



Johns Hopkins University Animal Care and Use Committee (ACUC)

Tumor Study Guidelines: Mice and Rats¹

Purpose

This document was developed to assist researchers in establishing criteria to ensure the welfare of animals involved in induced or spontaneous tumor studies.

Guidelines:

- **General:** All studies in which mice or rats will have experimentally induced or implanted tumors must specify in the protocol the expected size, clinical complications (e.g., ulceration or necrosis), experimental endpoints, and potential metastatic spread or development of ascites. Criteria for intervention and/or early termination must also be included. These details must also be included for tumors that are predicted to occur spontaneously in specific strains of rodents or genetically engineered rodents.
- **Tumor size:** The maximum allowable tumor size for a single spontaneous or implanted tumor that is visible without imaging is ~2 cm in any dimension in adult mice and ~4 cm in any dimension in adult rats.² In cases where there are multiple tumors, they should not exceed the maximum burden of a single tumor. Greater single or combined tumor burdens may be approved by the ACUC with sufficient scientific justification.

The maximum size that a tumor can grow at locations within the cranium, thoracic cavity, or behind the eye that are monitored through imaging is more limited. Tumors at these locations may interfere with vital functions of the animals and result in morbidity or mortality even though the size may be much less than cited above.

- **Monitoring:** Animals with developing tumors should be observed at least once a week (i.e., every 6 or 7 days) during the period when the tumor is not yet detectable. When a solid tumor in superficial tissues becomes palpable, the animal should be monitored at least two times a week (i.e., every 3 or 4 days). Monitoring frequency must be commensurate with the growth of the tumor and the condition of the animal. At later stages of tumor development, daily monitoring of the animals may be necessary to assure compliance with humane endpoints.

Although monitoring the development of solid tumors in superficial tissues is relatively straightforward, determining the tumor burden of internal or metastatic disease provides additional challenges. In these cases, reliance should be placed primarily on the general condition of the animal but may also include adjunct methods such as measurement of biomarkers or imaging techniques.

¹ Approved by JHU Animal Care and Use Committee on: January 17, 2002; revision approved October 20, 2005; revision approved 10/18/18, reviewed 1/15/21

² If a tumor volume has been specified in a protocol, it can be estimated by the following calculation: $\text{Volume} = (L \times W^2)/2$

- **Ulceration of skin overlying tumors and abrasions (overt open lesion or scabbed area).** Some tumor types are more prone to ulceration than others because of their aggressive and inflammatory nature. Ulceration in a tumor that is less in size than 2cm does not necessarily require euthanasia if the animal is healthy, but it will require more frequent monitoring and potential treatment. Some mice with ulcerated tumors, particularly those with scabbed ulcerations, and not wet seeping ulcers, may be permitted to continue as part of the experiment in an effort to reduce the need to transplant tumors into replacement animals. Ulceration may also reflect a positive, anti-tumorigenic response to therapy as responsive tissue becomes necrotic. Seepage of blood or body fluids from ulcerated tumors can predispose the animal to infection and will require treatment or euthanasia as per veterinary discretion.

Refer to the ACUC Guideline for using the ascites method for Monoclonal Antibody Production for additional information on ascitic tumors.³

- **Humane endpoints:** Common signs of pain and distress in rodents include: ruffled fur, discharge from the eyes, weight loss or weight gain, anorexia (lack of feces in the cage), dehydration (tenting of the skin), hunched posture, lethargy, reluctance to move, uncoordinated movements, being cool to touch (hypothermia), pale ears or feet, labored respiration, and blue-tinged mucous membranes (cyanosis). Furthermore, animals in pain or distress may not interact with their cage mates or cage mates may become aggressive towards them. The affected rodent may become uncharacteristically aggressive toward a familiar human handling it. Animals may squeal when picked up or when an affected area is touched. Persistent vocalization and crying indicates substantial pain or distress.

Metastatic spread of a tumor may be difficult to assess grossly. Signs of metastasis may include the signs of distress listed above, as well as limb paralysis, difficulty walking or climbing, or difficulty breathing. Moreover, metastatic tumor cell lines may form primary tumors at a slower rate than their non-metastatic counterparts. In some cases, a decrease in the size of a transplanted primary tumor can signal that the transplanted tumor cells are moving or metastasizing out of the transplanted site.

Because the growing tumor and potential buildup of ascites fluid contributes to overall body weight at a time when the host may actually be losing body mass, emaciation is a more reliable indicator of a serious condition than loss of body weight. Thus, in evaluating the physical condition of animals during tumor progression, it is useful to utilize a scoring system such as the Body Condition Score to assess overall health. The scoring system for both mice and rats is shown in the charts below.

Animals that exhibit signs described above in the presence of a tumor burden, have a body condition score ≤ 2 should be euthanized. If the skeletal structure is not easily visualized in a mouse with ascites, it may be necessary to pick up the mouse and palpate the vertebral column to assess emaciation

Regardless of the type of tumor, the overall health and condition of the animal must take priority over precise tumor measurements in decisions regarding euthanasia or other interventions.

³ JHU Animal Care and Use Committee Guidelines link to website: <http://web.jhu.edu/animalcare/policies/index.html>

Mouse

	<p>BC 1</p> <p>Mouse is emaciated.</p> <ul style="list-style-type: none"> • Skeletal structure extremely prominent; little or no flesh cover. • Vertebrae distinctly segmented.
	<p>BC 2</p> <p>Mouse is underconditioned.</p> <ul style="list-style-type: none"> • Segmentation of vertebral column evident. • Dorsal pelvic bones are readily palpable.
	<p>BC 3</p> <p>Mouse is well-conditioned.</p> <ul style="list-style-type: none"> • Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.
	<p>BC 4</p> <p>Mouse is overconditioned.</p> <ul style="list-style-type: none"> • Spine is a continuous column. • Vertebrae palpable only with firm pressure.
	<p>BC 5</p> <p>Mouse is obese.</p> <ul style="list-style-type: none"> • 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-...)

Rat

	<p>BC 1</p> <p>Rat is emaciated</p> <ul style="list-style-type: none"> • Segmentation of vertebral column prominent if not visible. • Little or no flesh cover over dorsal pelvis. Pins prominent if not visible. • Segmentation of caudal vertebrae prominent.
	<p>BC 2</p> <p>Rat is under conditioned</p> <ul style="list-style-type: none"> • Segmentation of vertebral column prominent. • Thin flesh cover over dorsal pelvis, little subcutaneous fat. Pins easily palpable. • Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure.
	<p>BC 3</p> <p>Rat is well-conditioned</p> <ul style="list-style-type: none"> • Segmentation of vertebral column easily palpable. • Moderate subcutaneous fat store over pelvis. Pins easily palpable with slight pressure. • Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.
	<p>BC 4</p> <p>Rat is overconditioned</p> <ul style="list-style-type: none"> • Segmentation of vertebral column palpable with slight pressure. • Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis palpable with firm pressure. • Thick fat store over tail base, caudal vertebrae not palpable.
	<p>BC 5</p> <p>Rat is obese</p> <ul style="list-style-type: none"> • Segmentation of vertebral column palpable with firm pressure; may be a continuous column. • Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis not palpable with firm pressure. • Thick fat store over tail base, caudal vertebrae not palpable.

If you have any questions or need assistance, please consult ACUC Office staff (443-287-3738) or an RAR veterinarian (410-955-3273). For evenings, weekends, and holidays, the veterinarian on clinical call can be paged (410-283-0929).