

Standard Operating Procedures for Rodent Irradiation & Bone Marrow Transplant Procedures (LabAn SOP 1)

The procedures described in this SOP have been reviewed and approved by the IACUC. If you intend to irradiate mice according to the procedure described in this SOP,

- **You must** state, in your IACUC protocol, your intention to irradiate mice and perform bone marrow transplants according to the procedures described in this SOP.
- **You must** describe in your IACUC protocol any deviations from the approved SOP.
- **You must** attach a copy of this SOP to your protocol when submitted for IACUC review.
- **You must** provide the following additional information in your IACUC Protocol

- An explanation of the purpose of the study
- The number of animals; including those irradiated as well as the bone marrow donors.
- A description of other procedures performed on these animals either prior or subsequent to irradiation and bone marrow transplant.

- **You must** list the number of animals that will be irradiated in Pain/Distress Category E.

Rodent Irradiation and Bone Marrow Transplant Procedures

1. Animal preparation for irradiation:
 - a. Acidified water – Animals to be irradiated will be placed on acidified water (pH 2.5-3.0) from 1 week prior to 2 weeks post irradiation.
 - b. Antibiotic treatment – Prior to irradiation animals will be treated with antibiotic with the intention of eliminating *Pseudomonas aeruginosa* from the gastrointestinal tract. The antibiotic will be selected by the investigator in consultation with the Attending Veterinarian. Directions for preparation and administration of the antibiotic will be provided by the Attending Veterinarian. Antibiotics will be chosen from the following list or will be specified in the IACUC Protocol
 1. Gentamycin 1-1.2mg/ml in the drinking water
 2. Neomycin 1.1 mg/ml in the drinking water
 3. Neomycin 1.1 mg/ml & Polymyxin B sulfate 1000 U/ml in drinking water
 4. Baytril .17 mg/ml in the drinking water
 5. Sulfamethoxazole (or Sulfadiazine) with Trimethoprim mixed with drinking water to a final concentration of between 0.65 and 1.6mg/ml

2. Rodent Irradiation – Exposure protocols

Last Approved by the IACUC 2/15/2016

RFP revision 4/24/2015

- a. Single exposure - Mice will be irradiated with 800-950 rads (exact dose is strain dependent) at a rate of approximately 80 rads/minute. This dose rate is achieved when the irradiator is used with out attenuators in turntable position 3 for a period of 10-12 minutes.
 - b. Split exposure – The total dose will be delivered in two 5-6 minute periods of exposure separated by 3 hours.
 - c. Reduced exposure – Strains such as SCID mice that are highly sensitive to irradiation will receive a reduced total dose of 350 to 400 rads.
 - d. Reduced exposure - When full reconstitution is not needed, such as adoptive transfer or transfer of leukemia stem cells.
3. Transplant procedures –
- a. Bone marrow donors will be euthanized with CO₂ for marrow collection.
 - b. Bone marrow cells in sterile media will be injected into the tail vein of the irradiated recipients. The number of cells will depend on the experiment and will range from 2×10^2 – 5×10^6 in 150-200 ul.
4. Post procedural Observation and Care – Whole body irradiation damages the bone marrow and potentially the GI tract leading to increased susceptibility to opportunistic infections. Although successful bone marrow transplant should be protective, it may be expected that the transfer will occasionally be unsuccessful.
- a. Potential signs of complication will be non specific and may include the following.
 - i. Poor body condition/weight loss
 - ii. Rough coat, inactivity, hunched posture
 - iii. Death without prior signs of illness
 - b. Monitoring
 - i. Animals will be monitored daily by the investigator following irradiation and bone marrow transplant.
 - ii. Mildly affected animals (rough coat, inactive, hunched posture) but still in good body condition will be monitored for further deterioration.
 - iii. More severely affected animals displaying obvious weight loss and poor body condition (body condition score of 2-) will be removed from the study. An Animal Resource Program veterinarian will be consulted for possible diagnostic testing.

Shepherd Cesium Irradiator

1. Location – Henning Building, room 24.
2. Irradiator Oversight – University Isotopes Committee, Jeff Leavey, Dr. Robert Paulson. The room is maintained by a security system. Access to the room and keys for the use of the irradiator are controlled by Dr. Robert Paulson.
3. Personnel – Only those personnel who have completed training provided by the University Isotopes Committee will be permitted to use the irradiator. At the

present time Robert F. Paulson, Laura Goodfield, Emily Finch, Bill Turbitt and Shawntanee Collins are approved users (April 2015).

4. Animal Housing – Animals will be housed in either static or ventilated microisolation housing.

Consideration of alternatives to whole body irradiation as a means of ablating host bone marrow prior to bone marrow transplant: The results of a literature search for alternatives are attached to this SOP and will be updated yearly.

- a. Sources used: [NOTE: At least two sources should be listed!]
PubMed and Agricola
- b. The date the search was completed:
August 2, 2016
- c. The years covered by the search:
PubMed – 1953-present
Agricola- 1960-present
Google Scholar 2015-present
- d. Keywords and/or the search strategy used:
Bone marrow Transplant And mouse
Myeloablation and Transplant and Mouse
Alternatives to Irradiation And Transplant and Mouse
- e. Brief statement summarizing the outcome of your research stating that no acceptable alternatives were found or why potential alternatives cannot be used.

The search revealed that irradiation as a method for myeloablative pre-conditioning prior to transplant is the standard procedure in the field. The potential alternatives have problems that make them less than ideal for an experimental system. One alternative is the use of mice that mutations in the Kit receptor. These mice have a stem cell defect that allows them to be transplanted without irradiation. The drawbacks of Kit mutant mice are that they are difficult to breed due to the fact that many alleles are also sterile. In addition you are limited to the strains that contain these mutant alleles (histocompatibility issues), which limits their usefulness. A second alternative is to use myeloablative drugs such as busulfan. The use of chemotherapy drugs does not have any advantage over radiation and introduces significant Biohazard issues as these chemical will contaminate bedding after treatment. A protocol for the use of Busulfan is presented at Peake et al. JOVE (2015) Apr 1;(98):e52553. A third option is low dose radiation. This technique could be used in some cases where full reconstitution is not needed, such as adoptive transfer of immune cells or leukemia cells. However when full reconstitution is needed, low dose radiations tends to cause mixed chimerism which would complicate the analysis of the experiments. High and low dose irradiation protocols are compared at Leon-Rico et al. Laboratory animals (2015) 49(9):132-141. Finally, several techniques have

been developed for the clinic such as blocking T cell responses. Although this procedure has advantages in the clinic, in an experimental setting the potential for mixed chimeras would complicate the analysis of the experiments.