



**THE UNIVERSITY OF TOLEDO
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

SUBJECT: Tumor Monitoring

DATE: March 19, 2025

Rodent Tumor Monitoring Guideline

The *Guide for the Care and Use of Laboratory Animals* states that tumor models require special consideration of humane endpoints. Tumors will inevitably impact animal welfare, often before the scientific endpoint is reached. Thus, humane intervention points should be developed to safeguard animal welfare by limiting the tumor burden and the severity of tumor-associated disease. An effective tumor monitoring plan in conjunction with the assessment of the animal's well-being should be used to achieve more objective and reproducible endpoints. This guideline was created to provide guidance to and establish expectation for researchers engaged in cancer experiments and applies to spontaneous as well as experimentally induced tumors.

Principles

1. The tumor size, clinical complications (e.g., ulceration), experimental endpoints, criteria for intervention and early removal of an animal from the study must be specified in the IACUC protocol.
2. Tumor ulceration is considered a humane endpoint unless the protocol includes adequate justification for keeping animals past the point of tumor ulceration. Animals with ulcerated tumors may experience impaired welfare due to loss of body fluids, infection, or increased pain and discomfort. Animals with ulcerated tumors must be monitored frequently and be provided appropriate supportive care. The protocol must specify euthanasia criteria after ulcer development such as the size of the ulcer, amount and type of discharge, inflammation around the wound, signs of self-mutilation, etc.
3. All cells/tumors (human or animal origin) should be tested for infectious agents and determined to be pathogen free prior to implantation into rodents.
4. The overall health and well-being of the animal take priority over precise tumor measurements in decisions regarding euthanasia or other interventions.
5. Unanticipated adverse events must be reported to the veterinarian and IACUC and may require modifications of the protocol intervention and end points.

Monitoring

1. Tumor measurement and health monitoring record must be used and should be described in or attached to the IACUC protocol.
 - a. Observations and measurements should include tumor dimensions and volume, body condition score, body weight, presence of tumor ulceration*, animal activity, mobility, overall health assessment, signs of pain (grimace), etc.
 - b. Health monitoring should include the objective scoring of signs of clinical illness where treatment is based on cumulative score. Treatment may include analgesia, nutritional supplements, parenteral fluids, and/or euthanasia.

- c. The monitoring record must be present in the animal room, or the data should be readily provided to the veterinarian upon request.
- d. *If tumor ulcerations are approved in the protocol, ulcer size, presence and character of discharge, bleeding, must also be recorded.
2. Rodents with inoculated tumors:
 - a. Animals must be observed, and assessments recorded by laboratory personnel at least once a week from the time of cell/tissue inoculation until a visible or palpable tumor is observed.
 - b. Following the noting of a visible or palpable tumor, Animals must be observed, and monitoring measurements recorded by laboratory personnel at least twice a week. Observations must be spread out in equal intervals within the week.
 - c. Once the tumor's largest diameter measurement reaches 10 mm in mice and 20 mm in rats, the tumor should be measured at least three times a week (every 2-3 days). If a rapid growing tumor line is being evaluated, then the frequency should be increased to daily.
3. Rodent strains that spontaneously develop tumors:
 - a. Animals must be observed, and assessments recorded by laboratory personnel at least once a week starting at the age when the spontaneous tumors typically develop until a visible or palpable tumor is noted.
 - b. Animals must be assessed, and measurements recorded by laboratory personnel at least twice a week following the noting of a visible or palpable tumor. Observations must be spread out in equal intervals within the week.
4. More frequent observations and measurements may be necessary based on tumor growth, study parameters, and general well-being of the animal. Health assessments and supportive care may be required multiple times per day. This includes weekends and holidays.

Humane endpoints:

1. Tumor size must not exceed 20mm (2.0cm) in any direction in an adult mouse and 40mm (4.0cm) in adult rats.
 - a. If multiple tumors are present in close proximity, the combination of the two largest diameters may not exceed 2.0 cm for mice and 4.0 cm for rats.
2. Tumor volume limit for mice is 2000 mm³ and for rats is 5000 mm³.
 - a. The same standard applies to multiple tumors: the combined total volume for mice is 2000 mm³ and for rats is 5000 mm³.
 - b. Tumor volume (mm³) = $d^2 \times D/2$ where d and D are the shortest and longest diameter in mm, respectively.
 - c. Figure 1. Example tumor volume:

d=15mm D=20mm		$15^2 \times 20/2 = 2250\text{mm}^3$	Humane endpoint reached 1) Volume > 2000mm ³ 2) D = 20mm
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3. Body weight and Body Condition Score (BCS)
 - a. Weight and BCS should be monitored and recorded at least once per week initially then at the time of each tumor measurement.
 - b. Depending on the tumor type and location, weight gain can occur due to tumor growth or ascites while body condition is decreasing so both need to be monitored.
 - c. Animals with a weight loss $\geq 20\%$ or BCS < 2 should be euthanized. See Figure 2 for an illustration of BCS.
 - d. For younger animals (depending on species and strain), failure to maintain weight gain to within 15% of untreated control animals should be considered as an indication of significant health deterioration.
4. Tumor ulceration (unless approved in the protocol)
5. Discharge or hemorrhage from tumor.
6. Tumor interferes with normal body functions, including but not limited to ambulation, eating, drinking, defecation, or urination; tumor negatively affects animal's gait or

- posture independent of tumor size.
7. Labored breathing
 8. Lack of movement
 9. Hypothermia
 10. Self-mutilation
 11. Positive pulmonary assessment of advanced metastasis (PAAM) score.
 - a. PAAM is a reliable noninvasive method to detect lung metastasis before the development of mortality. See Figure 3 for a step-by-step illustration.
 12. Animals that in the opinion of the University Attending Veterinarian require euthanasia for humane reasons.

References

1. *The Guide for the Care and Use of Laboratory Animals*, 8th Edition. National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US).
2. CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints. March 2022.
https://ccac.ca/Documents/Standards/Guidelines/CCAC_guidelines_scientific_endpoints.pdf
3. Ullman-Cullere, M. H. and C. J. Foltz. 1999. Body Condition Scoring: a rapid and accurate method for assessing health status in mice. *Lab Anim Sci* 49(3): 319-323.
4. Wallace, J. Humane Endpoints in cancer research. *ILAR Journal* 41: 87-93 2000.
5. OBSERVE: guidelines for the refinement of rodent cancer models. *Nature Protocols* 19: 2571-2596.
6. Mendoza A., Gharpure R, Dennis J, Webster JD, Smedley J, Khanna C. A Novel Noninvasive Method for Evaluating Experimental Lung Metastasis in Mice. *J Am Assoc Lab Anim Sci*. 2013 Sep;52(5):584–589.

Figure 2. Body condition scoring



BC 1

Animal is emaciated

- Skeletal structure extremely prominent
- Little or no flesh cover
- Vertebrae, ribs, and pelvic bones distinctly segmented



BC 2

Animal is under-conditioned

- Segmentation of vertebral column evident
- Dorsal pelvic bones are readily palpable or observable



BC 3

Animal is well-conditioned

- Vertebrae and pelvis not prominent
- Ribs palpable under slight pressure



BC 4

Animal is over-conditioned

- Vertebrae palpable only under firm pressure
- Noticeable fat deposits over spine



BC 5

Animal is obese

- Animal is smooth and bulky, abdomen is distended
- Bone structure disappears under flesh and subcutaneous fat

Figure 3. Step-by-step schematic description of the PAAM technique for detecting advanced pulmonary metastatic burden

