1. Applications for the development/induction/injection of tumors in mice and rats as scientific procedures at BRC must be submitted, reviewed and approved by the IACUC. To minimize the unnecessary pain and distress on these animals, all approved applications (protocols) will require close monitoring regarding their and the establishment of humane endpoints. Unless scientifically justified, **death as an endpoint for any animal is not accepted by the BRC IACUC**. Furthermore, best practices indicate that *endpoints earlier than moribund conditions* should always be used.

2. All scientific and facility staff should be familiar with both normal **and abnormal signs of health, behaviour and well-being** in mice and rats. In addition, they should be able to observe, assess and record these adverse changes due to tumor burdens. When abnormal and/or adverse cases are identified, the Principal Investigator (PI) (or his designate), BRC Veterinarians and respective BRC Head of Department (HOD) are notified. This is done through the BRC Veterinary Care Request form (can be obtained from any BRC Veterinary Staff), which is completed by the scientific or support staff and forwarded to the BRC Veterinarians. The PI is alerted on the animal's adverse health state and proposed follow-up procedure. It is then the PI's responsibility to work with BRC Veterinary staff and HOD for the well-being of these animals with abnormal/adverse signs.

3. In circumstances involving moribundity or unrelieved pain and discomfort, every attempt will be made to reach consensus with the PI bearing in mind the humane endpoints as described in the approved protocol. The final analysis and discharging of the BRC's animal care and use regulatory responsibility rests with the BRC Attending Veterinarian(s). All PIs are to update the emergency contact details with the IACUC as well as on the cage cards to facilitate communication between all parties.

4. Some tumors can cause significant changes in animal health. In particular, these **animals must be observed** for any indication of the following:

    - Presence of mass with ulceration
    - Restricted and/or abnormal mobility
    - Abdominal enlargement
    - Visual weight loss (consider using body condition score)
    - Decreased food/water intake
    - Changes in feces/urine
    - Lethargic/depressed activity
    - Restlessness
    - Vocalisation
    - Respiratory difficulty
    - Cranial deformity/neurological signs
    - Peri-anal soiling

- Rough/unkempt hair coat
- Hunched posture
- Skin pathology
- Jaw deformity/malocclusion
- Hypothermia

Observance of any of these changes in animal health by the scientific and/or facility staff must be documented in the BRC Veterinary Care Request form. Particular attention must be paid to the body system most likely to be affected by the tumor type (e.g. solid, ascitic, lymphoid, etc.) and organ system (e.g. skin, peritoneum, spleen, lymph node, etc.

5. All tumour bearing animals must be **observed on a scheduled basis** as described in the approved protocol. After a tumour or cell line has been injected, animals must be observed at least 3X/week. Once a tumour has reached 15mm in any dimension in mice, and 30mm in any dimension in rats, the animal must be **observed daily**, including weekends and holidays, and documented on the BRC "Watch Card". Although BRC monitors animals on a daily basis, it is equally important for researchers to monitor/observe these animals (i.e. mice with tumours greater than 15mm and rats with tumours greater than 30mm) more than 3X/week.

   For animals bearing more than one tumour, the application of humane end-points (as defined in Paragraph 21 in BRC/IACUC/002/F2) will be applied more critically with respect to euthanasia. No animal may have one or more tumours greater than 20mm in any dimension (for mice) and 35mm in any dimension (for rats).

6. The **site for injection** of solid tumours should be carefully chosen to permit room for tumour growth and to avoid unnecessary distress whenever possible (e.g. subcutaneous flank or back are considered to cause the least distress). Note: some tumor cells injected IP can grow SC, and animals should be monitored for skin ulcerations or other problems, such as impaired mobility.

7. The scientific staff must be aware of the parameters of the study, such as tumour growth potential and whether a tumour is likely to become ulcerated. Careful attention should be paid to any animal exhibiting an **ulcerated and/or necrotic tumor** especially for those on tumor regression studies. To deter cannibalization, any animal exhibiting an ulcerated or necrotic tumor should be separated immediately and singly housed until tumor regression is complete, or euthanized. A BRC "Watch Card" should be placed on each individual cage containing a mouse or rat with an open tumour, recording the date of the tumour opening on the card. Named Personnel on the approved protocols are responsible for ensuring adherence to:
   - IACUC approval regression timelines;
   - endpoints as described and approved in the animal study protocol;
   - IACUC Guidelines for Cancer Research in Rats and Mice; and

- Consideration of general human endpoints (see below) in animal study proposals (i.e. euthanizing the animal if the tumour becomes infected, interferes with ambulation/ eating/drinking, or the animal becomes otherwise debilitated).

8. All experiments must provide for a **humane endpoint**. As a general guideline, animals used in experimental procedures involving tumor development must be considered for euthanasia when anyone of the following conditions occur:

   - Tumour size (20mm in any dimension for a mouse and 35mm in any dimension for a rat) or metastatic growth interferes with animals' ability to exhibit normal behaviour such as grooming, moving, eating and/or drinking. Justification to exceed this size restriction must be approved by the IACUC in advance.
   - Tumor interferes with the animals' ability to exhibit normal behavior such as grooming, moving, eating and/or drinking.
   - Loss of 20% of their pre-study weight, or loss of more than 10% body weight in 24hrs
   - Emaciated appearance
   - Tumour becomes ulcerated, infected, or necrotic with break of overlying skin.
   - Palpation of tumor elicits a pain response
   - Animals become moribund, weak, comatose, unresponsive, or death appears imminent.
   - Animal shows signs of respiratory difficulty.
   - Animal shows signs of hypothermia (i.e. cold to touch, pale extremities)
   - Animal self-mutilates
   - Abscess(es) develops

9. The duration of the experiment should be as short as possible and defined in the IACUC protocol. The number of animals required for scientific and statistical evaluation of results should be kept to a minimum. Justification of animal numbers must be included in the IACUC protocol.

10. **The PI** must clearly define study parameters and humane endpoints in the Paragraph 21 in BRC/IACUC/002/F2". In order to facilitate monitoring process of the animals, there must be communication between the researchers and the BRC technicians, highlighting the humane endpoints to the BRC technicians. Failure to adequately monitor animals or to abide by the conditions in the approved study protocol will result in disciplinary action as determined by the IACUC.

11. All relevant study matters relating to husbandry and animal welfare involving tumour formation must be **highlighted** to BRC staff so that they are aware of these studies and be better informed to what humane end-points to look for.