Institutional Animal Care and Use Committee Clemson University

POLICY #19: INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
POLICY FOR STUDIES INVOLVING TUMOR FORMATION IN RODENTS

Purpose: The following policy has been developed to ensure compliance with the Animal Welfare Act and Public Health Service Policy, and to ensure humane use and care of rodents in studies involving tumor formation. This policy applies to all animal use protocols in rodents that are inoculated with tumor cells or in which the rodents may be expected to have a tumor burden as a result of the study. The Clemson University Institutional Animal Care and Use Committee (IACUC) has the responsibility and authority to oversee this policy.

1. Study Considerations

1.1 Tumor Kinetics – A detailed knowledge of the biology of the tumor model aids in the selection of appropriate humane endpoints.
1.2 Viral status – Cell lines or tumor tissues may be contaminated with viruses, which may serve as a source of infection for the animals in the study, as well as other colonies in the facility.
1.3 Solid tumor inoculation – Solid tumors should be minced into fine pieces or dispersed prior to transplantation to minimize trauma to the animal. For mice, transplantation of tumor fragments less than 1mm is preferred. Larger fragments may require anesthesia and a surgical procedure to implant.
1.4 Transplantation site – Whenever possible the tumor should be placed such that it can grow with minimal impact on the animal's ability to ambulate and perform normal bodily functions.

2. Conducting Tumor Studies

2.1 Notify Animal Research Personnel and Facility Manager each and every time a tumor study is started.
2.2 The following information must be recorded on the cage card provided by Research Services:
   - Date of injection
   - Animal weight on day of injection
   - Tumorigenic Agent/Cell line
   - Dose/Number of cells administered
   - Site of Administration/Injection
3. Humane Endpoints in Tumor Studies

All animal care and use proposals involving tumorigenesis must include criteria that establish the clinical endpoints at which animals are to be euthanized. Tumors may occur spontaneously, or be experimentally induced through transplantation, administration of carcinogens or other tumorigenic agents.

3.1 General criteria for consideration of euthanasia:
1. Maximum tumor size ≥ 10% of body weight on day of injection, calculated using the formula: Mass (mg) = Tumor volume (mm$^3$) = d2 x D/2 where d and D are the shortest and longest diameter in mm, respectively.
2. Mean tumor diameter exceeding 20mm in mice, 40mm in rats (Mean = (d + D)/2).
3. Ulceration of tumor regardless of size.
4. Tumor is interfering with movement, normal body functions, or function of vital organs.
5. Animal is seen to be in distress, with signs such as labored breathing, it should be euthanized regardless of tumor size.
6. Animal is unable to eat or drink.

3.2 Frequency of Monitoring Tumor Growth
1. Animals in which tumors are growing should be checked at least three times weekly, at intervals no greater than three days apart, by the PI or designee.
2. In the case of rapidly growing tumors or situations where the progress of clinical signs is likely to be rapid, animals should be checked daily.
3. Animals will be evaluated in consultation with veterinarians from Research Services when the tumor size approaches 10% of body weight, or any of the above criteria are observed. Any time a veterinarian determines that an animal is experiencing pain or distress, he or she has the authority to require euthanasia of that animal.

Tumor size references:

Table 1. Selected Clinical Observations Used in Cancer Research and Toxicological Studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>What to look for</th>
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<tbody>
<tr>
<td>General Appearance</td>
<td>Dehydration, decreased body weight, missing anatomy or fractured appendages. Abnormal posture, swelling of tissues or masses. Prolapses or paraphimosis, hypothermia</td>
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<tr>
<td>Skin and Fur</td>
<td>Discoloration, urine stain, pallor, redness, cyanosis, icterus, wound, sore, abscess, ulcer. Alopecia or ruffled fur.</td>
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<tr>
<td>Eyes</td>
<td>Exophthalmos, microphthalmia, ptosis, reddened eye, increased lacrimation or colored discharge. Opacity to the eye or cellular or blood accumulation in the eye.</td>
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<tr>
<td>Nose, Mouth, and Head</td>
<td>Head tilted, nasal discharge, malocclusion of teeth or jaw.</td>
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<tr>
<td>Respiration</td>
<td>Salivation or malodor associated with any orifice.</td>
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<tr>
<td>Urine</td>
<td>Sneezeing, dyspnea, tachypnea, rales</td>
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<tr>
<td>Feces</td>
<td>Discoloration, blood in urine, polyuria, anuria</td>
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<tr>
<td>Locomotor</td>
<td>Hyperactivity, hyperactivity, coma, ataxia, circling, muscle, tremors</td>
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