Protocol for Pre-Induction Transplants of Mice

Mouse irradiation:
- Irradiate mice 520 rads x 2
- 3 hours apart
- max. 20 animals in chamber at a time
- must be an approved user of irradiator and has access to the MSRB mouse facilities

Preparation of cells:
- Determine donor mice to be used for transplant
- Euthanize animals and take blood sample for CBC
- Remove spleen and take organ wt.
- Remove both upper and lower leg bones from the animal and put into 60mm dish containing 2%FBS/HBSS buffer, (FACS Buffer) and put dishes on ice
- Leg bones are separated by hyperextension at the knee joint with in a kimwipe and then removing excess tissue in the process.
- Make sure to remove the knee cap from either the femur or tibia that it separates with.
- Clean the bones very well by removing any tissue and cartilage by using a Kimwipe tissue. Cleaning of the bones will maximize the collection of the flushed bone marrow cells
- **Femur**: One the bone is cleaned and knee cap is removed then the access to the canal is available at the knee cap end for a syringe. At the head of the femur, snip the ball of the femur off with scissors to allow an exit for the flush
- Prepare a 60 mm dish with a cell filter for collection of BM flush.
- Wet the filter with a small amount of FACS buffer so cells won’t stick to the filter.
- Take a 3 ml syringe and fill with FACS Buffer. Take a 27g ½ inch needle connect it to the syringe and insert at knee joint end and flush the contents into the filter in the dish using all 3 ml of buffer. Make sure so use some to rinse the outside of the bone as well.
- **Tibia**: clean the bone well before flushing. Cut the ankle end of the tibia at the junction of the tibia and fibula. Take up a couple ml of buffer from the dish with filter and use that to flush the tibia out. Make sure so use some to rinse the outside of the bone as well.
- **REPEAT** these steps for the other set of leg bones. Using only the buffer from the dish.
• When done with the flushing, take an additional 1 ml of FACS buffer to rinse the filter out. Then remove the 4 ml of buffer and place it in a 5 ml FACS tube.
• Take another 1 ml of FACS buffer and then rinse the plate and place that in the FACS tube on ice.
• Once all of the donor leg bones have been flushed, cell counts are to be done using the following:
  o 10 ul of cell suspension from FACS tube
  o 40 ul of ACK buffer (cell lysis RBC)
  o Incubate for 5 min.
  o Add 50 ul of diluted Trypan blue (diluted 1:5 in PBS)
  o Add 100 ul of FACS buffer
  o Cells are in a dilution of 1:20
• Vortex and remove 20 ul and put on hemacytometer for counting.
• Count 4-4x4 squares and average. Calculate as follows:
  o Ave count x 20 x 10,000 = cells/ ml
  o Multiply - cells/ml x 5 ml volume of cells = total number of cells

Calculate the cell cocktail needed to insert 100 ul of cell suspension into each mouse for a 1:3 ratio of donor cells vs RPD cells

<table>
<thead>
<tr>
<th>Donor</th>
<th>Dose</th>
<th>RPD</th>
<th>Cells</th>
<th>#samples/final volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>#4605</td>
<td>200K</td>
<td>3.4 ml</td>
<td>510 ul</td>
<td>12/1200ul</td>
</tr>
</tbody>
</table>

*the volume of the RPD and donor Cells would be combined and then spun down at 1500 rpm for 5 min and then re-suspended at the 1200 ul volume for injection of 100 ul per mouse.

**Injection of Cells into irradiated mice:**
• Mice are taken from cage and put into glass drop jar containing .3 ml of isofluorane.
• Allow 15-20 sec of exposure to the anesthesia
• Remove the mouse and lay on blue pad in hood
• Pop eye out by pressing on both sides of the ocular cavity
• Insert needle from a 1cc tuberculin syringe in the left corner of the eye socket until you feel the bone of the eye socket and inject slowly making sure the cells do not come out of the eye.
• Mark the tail of the mouse to signify that he received cells.
• Mark the cage card with the date of transplant, donor mouse, genotype, cell number, Cage number, cell type
• If a cell injection was missed the mouse will die in approximately 7-10 days from bone marrow failure.
• Bleed in 4 wks to check for engraftment or treat with pIpC at this time