Subject: Monitoring Tumor Growth in Rodents

BACKGROUND

The Guide for the Care and Use of Laboratory Animals (the Guide, NRC 2011) states that tumor models require special consideration of humane endpoints (p. 27). This Standard Operating Procedure (SOP) provides detailed descriptions of procedures to be followed unless alternate procedures have been outlined in an IACUC Protocol Application. If an investigator wishes to deviate from the approved SOP, all changes must be outlined and justified in the protocol application (approval of the protocol indicates approval of the deviation from the SOP for that project only).

IACUC Procedure

Subcutaneous Tumors (visually monitored)

1. Prior to the approximate time when tumor growth becomes evident, animals are monitored on an occasional basis. The frequency will depend on how soon tumor growth will become evident following injection. In most cases, once a week should be adequate. If the approximate time of tumor growth is not known, the animals in the initial group are very carefully monitored (i.e. every other day).

2. Once tumor growth has been identified, animals are monitored at least every other day. Some tumor lines will require daily checks. It is best practice to maintain records of tumor measurements. Frequency of measurement is dependent on tumor volume doubling time and overall animal condition.

3. It is the laboratory’s responsibility to adhere to endpoints described in this SOP and approved in the protocol.

4. The monitoring frequency is such that animals are euthanized as soon as the following signs develop; death is not an acceptable endpoint. Any ONE of the criteria below could require euthanasia:
   - Total tumor burden approaches 5-10% of the animal’s body weight (tumor would be approximately 1.6 cm in diameter for a 20g animal or 1.8 cm for a 30g animal). The burden is additive for animals with multiple tumors.
   - Two of the most commonly used formulas for determining each individual tumor volume are:
     1. \( mg = \text{length (mm)} \times \text{width}^2 \text{ (mm}^2\) / 2
     2. \( \text{volume (V)} = \text{length (mm)} \times \text{width (mm)} \times \text{depth (mm)} \times \pi/6 \) (where V is equivalent to mg)
   - Body Condition Score (BCS) <2/5
   - Interferes with locomotion, eating or drinking
   - Ulcerated tumor*

*Ideally, studies should be designed so that data has been obtained prior to tumors developing ulcerations. However, in some cases, ulcerations are due to the cell’s origin type (e.g. papillomas, or ductal cell origin). Allowance for ulcerated tumors must be in the approved protocol – with justification and clearly defined endpoints. Mice bearing ulcerated tumors require close monitoring and assessment. Tumor burden for ulcerated tumors is limited to 1gm or 5% body weight, whichever is less.

5. For human tumors, if an animal dies unexpectedly, show signs or symptoms of illness or tumor ulceration is observed, refer to the WSU IBC Memo: Reducing Biohazard Exposure Risks When Working with Human Cell Lines and Human Tissue in Animal Projects.

6. Euthanize animals using the method as outlined in the approved IACUC protocol.
Internal or Orthotopic Tumors (cannot be visually monitored)

1. Prior to the approximate time when tumor growth begins, animals are monitored on an occasional basis. The frequency will depend on how soon tumor growth begins following injection. Monitoring can range from daily to weekly. If the approximate time of tumor growth is unknown, the animals in the initial group are very carefully monitored (at least every day is recommended).

2. Once the animal exhibits signs that can be attributed to the tumor, the animal is monitored with increased frequency. The frequency will depend on the rate of growth, organ affected, and type of tumor.

3. The monitoring frequency is such that animals are euthanized as soon as the following signs develop; death is not an acceptable endpoint. The criteria for euthanasia (one of the following):
   - Total tumor burden approaches 5-10% of the animal’s body weight (percentage depends on the tumor’s internalized location – higher limit needs justification provided in protocol)
   - Lethargy
   - Animal unable eat, drink or ambulate
   - Respiratory distress or abnormal respiratory pattern
   - Body Condition Score (BCS) <2/5

4. Euthanize animals using the method as outlined in the approved IACUC protocol.

SUMMARY
Careful monitoring of animals with tumor burdens is essential from the animal welfare aspect. In addition, valid data can be lost if animals succumb without the appropriate examination of the animals or tissue.