Policy 15 – Mouse Total Body Irradiation
Version 1.0
Approval Date: 11/14/12

Purpose - This policy describes mice exposed to total body irradiation (TBI) emanating from a Cesium 137 source (gamma radiation).

Background - Ionizing radiation causes breaks in the DNA helix, primarily affecting mitotically active cells such as those of the hematopoietic and gastrointestinal tracts. The degree of cellular damage depends on the dose of radiation, age, and strain of the mice. In general, C57Bl/6 mice are more radio-resistant than BALB/c mice. B6 mice can typically tolerate radiation doses of 1000 to 1100cGy, however, the LD50 of BALB/c mice is about 880cGy [2]. Ionizing radiation experiments are most commonly used in the fields of immunology and cancer biology.

Definitions –

- **Gamma irradiation** is one type of ionizing irradiation. Sources are typically Cesium 137, Cobalt 60, or high-energy X-rays. This policy refers to Cesium 137 irradiators.

- **Gray (Gy)** is the SI unit of absorbed radiation.

- **Rad** is a largely obsolete unit of absorbed radiation; 100 Rads = 1 Gray.

- **Fractionation of dose**: The total irradiation dose can be split into two or more equal parts separated by a time interval (usually 2-12hrs) in order to minimize morbidity and mortality.

Policy –

Requirements for animals on study exposed to irradiation -

- Irradiation must be scientifically justified in the IACUC protocol. Animals exposed to radiation must be monitored and findings documented daily on the post-procedural cards for the first 14 days. However, after protocols have been established and PI has experience with the particular strain of mice and dose, three times/week monitoring (with documentation) is acceptable. If animals experience morbidity or mortality daily checks are required.

- The planned dose of irradiation (dose range) and the frequency of the radiation must be specified in the IACUC application.

- Fractionated doses should be considered, if appropriate, to reduce morbidity and mortality.

- Unless literature references are available, a pilot study to determine the best dose is recommended if the PI is starting a new study or using a new strain of mice.

- IACUC policies 3 and 5 for humane endpoints must be followed. However, body weight loss up to 25% is acceptable during the first 2 weeks post-irradiation.

- The irradiation procedure is considered category “E” (USDA classification).
Effects of total body irradiation (TBI):

Irradiated animals experience 5 to 10 days of post-irradiation related sickness. Irradiated mice generally recover within 2-3 weeks [2].

**Appearance**: Mice may appear lethargic with a rough coat and assume a hunched posture due to radiation-induced tissue damage and inflammatory responses.

**Dehydration**: Early after irradiation mice can become dehydrated due to decreased water consumption and diarrhea that often develops from radiation-induced damage to the intestinal epithelium.

**Body weight loss**: Body weight loss up to 25% due to inappetance and diarrhea peaks at about 7 days post-irradiation. Depending on the dose and whether immune reconstitution had been provided, recovery will usually occur in 2 to 3 weeks. Mice may never regain their original, pre-irradiation body weight.

**Anemia**: Animals may appear pale, especially around the nose and paws.

**Intestinal bleeding**: Dark stool (melena) or blood stained perennial area may be present.

**Infection**: Severe bacteremia/septicemia may occur as a result of translocation of bacteria from the GI tract into the blood stream [3].

**Graft Versus Host Disease (GVHD)**: Successful survival of a bone marrow graft requires suppression of the host’s immune system. If the irradiation dose is too low, Graft Versus Host Disease (GVHD) will ensue. As in humans, older mice are more prone to develop GVHD.

**Graying of hair coat**: Black mice, such as C57BL, will frequently turn gray after irradiation.

**Development of secondary neoplasia**: The development of neoplasia after irradiation has been reported in humans and many large animal species. This may occur in mice on long-term studies as well.

**Incisor damage**: One non-neoplastic illness reported in mice is incisor damage and subsequent difficulty in eating. Giving softened food during the recovery phase is required.

Care of irradiated mice:

**PIs (or designated research staff) are responsible for care of irradiated animals.** Vivarium staff will provide special care in an emergency and in such a case the PI will be charged accordingly. For the first 14 days animals must be checked daily or 3X/week, with their condition and care documented on the rodent post-procedure monitoring card (blue cards). Care, especially during the first week, must ensure the animals are as comfortable as possible. This includes keeping them clean, hydrated, and having ready access to moistened food and Napa Nectar, if indicated. BCS or body weight
measurement should be performed and recorded in the Blue cards until mice return to normal condition, usually within 2-3 weeks.

**Use of antibiotics in the drinking water** - Administration of antibiotics in the drinking water may minimize bacterial contamination within the water source and potentially decrease the burden of gastrointestinal bacteria. Bacterial translocation from the intestinal tract after irradiation is a common source of systemic infection. PI is responsible for placing rodents on antibiotic water a few days before the scheduled irradiation in order for the animals to acclimate to the taste. Rodents are kept on antibiotic water for at least 14 days and up to 28 days post-irradiation.

**Making drinking water readily available** - Irradiated mice will suffer from radiation sickness and will not feel well for the first 7-14 days. It is important to provide easy access to water.

**Napa Nectar** must be provided on the bottom of the cage during the first 14 days if morbidity or mortality is observed. Napa Nectar is available in the animal room free of charge. Placing a new Napa Nectar in the cage daily can be done by research staff or by Vivarium staff for a fee. Napa Nectar becomes contaminated with fecal material quickly and must be replaced daily.

**Provision of softened food** - Giving softened food during the recovery phase is required. Powdered chow is available in the rodent housing rooms and should be mixed with water and served in a small Petri dish on the cage floor. Pellets moistened with water dry up easily and are not recommended. Placing a new moistened food dish in the cage must be done by the PI (or research staff) daily or can be done by Vivarium staff for a fee.

**Housing** - It is important to realize that even after bone marrow transplantation, lethally irradiated mice are severely immunosuppressed for the first two weeks and providing a completely sterile environment (cage, food, and water) is recommended [2] and is required if post-irradiation complications occur.

**Recommended antibiotic drugs and preparation (for water bottles):**

<table>
<thead>
<tr>
<th>drug</th>
<th>stock conc</th>
<th>dose</th>
<th>recipe</th>
<th>frequency</th>
<th>duration</th>
<th>final conc in bottle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>50mg/ml</td>
<td>134mg/kg/day</td>
<td>2.6ml stock +250ml H₂O</td>
<td>Change bottle every 3 days</td>
<td>14-28 days</td>
<td>0.52mg/ml</td>
</tr>
<tr>
<td>Baytril</td>
<td>22.7mg/ml</td>
<td>40mg/kg/day</td>
<td>1.7ml stock + 250ml H₂O</td>
<td>Change bottle every 3 days</td>
<td>14-28 days</td>
<td>0.15mg/ml</td>
</tr>
<tr>
<td>Sulfamethoxazole-Trimethoprim (SMX-TMP)</td>
<td>40/8 mg/ml</td>
<td>220/42 mg/kg/day</td>
<td>5.2ml stock + 250ml H₂O</td>
<td>Change bottle every 3 days</td>
<td>14-28 days</td>
<td>0.82 / 0.16 mg/ml</td>
</tr>
</tbody>
</table>

**Any exceptions to this policy must have IACUC approval**
References –

1. Boston University policy for Irradiation of Rodents:
http://www.bu.edu/orccommittees/iacuc/policies-and-guidelines/irradiation-of-rodents


4. RWJMS IACUC Policies 3 and 5