



BROWN

Institutional Animal Care and Use Committee (IACUC)

Rodent Tumor Policy

Date of IACUC Review and Approval: June 25, 2014

I. Purpose:

A. To provide guidelines for a tumor implantation and monitoring for mice or rats inoculated with neoplastic cells or toxic agents or animals that are genetically predisposed to develop tumors. This guideline is relevant to all investigators using models of neoplasia, including all subcutaneous, liquid, or non-palpable tumors; in addition, it applies to naturally occurring tumors. Humane interventions and endpoints should be determined and specified in the Animal Care and Use Protocol (ACUP) for all animals that will undergo tumor development as an expected part of the experimental protocol.

B. To describe the procedures for monitoring and documenting animals on protocols involving experimentally induced tumors.

C. To provide guidelines for evaluating the overall health of the animal and applying humane endpoint criteria.

TUMOR IMPLANTATION SITES

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, describe and justify in the ACUP.

- 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.¹
- Intramuscular implantation should be avoided to prevent inhibiting normal movement.
- Tumor implantation on the abdominal 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.

TUMOR/CLINICAL EVALUATION

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 below. Alternatively, for liquid tumors body weights may be used.
- Objective dimensional criteria (size)
- Anatomical location
- Incidence of multiple tumors
- Tumor ulceration

[The following guidance 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 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

(For the basis of this policy, tumors may be measured using the following formula:

$$TV = [(Width)^2 \times Length] / 2$$

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 150% (or 3000 mm³) of the volume compared with the volume of a single tumor. Please note that the limitation on any single tumor (2000 mm³ volume in mice) will still be valid.

Tumor Ulceration

- Ulceration (overt open lesion or scabbed area) of a tumor typically requires euthanasia UNLESS justified in the protocol and in consultation with the veterinarian, and will require at least daily monitoring.

Non-palpable or liquid tumors

Evaluating liquid tumors (e.g. leukemia) and tumors in central areas of the rodent's body (e.g. bone, brain and 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.^{1,2,3} 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 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 a veterinarian.

TUMOR MONITORING PROCEDURES

A. Principal investigator or designated lab member

- 1) Identify each cage at the time of injection of tumor cells, cage cards must be identified with an identifying tag. Tumor monitoring must begin at this time per 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 as determined by the veterinarian, based on tumor growth rate, study parameters, and general condition of the animal (possibly including weekends and holidays.) The overall wellbeing of the animal will take priority over precise tumor measurements in decisions regarding euthanasia or other interventions.
- 2) 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 animal care staff and veterinarians.)
- 3) A tumor monitoring sheet must be filled out for each protocol endpoint. The monitoring sheet must be filled out completely indicating:
 - protocol specific endpoints
 - monitoring frequency
 - Contact information for the person who is directly working with the animals
 - For each observation fill in date, observation code, cage identification numbers, and initials. For observations (U) ulcerated, (D) found dead and (E) euthanized, record number of animals with the observation code

B. Veterinary Services Staff

- 1) Inspect the tumor monitoring sheet at least once a week (same day each week).
- 2) Notify the laboratory, in writing, that “tumor monitoring sheet upkeep” is required **if not adequately completed** and needs to be completed in the next 24 hours.
- 3) Examine any animal of concern during the standard daily animal health checks and report at least the following:
 - any tumor reaches the size of a dime (18 mm)
 - any tumor which inhibits mobility
 - skin ulceration noticed at the tumor location
 - clinical signs including loss of body condition
- 4) Verify the tumor monitoring sheet for completeness and consistency with the protocol for the following:
 - laboratory contact
 - protocol number
 - cage identification number
 - tumor monitoring frequency

- protocol endpoint
- 5) Contact the responsible laboratory member as needed.
 - 6) Report any communication issues to the veterinarian

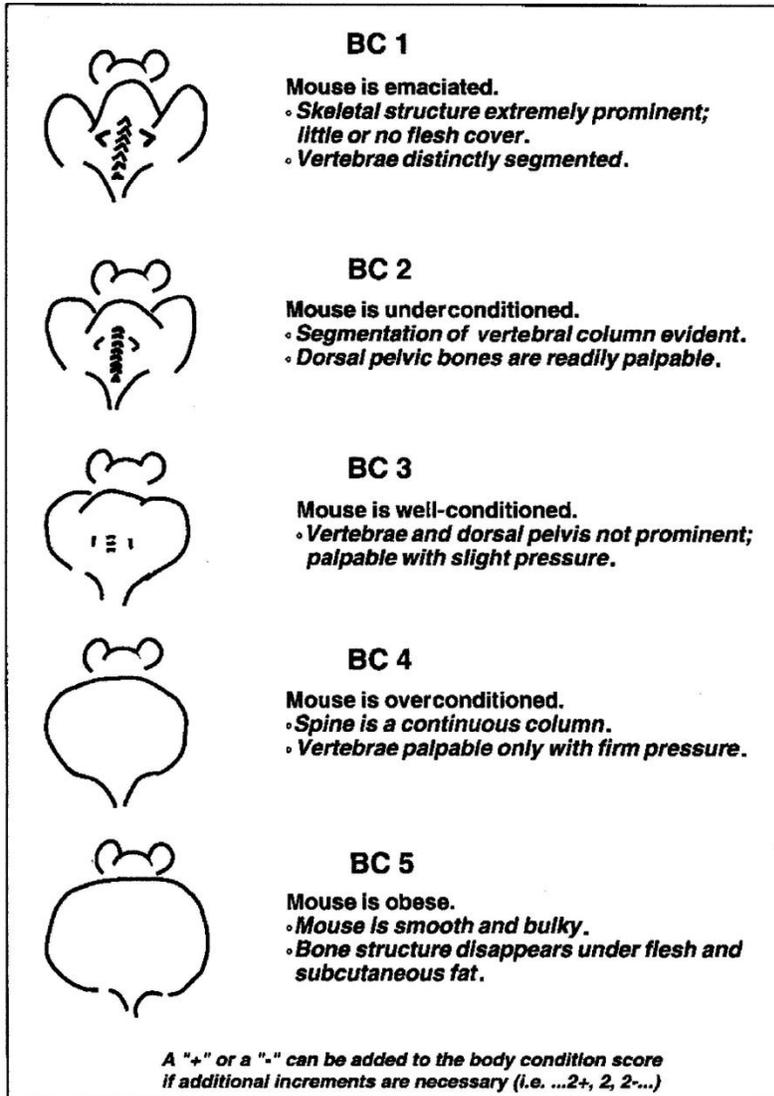
ANIMAL ASSESSMENTS

A. Body Condition Score (BCS)

The general physical condition of the animal is an important factor in effectively following the progression of tumors in rodents.³ Scoring systems from “1” (emaciated/wasted) to “5” (obese) are often used.^{4,5,6} 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 chemotherapy) 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:

- The body condition score is 1/5
- The body condition score is 2/5 and the mouse 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)
- veterinarian determines that the animal should be euthanized for humane concerns



ii. General clinical signs should 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 need to be reported to the veterinary staff.

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

4) Any animal which is found to be at protocol endpoint or which meets the guidelines for endstage illness must be euthanized.

¹ Wallace J. Humane endpoints and cancer research. ILAR J 2000;41:87-93.

² 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.

³ 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.

⁴ 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.

⁵ Paster EV, Villines KA, Hickman DL. Endpoints for mouse abdominal tumor models: refinement of current criteria. *Comp Med* 2009;59:234-241.

⁶ 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.