UNIVERSITY of VIRGINIA
OFFICE OF THE VICE PRESIDENT FOR RESEARCH
INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

06/22/2021

Dr. Kenneth Walsh
E0:MD-INMD CV Medicine
Delivered via email to kw9ar@virginia.edu

Dear Dr. Walsh,

Re: Protocol No. 4205
"EFFECTS OF CLONAL EXPANSION OF HEMATOPOIETIC CELLS CARRYING SOMATIC MUTATIONS IN VARIOUS DISEASE MODELS"

Initial Review Date: 01/12/2021
First Annual Review Date:
Second Annual Review Date:

The Animal Care & Use Committee (ACUC) is pleased to inform you that full approval has been given for:

___ New Protocol *[Approved for a three year term with annual reviews the month in which the initial approval was given becomes the anniversary month for Annual Review and Continuation after Three Years]*

_X_ Modification *[Approved 06/08/21 and added to protocol]*

___ Annual Review *[Approved each year in anniversary month during three-year term]*

___ Continuation After Three Years *[Approved for additional three year term with annual reviews]*

___ Minor Modification *[Approved and added to protocol]*

A copy of the approved protocol is provided for your reference. Notification from the ACUC Office to the Principal Investigator and to the Contact Person will be sent thirty to sixty days prior to the anniversary month. It is the responsibility of the Principal Investigator to ensure that if the protocol is to continue without interruption, Annual Review and Continuation after Three Years proposals must be approved before the end of the anniversary month. **Any change to or deviation from the research described in this protocol must be reviewed and approved by the ACUC prior to implementation!**

Sincerely,

Carl E. Creutz, Ph.D.
Chairman, Animal Care & Use Committee
Professor of Pharmacology
Harrison Professor of Medical Teaching in Pharmacology

Contact Person: Doviak, Heather (hd3be)

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- Removal of vital organs (e.g. heart, lungs, brain)
- Opening of the chest cavity to induce bilateral pneumothorax
- Cutting the major blood vessels to induce exsanguination (e.g. aorta, vena cava)
- Documented cardiac arrest (electrocardiogram)
- Absence of heart beat for 1 minute while using stethoscope

These procedures may not be performed in conscious animals without specific IACUC approval.

Main Procedure

Procedures, which require Humane Endpoints and Criteria for Euthanasia, to be performed on animals used in this procedure section:

- organ or system failure (diabetes mellitus)

Main Procedure Description:

The first step in this species procedure is to create the model of clonal hematopoiesis. This will be done using one of two strategies:

(1) BONE MARROW TRANSPLANT (BMT) with irradiation: The recipient animals will be transported to the irradiator in MR4 or Pinn Hall and placed in a sterile pie cage or other appropriate caging for irradiation. They will receive a total dosage of 1100 rads, in two doses, 550 rads approximately 3 hours apart. After irradiation, they will be returned to the housing room. Approximately 1 hour after the second irradiation dose the mice will be injected with ~200uL of donor bone marrow cells retro-orbitally. Recipient mice are 8 weeks of age or older so this volume is suitable for the size of the mouse at the time of the injection. For the injection, the mice will be anesthetized in an induction change with 5% isoflurane in medical air. After the retro-orbital injection, a drop of proparacaine will be put in the eye. Recipient mice will be maintained on antibiotics (pharmaceutical grade) supplemented in the water (5mM sulfamethoxazole, 0.86 mM trimethoprim) to protect them from infection for at least 2 weeks after the BMT. During this time the mice will also receive Clear H2O diet gel 76A.

(2) BONE MARROW TRANSPLANT (BMT) without irradiation: The non-irradiated recipient mice will be injected with ~200uL of donor marrow cells retro-orbitally on 3 consecutive days (best attempts will be made to alternate eyes for each injections). Recipient mice are 8 weeks of age or older so this volume is suitable for the size of the mouse at the time of the injection. For the injection, the mice will be anesthetized in an induction chamber with 5% isoflurane in medical air. After the retro-orbital injection, a drop of proparacaine will be put in the eye.

Once the clonal hematopoiesis model is created the mice will undergo the following procedures: DSS administration, blood withdrawal, and MRI imaging.

DSS administration: Mice in the UC groups will receive drinking water with 2% DSS for 7 days followed by a 14 day washout period. This cycle will repeat 5 times over 15 weeks.

MAGNETIC RESONANCE IMAGING (MRI): At baseline and at the 3 endpoints after the first DSS treatment mice may undergo MRI. The mice will be transported to the Molecular Imaging Core in MR4. The MRI procedure will be conducted in accordance with Dr. Stuart Berr's ACUC protocol #3539.

BLOOD WITHDRAWAL: Blood collection is required during these experiments to monitor blood cell counts after transplant of bone marrow and creation of the disease model. This data allows us to determine if the transplants were successful and to monitor certain cell counts (e.g. CD45.1, CD45.2, WBCs) during the duration of the experiment. Blood collection will start approximately 4 weeks after BMT and will be performed, at most, once every 2 weeks for the duration of the experiment. For each blood collection, ~100uL of blood will be withdrawn from the retro-orbital sinus. The mouse is anesthetized with isoflurane in medical air until there is no response to a toe pinch. A capillary tube is then used to collect the blood sample and transferred into an EDTA coated tube. The bleeding is stopped using gauze if needed and a drop of proparacaine is placed in the eye. Best attempts are made to alternate eyes for each blood sample.

Animals will be euthanized at 3 different time points after first receiving DSS (e.g. week 4, week 7, and week 15).

Post-Procedural Details:

- After bone marrow transplant in irradiated mice, the animals must be maintained on antibiotics and diet gel (as described above).

- We will screen animals for lack of bone marrow engraftment. If animals appear to be in distress or debilitated veterinary treatment will be started and if they do not improve they will be euthanized immediately.

- Mice will be weighed before starting DSS treatment. During the DSS treatment, mice will be weighed at least once a week. If a mouse has lost 15% body weight from baseline that particular mouse will be weighed daily.

- Mice are observed after blood collection to detect any signs of pain or distress. If an animal loses more than 10% of the total circulating blood volume (~0.7% of body weight; 210ul for a 30g mouse) it may become dehydrated. If this happens, the animal will be provided replacement fluids via IP or SQ injection of sterile saline.

- Fecal output will also be observed for the presence of blood. Feces will be tested using a product such as Seracult.

Humane Endpoints and Criteria for Euthanasia

What is your definition of humane endpoint(s) as it applies to these studies?
Be specific, e.g. greater than 15% weight loss from baseline or age matched controls, lack of food and/or water consumption for more than 48 hours, extreme lethargy, hindlimb paralysis, neurologic signs, etc.

The following humane endpoints will require veterinary intervention and if the mouse doesn't improve with treatment it will be euthanized:

- The mouse loses >20% body weight from the baseline weight

- Severe lethargy

- Pallor

- Dehydration

How frequently will animals be observed and assessed for these criteria?
If the frequency of observations will change over time (e.g. once weekly after tumor implantation, twice weekly after tumor growth is evident, and then daily when tumor reaches 50% of the approved size limit), this information must be provided.