INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
Advanced Sciences Research Center

POLICIES AND PROCEDURES

RODENT TUMOR AND CANCER MODELS

POLICY:
A. All injectable and/or implantable materials used for establishing tumors in animals must first be submitted for review according to the guidelines below. In addition, human tumor lines require IBC review and approval.
B. The visible size of the tumor is only one of the criteria used for determination of humane endpoint. The overriding consideration for humane endpoints of oncological experiments as well as spontaneous tumors must be the overall health of the animal. For subcutaneous tumors the maximum allowable size is 20 mm in diameter for a mouse or 40 mm diameter for a rat. If the animal is host to more than one tumor, this size is the maximum allowable size for all tumors combined.
C. All tumor-bearing animals must be observed on a scheduled basis (see I. D below) and findings documented to assess the progress of tumor growth and/or metastasis, and the general condition of the animal. Records must be kept and be available in the animal room with all pertinent information including time and frequency of monitoring sessions, the name of the person monitoring the animals, identification of the animals, protocol number, the number of animals displaying symptoms, types of symptoms, and any treatments given to the animals.
D. After a tumor or cell line has been injected animals must be observed and weighed at least once/week and findings documented. Once signs of morbidity have been identified or a tumor has reached 50% of the maximum allowable size or 10 mm in any dimension in mice and 20 mm in any dimension in rats, the animal must be observed daily, including weekends and holidays and findings documented.
E. The site of tumor implantation should be chosen to minimize damage to adjacent normal structures. Sites involving the special senses should be avoided. Clearly defined endpoints must be stated in the IACUC protocol if different from those stated in this policy.
F. In circumstances involving declining health status, morbundity, or unrelieved pain and discomfort, every attempt will be made contact the PI and to reach consensus with the PI bearing experimental endpoints in mind. However, the final analysis and discharging of the ASRC animal care and use regulatory responsibility rests with the Attending Veterinarian (AV).

GUIDELINE:
The Advanced Science Research Center Institutional Animal Care and Use Committee (IACUC) has developed the following guideline to help research investigators develop criteria for assessment of tumor burden on the welfare of rodents used in cancer experiments. This guideline is relevant to all investigators using models of neoplasia, including all subcutaneous, ascites-producing, liquid, or non-palpable tumors, particularly in rodent species. Humane interventions and endpoints should be determined and specified for all animals that will undergo tumor development as an expected part of the experimental protocol.

This guideline discusses the following topics:
- Monitoring and endpoints
- Implantable/Inducible tumors
- Evaluation of visible or palpable tumors
- Ascites produced by tumors
- Non-palpable or “liquid” tumors
MONITORING AND ENDPOINTS:
Animals that are on a tumor production study must be monitored as per policy. After a visible or palpable tumor is evident, the animals must be monitored by the laboratory group at least twice weekly. More frequent observations may be necessary as determined by the Attending Veterinarian (AV), based on tumor growth rate, study parameters, and general condition of the animal (possibly including weekends and holidays.) The overall wellbeing of the animal should take priority over precise tumor measurements in decisions regarding euthanasia or other interventions.

1. **Body Condition Score (BCS)** The general physical condition of the animal is an important factor in effectively following the progression of tumors in rodents. Scoring systems from “1” (emaciated/wasted) to “5” (obese) are often used. BCS is a helpful adjunct to assessment of overall health of the animal. It is important to note that treatments designed to affect tumor growth (such as chemotherapeutics) which are often part of tumor load studies, can lead to weight loss and poor body condition. Thus, the BCS becomes an important assessment tool in the tumor load experiments. Rodents must be euthanized if:
   a. The body condition score is 1/5; or
   b. The body condition score is 2/5 and the mouse has decreased activity/responsiveness; or
   c. The tumor affects the rodent’s gait or normal posture, ability to eat, urinate, or defecate independent of the size of the tumor; or
   d. An ASRC veterinarian determines that the animal should be euthanized for humane concerns.

2. An activity or adverse behavioral scoring system may also be effective, such as the Grimace Scale and nest-building, in placing objective measures for determination of humane endpoints in models with non-palpable tumors. If used, this should be discussed with the AV and included in the animal use protocol.

3. General clinical signs should be assessed. Any evidence of lethargy, change in ambulation, diarrhea, neurological signs (e.g. circling, head tilt) or increased respiratory effort should be reported by the protocol personnel to the vivarium staff immediately.

4. The known biology and effects of any individual tumor model should be described in the animal use protocol, including expected clinical signs, anticipated moribundity/mortality, interventions for the relief of pain and suffering, and objective criteria for the assessment of humane endpoints.

5. Quantitative measurements to determine tumor diameters and area are necessary.

6. Inspection and palpation to locate the sites of tumor growth, distension, ulceration and compromised mobility must be performed and documented.

7. Moribund animals must be euthanized immediately.

**IMPLANTABLE AND INDUCIBLE TUMORS**

**Rodent Pathogen Testing**
Because transplantable tumors, hybridomas, cell lines, and other biologic materials can be sources of murine viruses that can contaminate rodents (Guide), all transplantable murine tumors must be assayed for contamination with adventitious murine viruses to prevent the possible spread of pathogens into our rodent colonies.
IDEXX RADIL (http://www.idexxradil.com/) PCR Profile Impact II (mice) or Impact VI (rats) is required prior to the approval to inject rodent cells or implant rodent cells into recipient rodents. Please submit materials as part of the protocol application or directly to the AV to be reviewed prior to final approval by IACUC.

**Implantation Sites**

Tumor implantation sites should be chosen to minimize damage to adjacent normal structures. The IACUC recommends implanting tumors on the dorsum or flank of an animal, as these areas will likely have the least amount of site-related morbidity. If other sites are to be used, they should be described and justified in the animal use protocol.

- Sites involving the face, limbs or perineum should be avoided as there is little to no space for tumor growth and expansion, and they may interfere with eating and drinking. 6
- Intramuscular implantation should be avoided as this is considered to be painful due to the distension of the muscle by the tumor.
- Tumor implantation on the ventral surface of the body should also be avoided due to the risk of irritation to the tumor site in contact with the bedding and floor of the cage.

**Induction Agents**

Drugs used to induce tumors (for example, doxycycline in drinking water) are to be listed in the animal use protocol. Non-pharmaceutical grade drugs are to be identified (e.g. tamoxifen) and their use must be justified.

**EVALUATION OF VISIBLE OR PALPABLE TUMORS**

Evaluating tumor burden based only on a percentage of body weight is generally not accurate—while the growing tumor(s) may cause an increase in body weight, the general condition of the rodent may be decreased (loss of lean body mass), resulting in a relatively stable body weight but an unhealthy animal.

Tumor burden should be determined by evaluating the following:

- Body condition score (BCS). See previous section on “Monitoring and Endpoints.”
- Objective dimensional criteria (size)
- Anatomical location
- Incidence of multiple tumors
- Tumor ulceration

The guidance below assumes that a normally sized adult rodent will be studied (a ~25 g mouse or a 250+ g rat). The allowable sizes of tumors will be decreased if the tumors are injected into immature or genetically small mice.

**Tumor Size and Location**

The concern of size for individual tumors is related to central necrosis, ulceration of skin overlying tumors, and abrasions. When on the dorsum or flank of adult rodent, tumors may be allowed to grow to a diameter of 2.0 cm (or 4.2cm$^3$) in mice and 4.0 cm (33.5cm$^3$) in rats (NIH ARAC-(http://oacu.od.nih.gov/ARAC/documents/ASP_Endpoints.pdf) otherwise healthy.
Multiple Tumors
Multiple tumors that are individually smaller than the single tumor limit may not have the same negative sequelae as a single tumor. Please note that the limitation on any single tumor (2.0 cm diameter in mice) will still be valid.

Tumor Ulceration
Ulcerated, necrotic tissue is one of the most common findings in tumor models. Ulcerated or necrotic tissue may result in a continuous seepage of body fluids and predisposes the rodent to infection. However, an overtly open lesion or scabbed area of a tumor does not necessarily require euthanasia, if this is the phenomenon under study. If this is the case, it does require more frequent monitoring and potentially treatment as defined below. The level of follow-up care for ulcerated tumors is based on both the size of the ulceration and the clinical judgment of the AV.

- **Pinpoint (≤ 1mm) ulcerations** at the site of tumor injection must be monitored at least 2 times per week for worsening of the ulceration site.
- **Ulcerations (> 1mm)** of the surface area of the tumor shall be monitored at least 3 times per week and must be reported to the AV for evaluation and potential treatment.

*It is inconsistent with sound research to allow the tumor to proceed to the point of ulceration and necrosis unless this is the phenomenon under study.*

ASCITES PRODUCED BY TUMORS
In cases where tumors are expected to grow with accumulation of ascites, rodents must be weighed prior to inoculation and subsequently be followed by weight measurements at regular intervals—described in the protocol and based on the expected rate of fluid accumulation. When the body weight exceeds 120% of initial weight, the rodents must be euthanized or abdominocentesis (“abdominal tap”) must be performed. Juvenile animals that are maturing (those < 8 weeks of age) that develop ascites must be monitored based upon the above expectations; however, their growing rate must be compared to age-matched control animals or published growth curves for the background strain (see www.jax.org for more information).

Ascites pressure must be relieved before abdominal distension is great enough to cause discomfort, increase respiratory rate, or interfere with normal activity. The abdominal “tap” should be performed by trained personnel using proper aseptic technique, with manual restraint or anesthesia, and by using the smallest needle possible (e.g. 22 gauge) that allows for adequate flow (NIH ARAC [http://oacu.od.nih.gov/ARAC/documents/Ascites.pdf](http://oacu.od.nih.gov/ARAC/documents/Ascites.pdf)). In addition to weight measurement, BCS needs to be part of the evaluation of the animals as described above.

NON-PALPABLE OR LIQUID TUMORS
Evaluating liquid tumors (e.g. leukemia- effects hematopoiesis and interferes with normal health because of anemia, cachexia and weight loss without any other clinical signs) and tumors in central areas of the rodent’s body (e.g. bone, brain, lungs) can be challenging. Tumor size will likely not be useful due to inability to measure size or because of the sensitivity of areas to compressive lesions. 1,6,7 For these models, the BCS and clinical evaluation of the animals take priority regarding decisions on humane endpoints. The expected clinical signs and the humane endpoints of those signs must be clearly described in the protocol. A scoring system (as mentioned above in this document) may be most helpful in this scenario. The evaluation of clinical signs in an animal with a tumor burden of this type should include consultation with the AV.
REFERENCES/Regulatory Authority


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Revised: