UNIVERSITY STANDARD

Title

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
STANDARD ON TUMOR PRODUCTION AND CANCER RESEARCH IN MICE AND RATS

Introduction

PURPOSE
The standards and procedures described below provide guidance to all researchers and animal handlers for requirements for humane endpoints for mice and rats in cancer studies.

SCOPE OF APPLICABILITY
All personnel engaged in research involving tumor burden in mice and rats. The standards described in this document apply to all research involving spontaneous and experimentally induced cancer studies in mice and rats.

The UNC-CH IACUC expects that anyone involved in animal work at the University will comply with this Standard. Requests for exceptions to this Standard must be reviewed and approved by the IACUC and/or DCM Management.

Standard

The purpose of this policy is to provide investigators the requirements and humane endpoints for conducting spontaneous and experimentally-induced cancer studies in mice and rats. Consideration when designing these studies should include tumor size, potential clinical complications of tumor burden, monitoring of animals, and criteria for intervention and/or euthanasia. Investigators producing or passaging tumors in mice or rats should use the information contained in this document as a reference when preparing their IACUC protocol. Any exceptions to these policies must be scientifically justified and approved by the IACUC.

Careful consideration should be given to the following items:

- scientific requirements of the study
- methods of reducing pain and distress
- clinical signs associated with tumor development
• frequency of monitoring
• humane endpoints and the overall health of the animal
• documentation

1. Pathogen testing of tumor cell lines:

• All cells/tumors implanted or injected into rodents should be tested for infectious agents and determined to be pathogen free. Inoculation with human cell lines or tumors must be included in the IACUC protocol, the laboratory safety plan and in a Schedule F form. Note that most companies selling human cell lines require that UNC’s Biosafety Officer or the Institutional Official approve the order. Please contact DCM (919-962-5335) for further information regarding pathogen screening.

2. Tumor size, location and number:

• **Tumor size**: The maximum allowable tumor size for a single spontaneous or implanted tumor should not exceed 2.0 cm in any dimension in an adult mouse and 4.0 cm in any dimension in an adult rat.

• As tumors located within the cranium, thoracic cavity, mouth, or behind the eyes may interfere with vital functions and result in morbidity or mortality, the maximum tumor size should be considerably smaller with assessment of overall health status taking priority. As the use of imaging or biomarkers may detect tumors before they are visible or can be palpated, consideration should also be given to the use of imaging in studies where the tumors are located at sites not readily observable.

• **Tumor location**: Tumor implantation sites should be chosen to minimize the impact on the animal’s health status. The animal’s back or flank is the preferred site for tumor implantation and is thought to cause the least pain and distress. Sites involving the face, limbs and perineum should be avoided as even minimal tumor growth in these areas can interfere with basic bodily functions, including eating, drinking and urinating. In many studies, however, the implantation site is selected on the basis of clinical relevance. If locations other than the back or flank are selected, they should be scientifically justified in the protocol and the humane endpoints clearly defined.

• **Multiple tumors**: Individual tumors that are smaller than the single tumor maximum size may not have the same adverse health consequences as a single, large tumor. Nevertheless, even some relatively small tumors can
interfere with basic bodily functions, especially when located in the face or perineum. When multiple tumors are present, the assessment of overall health status should take priority. Under no conditions, however, should any single tumor exceed 2.0 cm in any dimension in mice and 4.0 cm in any dimension in rats. When 2 or more tumors are present, no 2 tumors should exceed 1.5 cm apiece in mice or 3.0 cm apiece in rats. Cases involving multiple tumors that alter the health status of the animal should be assessed by Vet Services.

3. **Humane endpoints:**

- Under conditions in which pain or distress is an unavoidable component of the study, humane endpoints must be identified and clearly defined. The humane endpoint is defined as the earliest point at which pain or distress in an experimental animal is prevented, terminated, or relieved.

**Clinical signs which may be associated with tumor progression:**

- Decreased eating or drinking
- Persistent hypothermia
- Visible signs of anemia (pale)
- Progressive dehydration
- Blood stained discharges
- Lethargy
- Labored respiration
- Weight loss
- Ulceration of tumor site
- Rough hair coat
- Reluctance to move
- Hunched posture
- Vocalization
- Abdominal distension

**Table 1. Selected Clinical Observations**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>What to look for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>General appearance</td>
<td>Dehydration, weight loss, abnormal posture, hypothermia, abnormal appearance of limb, swelling, tissue masses</td>
</tr>
<tr>
<td>Skin and fur</td>
<td>Discoloration, urine stain, pallor, redness, blueness, jaundice, wound sore, abscess, ulcer, bald spot, ruffled fur</td>
</tr>
<tr>
<td>Eyes</td>
<td>Enlarged eyes, microphthalmia, droopy lids, red-eye, tears, discharge, opacity</td>
</tr>
<tr>
<td>Nose, mouth, and head</td>
<td>Head tilted, nasal discharge, malocclusion, drooling</td>
</tr>
<tr>
<td>Respiration</td>
<td>Sneezing, rattle, abnormal breathing</td>
</tr>
<tr>
<td>Urine</td>
<td>Discoloration of back/ventral fur, blood in urine, excessive or no urination</td>
</tr>
<tr>
<td>Feces</td>
<td>Discoloration of back/ventral fur, blood in the feces, softness/diarrhea</td>
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<tr>
<td>-----------------------------</td>
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<tr>
<td>Genital area</td>
<td>Prolapses, paraphimosis</td>
</tr>
<tr>
<td>Locomotion</td>
<td>Hyperactivity, hypoactivity, impaired movement, poor coordination, circling, tremors</td>
</tr>
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</table>


- **Body Condition Score.** Assessment of the overall health of the animal should take priority over assessment of tumor size or loss of body weight. In some cases, tumor growth may result in an increase in body weight and a decrease in lean body mass (close assessment of tumor size and body weight is especially important for younger, growing animals as failure to maintain weight gain comparable to untreated control animals may indicate adverse tumor effects). The body condition scoring system (BCS), developed by Ullman-Cullere and Foltz (*Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice. Lab. Animal Science; 49, 319-323, 1999*), has proven to be a reliable indicator of general health and utilizes a scoring system that ranges from “1” (emaciated /wasted) to “5” (obese). The BCS offers a useful, rapid and objective assessment of an animal’s health, especially in cases where treatments designed to affect tumor growth (e.g., radiation, chemotherapy) lead to poor body condition. This system quantitatively assesses a number of indicators of health status, including body weight, physical appearance, measurable clinical signs, unprovoked behavior and response to external stimuli. The use of this method, however, does not preclude other criteria for euthanasia prior to study endpoint, including, but not limited to the ability to eat or drink, labored breathing, ulceration (a breakdown of the skin cells resulting in exposure of the underlying tissue) and necrosis (death of cells in an organ or tissue due to disease, injury, or failure of the blood supply) at the tumor site. (See next page)
Appendix I: Body Condition Scoring (BCS) Guide.

**BCS 1**
Animal is emaciated
- Skeletal structure extremely prominent; little or no flesh cover
- Vertebrae distinctly segmented

**BCS 2**
Animal is under conditioned
- Segmentation of vertebral column evident
- Dorsal pelvic bones are readily palpable

**BCS 3**
Animal is well conditioned
- Vertebrate and dorsal pelvis not prominent; palpable with slight pressure

**BCS 4**
Animal is well over conditioned
- Spine is a continuous column
- Vertebrae palpable only with firm pressure

**BCS 5**
Animal is obese
- Animal is smooth and bulky
- Bone structure disappears under flesh & SC fat

A “+” or a “-” can be added to the body condition score if additional increments are necessary (i.e. ...1+, 2, 2-)
• Any tumor-bearing animal must be humanely euthanized when the following criteria are met (unless scientifically justified in the protocol and specifically approved by the IACUC):
  - the BCS is <2
  - the tumor meets the tumor size limitations*
  - the tumor becomes ulcerated or necrotic
  - the animals ability to eat or drink is significantly impaired
  - the animals breathing is labored
  - investigators should consult with a DCM veterinarian about appropriate endpoints and must have a plan for euthanasia or treatment that is based on clearly defined endpoints

* Possible exceptions to this policy: If the tumor reaches the maximum size limit and there are no observable health issues, investigators should consult with a DCM veterinarian to determine if euthanasia or treatment of the animal is required. If the veterinarian determines that the health status of the animal is not being adversely impacted by the tumor and that continued use of the animal is critical for meeting the study’s objective, an amendment must be submitted that includes an Exception request that describes the revised criteria for euthanasia (e.g., maximum tumor size, changes in BCS, changes in body weight, etc.) and a revised frequency of monitoring.

4. Monitoring:

• Frequency of monitoring: All animals involved in tumor studies must be monitored for tumor size, health status, and pain/distress/suffering by qualified laboratory personnel. The monitoring schedule listed below applies to weekdays, weekends as well as holidays.
  - After implantation of tumor cells or in animals being utilized for the development of spontaneous tumors, animals must be monitored at least twice per week at intervals no greater than three days apart.
  - After a visible or palpable tumor is evident, the animals must be monitored at least three times weekly at intervals no greater than three days apart.
  - If tumor growth is expected to be rapid, twice daily monitoring may be necessary.
  - More frequent observations are necessary when the general health of the animal show signs of deterioration. Animals that are approaching humane endpoints must be monitored at least daily.
5. **Documentation:**

- The PHS policy requires proper documentation of animal care and use to assess compliance with research protocols and clinical care procedures.

- Animal ID, tumor size and body weight, surgical procedures, post-surgical evaluations, euthanasia, and administration of anesthesia and pain medications must be recorded. All records must be available for review at any time by IACUC and external regulatory officials.

6. **Assignment to USDA pain categories:**

- Most subcutaneous implanted tumors are thought to cause no pain or discomfort and thus should be classified as USDA Category C. Other types of tumors (e.g., spontaneously developing, virus-induced, metastatic) may have a different pain profile and thus alternate pain categories should be considered.

- In cases where 1) palliative treatments used specifically to relieve pain and discomfort (e.g., anesthetics, analgesics) or 2) euthanasia when humane endpoints are reached, will be used when the tumor burden is expected to cause disruption of normal activity and pain/distress, the animals should be classified as USDA Category D.

- In cases where the tumor burden is expected to cause disruption of normal activity and pain/distress, but scientific justification has been provided for either withhold palliative treatments (e.g., anesthetics, analgesics) or to exceed humane endpoints, the animals should be classified as USDA Category E.

**EXCEPTIONS**

Requests for exceptions to this Standard must be reviewed and approved by the IACUC and/or DCM Management.

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**Definitions**

- **IACUC:** Institutional Animal Care and Use Committee
- **DCM:** Division of Comparative Medicine
- **University Standard:** The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC.
- **Humane endpoint:** The earliest point at which pain or distress in an experimental animal is prevented, terminated, or relieved
Related Requirements

EXTERNAL REGULATIONS AND CONSEQUENCES

UNIVERSITY POLICIES, STANDARDS, AND PROCEDURES

For more detailed guidance, please refer to the University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes.

Contact Information

<table>
<thead>
<tr>
<th>Subject</th>
<th>Contact</th>
<th>Telephone</th>
<th>Email</th>
</tr>
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<tbody>
<tr>
<td>DCM Veterinary Services</td>
<td>DCM</td>
<td>919-966-2906</td>
<td></td>
</tr>
<tr>
<td>IACUC Protocol</td>
<td>OACU</td>
<td>919-966-5569</td>
<td><a href="mailto:iacuc@med.unc.edu">iacuc@med.unc.edu</a></td>
</tr>
</tbody>
</table>

Important Dates

- Effective Date and title of Approver: 07/26/2002; UNC IACUC
- Revision and Review Dates, Change notes, title of Reviewer or Approver: Revised 07/26/2002; 04/26/2006, 11/14/2008, 02/13/2009, 06/12/2009, 11/09/2012, 04/2014, 11/14/2014, 10/2015, May 2018, March 2019 added ‘When 2 or more tumors are present, no 2 tumors should exceed 1.5 cm apiece in mice or 3.0 cm apiece in rats.’; UNC IACUC

Approved by: UNC IACUC

Dr. Roland Tisch
UNC IACUC Chair

03/2019