



Institutional Animal Care and Use Committee (IACUC) Directive 14.0: Rodent Tumors

1.0 Directive Purpose

The purpose of this directive is to provide guidelines for tumor implantation and monitoring for rodents inoculated with Neoplastic cells or toxic agents and for rodents that are genetically predisposed to develop tumors.

2.0 To Whom the Directive Applies

This directive applies to all individuals using live rodents at Brown University under an approved IACUC animal use protocol.

3.0 Directive Statement

Tumor implantation sites should be chosen to minimize adjacent tissue damage or disrupting normal physiology. 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, the Principal Investigator (PI) must describe and justify the site within the animal use protocol. In general, the following sites should be avoided:

- Sites involving the face, limbs or perineum, as there is little to no space for tumor growth and expansion, and resulting tumors may interfere with eating and drinking.
- Intramuscular implantation to prevent inhibiting normal movement.
- Abdominal surface of the body due to the risk of irritation to the tumor site in contact with the bedding and floor of the cage.

The known biology and effects of any individual tumor model must 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.

3.1 Tumor/Clinical Evaluation

Evaluating tumor burden based exclusively on a percentage of body weight is generally not acceptable. While the growing tumor(s) may cause an increase in body weight, the general condition of the rodent may be decreased (*i.e.*, loss of lean body mass), resulting in a relatively stable body weight but an unhealthy animal. Therefore, tumor burden should be determined by evaluating all of the following:

- Body Condition Score (BCS) (see section 3.7 for more details);
 - For liquid tumors, body weights may be used in addition to or instead of BCS (see section 3.5 for more details);
- Objective dimensional criteria (size);
- Anatomical location;
- Incidence of multiple tumors; and

- Tumor Ulceration.

General clinical signs must also be assessed. Any evidence of lethargy or other change in behavior, change in ambulation, diarrhea, neurological signs (*e.g.*, circling, head tilt), or increased respiratory effort must be reported to the veterinary staff.

3.2 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 an adult rodent, tumors may be allowed to grow to the following volumes as long as the rodent remains otherwise healthy:

- Mice: 2000 mm³ in size (which is roughly 10% baseline body weight)
- Rats: 5000 mm³ in size

Tumors should be measured using the following formula: $TV = [2(\text{Width}) \times \text{Length}] / 2$

This guidance assumes a normally sized adult rodent (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.

3.3 Multiple Tumors

Multiple tumors that are individually smaller than the single tumor limit may not have the same negative sequelae as a single tumor. Multiple tumors may be allowed to grow up to 150% (or 3000 mm³) of the volume compared with the volume of a single tumor. The limitation on any single tumor (2000 mm³ volume in mice) still applies.

3.4 Tumor Ulceration

Ulceration of a tumor typically requires euthanasia **unless** Ulceration without euthanasia is justified in the animal use protocol and in consultation with a veterinarian. If Ulceration without euthanasia is approved, a minimum of daily monitoring of the animal will be required.

3.5 Non-Palpable or Liquid Tumors

Evaluating liquid tumors (*e.g.*, leukemia) and non-palpable tumors in central areas of the rodent's body (*e.g.*, bone, brain and lungs) can be challenging. Tumor size will likely not be a useful parameter to monitor due to the inability to measure size or because of the sensitivity of areas to compressive lesions. For these models, the BCS **and/or** body weight along with 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 animal use protocol. A scoring system (see Section 3.7 for details) may be helpful in this scenario. The evaluation of clinical signs in an animal with a tumor burden of this type must include consultation with a veterinarian.

3.6 Tumor Monitoring

At the time of injection of tumor cells, each cage must be identified with an identifying tag on the corresponding cage card. The PI or a designated lab member must provide each cage with a unique cage number on the blue flag using a permanent marker. This is intended to facilitate communication between the research laboratory and the Center for Animal Research and Education (CARE) staff and veterinarians. Tumor monitoring must begin at this time in

accordance with protocol-specific frequency, or at least once per week, whichever is more frequent. After a visible or palpable tumor is evident, the animals must be monitored at least twice weekly. More frequent observations may be necessary, possibly including on weekends and holidays, as determined by a veterinarian. The overall wellbeing of the animal will take priority over precise tumor measurements when making decisions regarding euthanasia or other interventions.

A tumor monitoring sheet must be completed by the research laboratory for each protocol endpoint. The monitoring sheet must be filled out completely indicating all of the following:

- protocol-specific endpoints;
- monitoring frequency;
- contact information for the person who is directly working with the animals;
- date, observation code, cage identification numbers, and initials for each observation;
- number of animals with the following conditions using the respective observation codes, when applicable: (U) ulcerated, (D) found dead, or (E) euthanized.

3.7 Body Condition Score

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 (see Appendix A for detailed descriptions and corresponding images). 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 any of the following applies:

- The body condition score is 1/5;
- The body condition score is 2/5 and the rodent has decreased activity/responsiveness;
- The tumor affects the rodent’s gait or normal posture, ability to eat, urinate, or defecate (independent of the size of the tumor); or
- A veterinarian determines that the animal should be euthanized for humane concerns.

4.0 Definitions:

For the purpose of this policy, the terms below have the following definitions:

Neoplastic: Caused by the formation or presence of a new, abnormal growth of tissue.

Ulceration: The formation of a break on the skin or on the surface of an organ.

5.0 Responsibilities

All individuals to whom this directive applies are responsible for becoming familiar with it and following this. Animal research program stakeholders (IACUC, CARE, ARC) are responsible for promoting the understanding of this document and for taking appropriate steps to help ensure adherence to it.

6.0 Consequences for Violating this Document

Violation of this document may be considered a serious event of noncompliance that is reportable to the IACUC, funding and accrediting agencies, as well as other regulatory agencies. Violations of this

document are a serious matter that may adversely affect both the ability to perform animal work and acquire funding sources.

7.0 Related Information

Brown University is a community in which employees are encouraged to share workplace concerns with University leadership. Additionally, [Brown's Anonymous Reporting Hotline](#) allows anonymous and confidential reporting on matters of concern online or by phone (877-318-9184).

The following information complements and supplements this document. The information is intended to help explain this document and is not an all-inclusive list of policies, procedures, laws and requirements.

7.1 Related Policies/Directives/SOPs: N/A

7.2 Related Procedures: N/A

7.3 Related Forms: N/A

7.4 Frequently Asked Questions (FAQs): N/A

7.5 Other Related Information:

- Hickman DL. Use of body condition scoring as an adjunct endpoint for tumor growth studies. *Journal of the American Association for Laboratory Animal Science* 2007;46:111-111.
- Paster EV, Villines KA, Hickman DL. Endpoints for mouse abdominal tumor models: refinement of current criteria. *Comp Med* 2009;59:234-241
- Redgate ES, Deutsch M, Boggs SS. Time of death of CNS tumor-bearing rats can be reliably predicted by body weight-loss patterns. *Lab Anim Sci* 1991;41:269-273.
- Ullman-Cullere MH, Foltz CJ. Body condition scoring: a rapid and accurate method for assessing health status in mice. *Lab Anim Sci* 1999;49:319-323.
- Wallace J. Humane endpoints and cancer research. *ILAR J* 2000;41:87-93
- Workman P, Aboagye EO, Balkwill F, et al. Guidelines for the welfare and use of animals in cancer research. *Br J Cancer* 2010;102:1555-1577

8.0 Document Owner and Contact

8.1 Owner: IACUC

8.2 Approved by: IACUC

8.3 Subject Matter Contact: Brown University Animal Research Compliance (ARC)

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9.0 Document History

9.1 Effective Date: June 25, 2014

9.2 Last Reviewed: June 2, 2023

9.3 Update/Review Summary: This document is not new; it is transferred to the directive format and reviewed by the IACUC at the June 2, 2023 convened meeting.

- Section 8.3 was updated on December 2, 2020, to reflect a new office title. This policy is not new; it was converted to the University's new policy template and re-reviewed by the IACUC at its convened meeting on June 5, 2020.

Appendix A: Body Condition Scoring

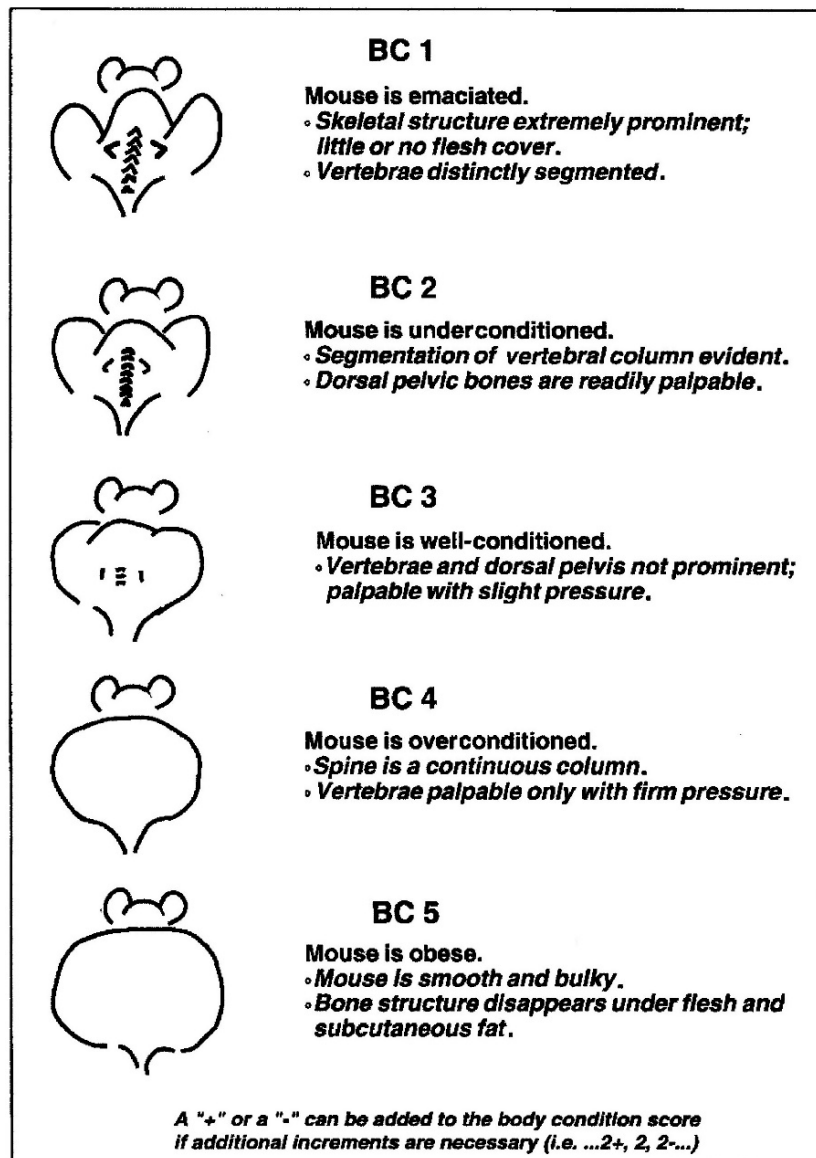


Figure 1: Body Condition Score Chart for a Mouse