protocol is not unnecessary duplication of previous research:** ☑

# Breeding

1. *** Describe the objectives and justifications for this breeding activity:**
   We expect that we will obtain only a small number of CV humanized hPXR/hCYP3A4 mice and CV humanized hCYP3A4 mice (e.g., one pair of each mouse model) and these mice are not commercially available. Therefore, to conduct germ-free rederivation and our proposed animal studies, we need to increase the number of CV and GF mice of these two mouse models through in-house breeding.
2. **Describe the breeding scheme:**
   The standard IACUC breeding scheme will be used. We will use trio breeding (one male and two females for each strain, humanized hPXR/CYP3A4 or humanized CYP3A4). This breeding scheme maximizes cage space for breeding and mothers assist in raising each other's pups. Leave trio together continuously, whenever possible. Mating age: 6-8 weeks of age; Gestation: ~19-21 days; Wean age: ~21 days.
3. *** Describe the genotyping methods you will use:**
   We will use quantitative real-time PCR of tail DNA to determine the expression of human PXR and human CYP3A4. Tail DNA will be isolated from tail tip (1-2 mm). If we can detect both human PXR and human CYP3A4 from a mouse, this mouse should be a humanized hPXR/hCYP3A4 mouse. If we detect only human CYP3A4, then the mouse should be a humanized hCYP3A4 mouse.
4. **For mouse tailing, if sample is greater than 3 mm or collected from mice greater than 28 days of age, include anesthetic and analgesic protocol below:**
   In case that genotyping is needed, tail tip (1-2 mm) will usually be collected from mice before weaning (21 days of age). If tissues for genotyping have to be collected from mice greater than 28 days of age, ear punch will be done to obtain tissue for genotyping. No anesthesia or analgesia is necessary with an ear punch.
5. **Identify any other protocols or research institutions to which you will supply animals bred from this protocol and explain:**
   N/A
6. **Describe the disposition of non-experimental animals (e.g., use as controls, euthanasia, etc.):**
   **Note: Be sure to include any plan for euthanizing offspring in the procedures section.**
   We will use all mice for our proposed animal studies. In case we have non-experimental mice, these mice will be euthanatized with $CO_2$ using a standard procedure and then disposed through DCM.

# Housing and Use

Housing and use outside of the vivarium is not allowed without strong scientific justification.

## 1. Identify each location where animals will be housed:

| | Facility | Species | Justification for Housing Outside Vivarium |
|---|---|---|---|
| View | ARCF ABSL1 | Mice | N/A |
| View | ARCF Gnotobiotic ABSL1/2 | Mice | N/A |

## 2. Identify each location where animals will be used:

| | Facility | Use | Species | Justification for Use Outside Vivarium |
|---|---|---|---|---|
| View | H153A Vivarium ABSL1 | H153 will be used for all procedures including collection of blood and tissues. | Mice | N/A |

# Disposition

1. **Disposition plans for the animals when this research is complete:**
   (check all that apply)
   Euthanasia
2. **If other, provide an animal disposition description:**
   N/A
3. **If protocol involves fixing tissues, list agents (e.g., paraformaldehyde, formalin):**
   N/A

# Refinement, Replacement and Reduction

1. **Describe below how the three R's (refinement, replacement and reduction) have been employed on this project. Include alternatives that were considered for the procedures above that cause pain or distress:**

   *** Refinement (use of methods to decrease animals' sensitivity to pain)**
   As explained in this protocol, we will use routine methods for blood and tissue collection that will not cause much pain in mice, namely, mice will be anesthetized for blood and tissue collection.

   *** Replacement (include in vitro tests, use of less sentient animals)**
   Such studies must be done in the presence (conventional) and absence (germ-free) of the gut microbiome and therefore cannot be replaced by in vitro tests.

   *** Reduction (use of fewer animals to attain statistical significance)**
   We did power analysis to estimate the number of mice to be used in our studies based upon data published in the literature. In the future, if we are able to develop more sensitive assays to quantify the expression or activity of human CYP3A4, we will likely reduce the number of mice needed for our studies. This is something we will try.

2. **Describe the rationale for using animals and the appropriateness of the species proposed:**
   Our proposed animal studies will utilize humanized PXR and CYP3A4 mouse models. These studies will elucidate the mechanisms by which the gut microbiome modulates host metabolism of drugs by altering the expression of human CYP3A4 in vivo. Such mechanisms cannot be convincingly demonstrated by in vitro or clinical studies. Therefore, humanized mouse models must be used.

# Supporting Documents

1. **Attach supporting files:**

| Document Name | Date Modified |
|---|---|
| There are no items to display | |

# Procedures Appendix:




## Procedure Identification: Mao: Exsanguination via Cardiac Puncture, Under Isoflurane Anesthesia, Anesthesia Machine

1. *** Name of the procedure or surgery:**
   Mao: Exsanguination via Cardiac Puncture, Under Isoflurane Anesthesia, Anesthesia Machine
2. *** Select procedure type:**
   Euthanasia
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

**If yes,**

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

# Euthanasia

1. *** Method of euthanasia:**
   Other - Physical
2. **Describe procedure:**
   The mouse is placed in an induction chamber and 1-5% isoflurane (pharmaceutical grade) is administered until the mouse is recumbent. If more than momentary anesthesia is required, the mouse is removed from the chamber and positioned in a nosecone with 1-5% isoflurane administered to maintain anesthesia.

   A surgical plane of anesthesia is confirmed by lack of response to toe pinch, change in respiratory character and rate.

   Terminal cardiac puncture is performed by inserting a needle with attached syringe into the heart percutaneously from either under the sternum or from the left lateral thoracic wall near the point of the flexed elbow. Maximum blood volume is collected.

   If less than 500uL of blood is collected, a secondary method of euthanasia will be used. The secondary method of euthanasia will be one of the following: placed in a bag filled with CO2, decapitation, thoracotomy/tissue collection, cervical dislocation by certified personnel.

   Isoflurane is administered using an anesthesia machine that has been adequately tested and certified. Waste gas is scavenged using either an activated charcoal canister (e.g., F/Air), active scavenging system, or by conducting the work within a certified fume hood.
3. *** Will anesthesia be used?** **Yes** No
4. **Describe how death will be confirmed:**
   Death will be confirmed by lack of respirations and heartbeat.
5. **Is this method approved by the AVMA Guidelines on Euthanasia (2013)?**
   **Yes** No

# Procedure Documents

1. **Supporting documents:**

| Document Name | Date Modified |
|---|---|
| There are no items to display | |





# Procedure Identification: CO2 Followed by Secondary Methods (>10 days of age)

1. *** Name of the procedure or surgery:**
   CO2 Followed by Secondary Methods (>10 days of age)
2. *** Select procedure type:**
   Euthanasia
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

**If yes,**

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

# Euthanasia

1. *** Method of euthanasia:**
   CO2 Overdose
2. **Describe procedure:**
   CO2 will be administered from a compressed commercial cylinder utilizing a flow meter to deliver 30-70% of the chamber volume/minute. Total gas exposure will be at least 5 minutes, with gas flow being maintained for at least 1 minute after apparent clinical death. A timer will be used to ensure adequate length of exposure.

   Secondary method of euthanasia will be one of the following: placed in a bag filled with CO2, decapitation, exsanguination, thoracotomy/tissue collection.
3. *** Will anesthesia be used?** Yes **No**
4. **Describe how death will be confirmed:**
   Death will be confirmed by lack of respirations and heartbeat.
5. **Is this method approved by the AVMA Guidelines on Euthanasia (2013)?**
   **Yes** No

# Procedure Documents

## 1. Supporting documents:

| Document Name | Date Modified |
| --- | --- |
| There are no items to display | |

1. *** Select the funding organization:**
   National Institute of General Medical Sciences (NIGMS)

   **If Other was selected in question 1, provide Funding Organization:**

2. *** All animal use projects must be reviewed for scientific merit prior to initiating animal use. Choose the required reviews for this project:**
   Will be conducted by a funding agency prior to the start of the project

3. **Provide name of the committee or the department reviewer** (Required if "Has been conducted by my department or school and has been found to be scientifically meritorious" was selected):

4. **eGC1 Number(s):**(assigned internally)

# Experiments Appendix:

## Germ-free rederivation of humanized hPXR/hCYP3A4 and hCYP3A4 mice

1. *** Experiment name:**
   Germ-free rederivation of humanized hPXR/hCYP3A4 and hCYP3A4 mice
2. *** Species:**
   Mice
3. **If other was selected, provide a species:**
4. **What is the scientific goal of this experiment:**
   This study will first create germ-free (GF) humanized hPXR/hCYP3A4 and hCYP3A4 mice and then compare the expression and activity of human CYP3A4 between these conventional (CV) and GF mice.
5. *** Describe the animal experience in the experiment, from enrollment in the study to the final endpoint, including all procedures in chronological order and the minimum time between procedures. We encourage using bullet points, timeline, table, or a flow chart as appropriate:**

   ***Germ-free rederivation***. We will obtain CV humanized *hPXR/hCYP3A4* and *hCYP3A4* mice from a collaborator at the University of Pittsburgh. CV humanized *hPXR/hCYP3A4* mice express human PXR and human CYP3A4, but are deficient in the mouse *Pxr* and *Cyp3a* genes. CV humanized *hCYP3A4* mice express human CYP3A4 alone, but are deficient in the mouse *Pxr* and *Cyp3a* genes. Germ-free rederivation of CV humanized *hPXR/hCYP3A4* and *hCYP3A4* mice will be carried out at the University of Washington (UW) Gnotobiotic Animal Core (GNAC). Germ-free rederivation requires 8-12 females and 4-6 males of each mouse strain. In case that genotyping is needed, tail tip (1-2 mm) will usually be collected from mice before weaning (21 days of age). If tissues for genotyping have to be collected from mice greater than 28 days of age, ear punch will be done to obtain tissue for genotyping.

   ***Animal studies***. All mice (12-14 weeks old) will be sacrificed and tissues (liver and intestinal sections and other tissues) will be collected between 9:00am and noon to minimize variations in *CYP3A4* gene expression due to the circadian rhythm. Briefly, under anesthesia (isoflurane), blood will be collected via cardiac puncture. Then, intestine will be removed. To collect intestinal sections, small and large intestinal contents (SIC and LIC) will be flushed using PBS and centrifuged at 4°C to isolate the solid intestinal content pellets. Intestinal tissues will then be separated into duodenum, jejunum, ileum, and large intestine (colon and cecum). Liver and other tissues (e.g., brain and kidney) will also be isolated. Liver, intestinal sections, intestinal contents, plasma samples and other tissues will be snap-frozen in liquid $N_2$ and stored at -80$^{o}$C until further analysis to determine the expression and activity of human CYP3A4 and other related analysis (e.g., untargeted metabolomics analysis of plasma samples). CV and GF humanized *hPXR/hCYP3A4* and *hCYP3A4* mice aged 12-14 weeks will be used. Male and female mice will be analyzed separately.

   In case that we need to dispose any non-experimental mice (for example, if we get more mice than we need for our studies from breeding), these mice will be euthanized with CO2 followed by secondary methods using a standard procedure and disposed through DCM.

Animal Sex:
Female
Male

Animal Ages:
7-10 weeks for breeding and 12-14 weeks for animal studies.

Animal Size:
up to 20 g

**6. Select experimental procedures:**

| Name | Type | Version | Scope |
|---|---|---|---|
| CO2 Followed by Secondary Methods (>10 days of age) | Euthanasia | 2 | Standard |
| Mao: Exsanguination via Cardiac Puncture, Under Isoflurane Anesthesia, Anesthesia Machine | Euthanasia | 1 | Team |

**7. Monitoring protocol, including frequency and specific behavioral and clinical signs to be monitored. Include humane endpoints (criteria for euthanasia):**
For the breeding portion, animals are checked once per week by laboratory staff and every day by animal husbandry staff. Any animal exhibiting signs, such as >20% weight loss, body condition score of 2 or less, inability or reluctance to move when stimulated, or moribund condition, impairment of ability to eat, drink, or ambulate
normally, or labored breathing will be euthanized.

For blood collection, mice will be anesthetized before blood withdraw and tissue collection. Mice will be monitored closely for depth of anesthesia (good, too light or too deep), respiration (good, too fast or too slow), mucous membrane (pink, reddish or bluish), and activity. Veterinary staff will be consulted if necessary.

## 8. If there is expected mortality (spontaneous death) in this experiment:

**a.** Procedure/condition associated with mortality:
None expected

**b.** Estimated mortality rate, i.e. percentage of animals expected to die spontaneously (not via euthanasia) or need to be euthanized as a result of the procedure. (Be sure to account for this in your animal number calculations):
N/A

**c.** Explain why euthanasia is not possible or appropriate:
N/A

**9. Will some animals live out their natural lifespan as part of this experiment? If so, indicate their use and describe the monitoring plan for aged animals (e.g., rodents >18 months of age), including frequency, behavioral and clinical**

**signs to be monitored and criteria for euthanasia.**

N/A

**10. * Total number of animals used in this experiment:**(including all the animals to be produced)

111

**a. Justify total number of animals used in this experiment:**

1) For germ-free rederivation, we need 12 female CV and 6 male CV mice of each mouse model (humanized hPXR/hCYP3A4 and humanized hCYP3A4). Therefore, we need 36 mice for germ-free rederivation.

2) For animal studies, according to means ± SEM of *Cyp3a11* mRNA and the difference in mean between CV and GF mice [1], we performed power analysis and found that 6 mice per group will be needed to detect a difference between CV and GF mice at a 0.05 significance level with power of 0.8. Therefore, 6 male CV, 6 male GF, 6 female CV, and 6 female GF humanized *hPXR/hCYP3A4* mice will be needed for this analysis (in total 24 for CV and GF humanized hPXR/hCYP3A4 mice). The same number (24) of CV and GF humanized *hCYP3A4* mice will be needed. In addition, 2 male GF mice and 4 female GF mice of each mouse model as well as 2 male CV and 4 female CV mice of each mouse model will be needed for breeding. Therefore, we need 60 mice for animal studies (48 experimental; 12 for breeding).

[1] Selwyn FP et al. RNA-Seq Quantification of hepatic Drug Processing genes in Germ-free Mice. Drug Metabolism and Disposition 43:1572-1580, 2015.

3) A small number of mice (5) will be requested for personnel training. Personnel training may include procedures such as anesthesia with isoflurane and collection of intestinal contents and tissues. In addition, we request 10 mice to cover other needs. Other needs may include the situation that more mice may be needed to reach a statistically significant difference between GF and CV mice if our power analysis underestimates the number of mice needed. We will also perform a number of analysis of the tissues and plasma samples collected such as quantitative proteomics of the liver and intestinal tissues and untargeted metabolomics analysis of plasma samples. Thus, if the tissues and plasma samples collected from 6 mice per group are not sufficient for all the analysis, we need more mice.

Together, we need 111 mice for germ-free rederivation and animal studies.

**11. Number of animals by pain and distress category:**(include each animal only once in the highest pain category)

**B:** 48
**C:** 63
**D:** 0
**E:** 0

**a. Justify the need for any animals in pain category E:**

N/A

**12. * Identify husbandry exceptions:**

| | Exception Type | Description and Justification |
|---|---|---|
| View | Mice - No husbandry or enrichment exceptions. | N/A |

**13. Supporting documents:**

| Document Name | Date Modified |
|---|---|
| There are no items to display | |

1. *** Exception type:**

   Mice - No husbandry or enrichment exceptions.

2. **Description and justification:**

   N/A

## 1. * Identify the location where animals will be used:

**a. For locations that are lab managed, provide justification for housing outside of the vivarium:**

N/A

## 2. * What species will be housed in this location?

| Common Name | Scientific Name |
|---|---|
| Mice | Mus |

**1. * Identify the location where animals will be used:**

ARCF Gnotobiotic ABSL1/2

**a. For locations that are lab managed, provide justification for housing outside of the vivarium:**

N/A

**2. * What species will be housed in this location?**

| Common Name | Scientific Name |
|---|---|
| Mice | Mus |

**1. Campus:**

Vivarium

**2. Vivarium:**

ARCF (Animal Research & Care Facility)

**3. * BSL Level:**

ARCF Gnotobiotic ABSL1/2

**1. * Identify the location where animals will be used:**

H153A Vivarium ABSL1

**a. For locations that are outside of the vivarium, provide justification for the use of this space:**

N/A

**2. * What species will be used in this location?**

| Common Name | Scientific Name |
|---|---|
| Mice | Mus |

**3. Describe how this location will be used:**

H153 will be used for all procedures including collection of blood and tissues.

**4. * If animals are left unattended in this location, provide an explanation and include maximum duration:**

N/A

**5. Describe how animals will be transported to and from this location, including container and route. (Note: use of private vehicles requires IACUC approval):**

Transportation of mice will occur at the time of arrival of CV mice to DCM from our collaborator at the University of Pittsburgh. GF mice also will need to be transported from UWGNAC facility to H153 for procedures. We will use cages covered by paper towels and the cages will be placed in a drape-covered cart for transportation of mice from ARCF to H153.

View: UW IACUC Select Room Level

1. **Campus:**
   Vivarium
2. **Vivarium:**
   H153A Vivarium
3. *** BSL Level:**
   H153A Vivarium ABSL1

**From:** Emily W. Clark <ewilkins@uw.edu>
**Sent:** Friday, October 9, 2020 2:18 PM
**To:** Qingcheng Mao
**Subject:** Re: IMPORTANT: Major HoverBoard upgrade in November

Hi Qingcheng,

Thanks for your message. All animal transfers/imports go through the Animal Operations system, which is part of DCM's process. If you have questions about the transfer, you can contact Animal Purchasing, animals@uw.edu.

As far as new locations and personnel, you can use the Create Amendment activity to add this information. These types of amendments are generally administrative and approved quickly.

Hope this answers your questions, but let me know if anything else pops up.

Thanks, and have a great weekend!
Emily

> On Oct 9, 2020, at 1:55 PM, Qingcheng Mao <qmao@uw.edu> wrote:
>
> Hi Emily:
>
> As you know, I have a new protocol that has just been approved. In the new protocol, we propose to create germ-mice. The conventional mice will be requested from University of Pittsburgh. Now I have two questions:
>
> 1) Who in the UW Office of Animal Welfare I should contact to complete the MTA for request of animals and coordinate transfer of mice from University of Pittsburgh to UW? I guess there is a lot of paper work to do for animal transfer.
> 2) In the approved protocol, I said I will use H153A (a room belongs to my department) for animal housing. However, I now think I should use the UW CompMed SPF animal facility to maintain the animals. How do I modify the protocol? I would also like to add some staff at UWGNAC to my protocol as they will help us for GF rederivation.
>
> I greatly appreciate your help. Best regards, Qingcheng

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**From:** Emily W. Clark [mailto:ewilkins@uw.edu]
**Sent:** Monday, August 31, 2020 10:46 AM
**To:** Emily W. Clark
**Subject:** IMPORTANT: Major HoverBoard upgrade in November
**Importance:** High

Hello!

As your liaison in the Office of Animal Welfare, I'm contacting you to let you know that **HoverBoard will undergo a major upgrade in early November.** The

expected system downtime is **11/6-11/9/2020**. OAW will also be short-staffed beginning in mid-September due to extended leave for two liaisons/reviewers.

What does this mean for you? While our office is making efforts to minimize any disruption to response and review time, it is possible that these combined events will increase the time needed to create and review IACUC submissions, including new protocols, triennial reviews, and amendments to existing protocols. **Please take some time now to think ahead as much as possible regarding your plans with your IACUC protocol(s).** If you plan to submit any new protocol items, please plan to work on those items and have them submitted well in advance of when approval is needed (at least 4 weeks for amendments, at least 8 weeks for new protocols and triennial reviews).

Please don't hesitate to get in touch with questions or if I can be of assistance.

Thank you,
Emily

**Emily W. Clark, PhD**
Review Scientist
Office of Animal Welfare Research Support Services

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<image002.jpg>

UNIVERSITY of WASHINGTON

APPROVAL OF NEW PROTOCOL SUBMISSION

September 8, 2020

Dear Qingcheng Mao,

This email serves as written notice of animal use approval by the Institutional Animal Care and Use Committee (IACUC).

*To help us better serve you, please take this **3 question survey** about your experience with the review process.*

| | |
|---|---|
| Type of Review: | Designated Member Review |
| Short Title of Protocol: | 4035-07: Germ-free hPXR/hCYP3A4 mice |
| Investigator: | Qingcheng Mao |
| HoverBoard ID: | PROTO202000114 |

Please note the approval and expiration date listed. All animal use protocols must be renewed annually from the date of IACUC approval, independent of project or funding dates. Please refer to the assigned protocol number for all animal orders and future correspondence with the IACUC.

**Protocol Approval Dates:** 9/8/2020 to 9/7/2023.

**Next Triennial Expiration Date:** 9/7/2023

If you have any questions, contact OAWRSS at oawrss@uw.edu.

Sincerely,

Office of Animal Welfare



OFFICE OF ANIMAL WELFARE