Policies and Procedures

SECTION: IACUC
NUMBER: 7.25

CHAPTER: Miscellaneous Experimental Animal Use Policies

POLICY: Tumor Policy for Mice and Rats

Purpose

The purpose of this policy is to provide guidelines for mice and rats involved in tumor studies.

I. Application

A. All injectable and/or implantable materials used for establishing tumors in animals must be reviewed for hazardous agents by the appropriate committees and departments. In addition, documentation checking for human pathogens must be provided for all human tumor lines.

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 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 3X/week and weighed at least 2X/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 to contact the PI and to reach consensus with the PI bearing experimental endpoints in mind. However, the final analysis and discharging of the animal care and use regulatory responsibility rests with the Attending Veterinarian.
II. Experimental Tumor Models

A. Injecting tumor cells in rodents, usually mice, is an accepted experimental procedure for the purpose of either propagating a tumor line or for studying various cancers and cancer treatments.

B. Mutant or genetically engineered mice with predisposition for developing a certain tumor are used in order to study specific cancers.

C. Chemically induced tumors.

III. Spontaneous, Naturally Occurring Tumors and Masses

Naturally occurring tumors or masses are also found in laboratory rodents, as well as in other animals including humans. These may include benign masses such as lipomas (usually benign unless interfering with a physiologic function), but can also be cysts, hematomas, abscesses or masses of various etiology. Animals being diagnosed with a non-experimental tumor or mass must be evaluated as soon as possible. Depending on the findings and the condition of the animal, the PI may decide to euthanize the animal. Humane Endpoints as listed in this policy are determinants for euthanasia.

Rats and mice sometimes develop tumors as part of their genetic predisposition. Examples of these are mammary tumors in both rats and mice, pituitary tumors in aging Sprague-Dawley rats and interstitial cell tumors in male Fisher-344 rats. There are many others, most frequently seen as the animal gets older.

IV. Induction of the Animal Cancer Model

Transplantable tumors may be induced orthotopically, in the tissue or site of origin, or ectopically, usually subcutaneously in the flanks or by intravenous injection. Changing the inoculation site may change the growth characteristics of the tumors. Knowledge of the origin, incidence, and time of onset of spontaneously developing tumors in the host animal is necessary if experiments or animal welfare are not to be compromised.

To promote the engraftment of some experimental tumor lines, it may be necessary to modify the recipient’s immunologic or physiologic status. Low-level whole body irradiation or immunosuppressive agents are frequently used to further suppress the immune response of immunodeficient rodents before inoculation with human tumor xenografts.
V. Clinical Presentations of Animals on Oncology Study

Many tumors grow rapidly and can compromise the health and well-being of the animal. If the tumor is subcutaneous or on the skin’s surface, it can become large enough to interfere with the animal’s locomotion, grooming and ability to eat and drink or other physiological functions or become a metabolic burden. In addition, tumors can ulcerate and become necrotic, thus giving rise to secondary bacterial infections and/or cause fluid drainage. If the tumor metastasizes into a major body cavity, either the abdominal, the chest cavity or cranium it can compromise respiration, digestion, urination or defecation and in some cases, and locomotion. Though the specific tumor biology, implantation site, host status, mode of growth, and the nature of associated treatments preclude development of precise guidelines for the upper limits of tumor size, the following general guidelines should be followed:

- “Detailed knowledge of the growth characteristics and biology of the proposed tumor model and the onset and nature of any adverse effects on the animals is crucial to the establishment of both scientific and humane endpoints”.

- “Solid and non-solid tumors give rise to different clinical presentations of animal morbidity and mortality. Leukemia, for example, effects hematopoiesis and interferes with normal health because of anemia, cachexia and weight loss without any other clinical signs”.

- “Frequency of monitoring will be determined by the growth characteristics of the tumor and the onset of critical phases in the experimental process or development of the tumor. Animals with newly transplanted tumors may require only routine observation [3x/week] during the early stages of tumor development; however, animals in the terminal stages of tumor-associated disease or drug toxicity may require monitoring several times a day”.

- “The incidence and growth rate of spontaneous tumors, some chemically induced tumors, or those arising in transgenic animals may be difficult to predict. When grown as ascites, some tumors such as the mouse L1210 leukemia (Kline et al. 1972) and the Leydig cell rat tumor (Cooke et al. 1979) are rapidly lethal when growth is uncontrolled”.

- “Tumors may be solid and localized or may be solid and metastasizing to produce secondary disease in other tissues. Solid tumors may develop either in the superficial tissues or internally. Certain types of malignancies may arise in the blood, bone marrow, or lymphatic system. In contrast to the development of clinical cancer, experimental tumor systems in animals are frequently characterized by a relatively short latent period and rapid tumor growth. The incidence, site of origin, and growth rate of an experimental
### Policies and Procedures

<table>
<thead>
<tr>
<th>SECTION: IACUC</th>
<th>NUMBER: 7.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAPTER: Miscellaneous Experimental Animal Use Policies</td>
<td>ISSUED: 7-27-15</td>
</tr>
<tr>
<td>POLICY: Tumor Policy for Mice and Rats</td>
<td>REV. D:</td>
</tr>
</tbody>
</table>

Tumor and the onset and nature of the adverse effects on the host will vary with (1) methods used to induce the tumors, (2) the biology of the tumor, (3) the site of development and the issues involved, (4) any associated experimental challenge, and (5) the response of the host.”

- “Leukemias can be detected in the living animal by examining blood samples for the presence of circulating cancer cells or changes in the cellular constituents of the blood. Increases in circulating tumor cells or changes in blood constituents can forecast the onset of clinical symptoms. When human leukemia cells are engrafted into immunodeficient mice, the number of circulating human cancer cells is less predictive of either the onset or severity of clinical symptoms. In the absence of reliable laboratory-based assays, animals with leukemia or lymphomas should be observed for early clinical signs such as anemia, loss of condition or weight, and enlargement of the spleen and lymph nodes. Scientific end-points should precede limiting clinical signs such as consistent weight loss, clinical anemia, apathy, impaired respiration, or death”.

- “Ascitic Tumors Abdominal distension, anemia, solid tumor deposits, and loss of condition are associated with the development of ascites. When the L1210 mouse leukemia is grown as ascites, it is lethal approximately 8 days after inoculation. The terminal period is associated with abdominal ascites, dyspnea, and piloerection. During the terminal phase, the animals should be inspected and continually assessed for early termination several times each day. Tumor cell survival assays may be used as surrogate endpoints in place of limiting clinical signs or death of the host”.

- “Spontaneous tumors can arise in most tissues, and their development most closely resembles the clinical course. Typically, spontaneous tumors are characterized by a variety of histologic types, slow development, and cell turnover; if malignant, they may be poorly differentiated”.

### VI. Training of Personnel and Research Staff

Scientific staff responsible for monitoring the animal on tumor study should not only be familiar with normal animal health and behavior, but must also be able to observe adverse changes in health, behavior, or tumor burden. Specifically, since there are differences in normal behavior between different mouse and rat strains, the responsible research staff must be familiar with the animal(s) on study BEFORE the experiment begins.
VII. Tumor Assessment Techniques and Documentation

A. Observation of the overall condition of the animal including appearance, posture, behavior and physiological responses, food and water intake must be done and documented on a regular basis and at least 3X/week until signs of morbidity and mortality have been observed, when daily observation and documentation is required.

B. Caliper measurements to determine tumor diameters and area are helpful.

C. Animals must be weighed regularly and the body weight documented at least 2X/week to determine changes in body weight.

D. Inspection and palpation to locate the sites of tumor growth, distension, ulceration, and compromised mobility must be performed and documented on regular basis and at least 3X/week.

VIII. Husbandry

A. The development of immunodeficient mouse models engrafted with human tumors: These animals may be naturally immune suppressed such as the nude mouse or the SCID mouse, or they may have been made immunosuppressed by irradiation or administration of immunosuppressant agents.

B. Once animals become sick with the tumor it may be necessary to separate them and allow them more space to avoid cannibalism by cage mates. They may require supportive care such as food on the cage floor.

C. Nursing care may be given as indicated by the animal’s condition and consistent with the research goals.

IX. Humane Endpoint Criteria

Experiments should be completed before tumor development or tumor-associated disease causes death or a significant deterioration in the host.

A. 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.

B. An animal in chronic pain or distress that cannot be relieved by analgesics, must be euthanized unless prior scientific justification for these humane endpoints are approved by the IACUC.

C. Some tumors can cause significant changes in animal health and well-being. In particular, animals must be observed as often as needed, and be monitored at least three times per week for any indication of the following:

If there is indication of diminished health or morbidity, then the Attending Veterinarian must be consulted. The presence of one or more of the criteria below is indication for immediate euthanasia:

- Impaired mobility (the inability to reach food and water)
- Restlessness/Unable to get comfortable
- Inability to remain upright
- Unconsciousness with no response to external stimuli
- Interference with a vital physiological function: This includes respiration, mastication, swallowing, urination, defecation or locomotion
- Location of the tumor on the animal’s belly or its inner leg causing the tumor to be abraded or interfering with locomotion
- Hunched abnormal posture for > 48 hours
- Labored breathing and cyanosis [bluish pinnae (ears) or feet or mucous membranes]
- Clinical dehydration and/or prolonged decreased food intake
- Muscle atrophy and signs of lethargy and lack of physical activity
- Weight loss/Body condition score < 2 (see Figure 1)
- Chronic diarrhea or constipation for more than 48 hours
- Hematological or biochemical values that indicate organ failure
- Severe anemia [pale pinnae (ears) or feet or mucous membranes]
- Bloodstained or mucopurulent discharge from any orifice
- Self-mutilation; Lack of grooming behavior/Rough/Unkempt hair coat for >48 hours
- Enlarged lymph nodes or spleen
- Significant abdominal distension
- Cranial deformity/Neurological signs
- Exophthalmos (bulging eye)
• Skin pathology including ulceration or necrosis of tumor requires euthanasia within 72 hours. Ulcerated or necrotic tissue may result in a continuous loss of body fluid and/or infection.

Comments:

• 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 to infection. 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.

• Weight loss/cachexia: Implanted or naturally occurring tumors may cause weight loss in the host animal due to their nutritive demands or due to loss of well-being causing anorexia. A recommended humane endpoint is a body weight loss of no more than 20% of pre-procedural weight, in adult rodents. In the live animal, this has to be estimated, since the tumor cannot be weighed apart from the host. The BCS score may be the most accurate assessment of body mass loss. Animal on tumor studies must be weighed at least weekly and documented and records available for veterinary staff.

• Restlessness/Unable to get comfortable is an indication of severe pain and requires immediate attention either with administration of analgesics or euthanasia.

• Self-mutilation; lack of grooming behavior/rough/unkempt hair coat is an indication that the animal is not well and requires daily monitoring and attention

• The professional judgment and decision of the Attending Veterinarian is final.

References

1) Boston University IACUC Policy on Tumor Guidelines for Rats and Mice
2) NCI Frederick ACUC Guidelines Involving Experimental Neoplasia Proposals in Mice and Rats, 2006.
**Policies and Procedures**

<table>
<thead>
<tr>
<th>SECTION: IACUC</th>
<th>NUMBER: 7.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAPTER: Miscellaneous Experimental Animal Use Policies</td>
<td>ISSUED: 7-27-15</td>
</tr>
<tr>
<td>POLICY: Tumor Policy for Mice and Rats</td>
<td>REV. D:</td>
</tr>
</tbody>
</table>

Figure. 1 Chart for assessing body condition score (BCS)

- **BC 1**: Mouse is emaciated.  
  - Skeletal structure extremely prominent; little or no flesh cover.  
  - Vertebrae distinctly segmented.

- **BC 2**: Mouse is underconditioned.  
  - Segmentation of vertebral column evident.  
  - Dorsal pelvic bones are readily palpable.

- **BC 3**: Mouse is well-conditioned.  
  - Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.

- **BC 4**: Mouse is overconditioned.  
  - Spine is a continuous column.  
  - Vertebrae palpable only with firm pressure.

- **BC 5**: Mouse is obese.  
  - Mouse is smooth and bulky.  
  - Bone structure disappears under flesh and subcutaneous fat.

* A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)