

**MASSACHUSETTS GENERAL HOSPITAL (MGH)
INSTITUTIONAL ANIMAL CARE AND USER COMMITTEE (IACUC)**

POLICY ON SPONTANEOUS AND INDUCED TUMOR PRODUCTION IN RODENTS

GENERAL POLICY

Special consideration is required for humane endpoints based on known tumor biology for studies that employ tumor models, according to the *Guide for the Care and Use of Laboratory Animals* and the “Guidelines for the Welfare and Use of Animals in Cancer Research” adopted by AAAALAC International.^(1,2) Predictable indications of pain, distress or significant deviation from normal behavior should be considered.

INDUCTION SITE CONSIDERATIONS

The method and the site for implantation of transplantable or induced solid tumors** requires considerable care to minimize trauma to the host animal. Sites should be chosen that minimize damage to adjacent normal structures and will not interfere with normal body functions such as ambulation, eating, drinking, defecation, and urination. Implantation of tumors in the muscle, footpad, tail, brain and eye are discouraged and will require scientific justification (note: metastatic tumor cells may be administered by IV injection into the tail).

For spontaneous and transplanted tumors, important features include growth rate, invasion, distension, ulceration, metastases, and production of cachectic factors. All of these factors can differ with tumor type and may impact both the frequency at which the animals must be observed and the duration of the study.

In the case of leukemias, internal, disseminated, metastatic or other occult tumors, determination of the tumor burden may be difficult. The development and/or use of appropriate biochemical and pathological laboratory methods to determine the onset of these tumors may be required. Careful monitoring of the animal’s overall clinical condition is necessary in these situations.

The technical staff must be aware of the parameters of the study, such as tumor growth potential, whether a tumor is likely to become ulcerated and/or appropriate biochemical or pathological endpoints. The Principal Investigator (PI) must clearly define study parameters and endpoints in their IACUC Approved Protocol.

Particular care should be taken with monitoring the development of spontaneous tumors in all transgenic animals and especially those strains that are known to be cancer laden. Careful and regular clinical examination should be carried out to allow for the detection of both predicted and unexpected sites of tumor development.

***Contamination of tumor cell lines with human and/or rodent viruses and other microorganisms may compromise experimental results, as well as cause an outbreak of disease among laboratory animals. Screening of cell lines for rodent viruses and mycoplasmas is required.*

Studies involving the use of primary human tumors or tissues with uncharacterized blood-borne pathogens must first be reviewed and approved by the Partners Biosafety Committee (PIBC).

MONITORING GUIDELINES

The IACUC emphasizes the need for frequent monitoring during tumor development to allow for appropriate intervention before significant deterioration of animal health or death occurs. Effective monitoring systems and endpoints should include defined limits on the tumor burden and severity of tumor-associated disease. The use of altered physiological, biochemical, and other biomarkers are encouraged as potentially additive objective and reproducible endpoints than clinical signs.

All tumor-bearing animals must be directly observed at an established frequency to assess their physical condition, tumor growth, and /or metastasis in accordance with your protocol. The monitoring plan should be based on known information about growth characteristics and biology of the proposed tumor model and onset and nature of any adverse effects on the animals. The IACUC requires that tumor-bearing animals be observed 1-2 times weekly, but more frequent monitoring may be required depending on tumor size, growth rate and associated disease. If information is unknown regarding the proposed tumor model, frequent observations should be planned until tumor growth and animal welfare impact is characterized.

Records must be kept with all pertinent information in the Individual Animal Medical Record, Rodent Record Card, laboratory notebook or equivalent, in accordance with the IACUC Policy on Animal Observation and Record Keeping. Information that should be documented includes protocol number, time and frequency of monitoring, the name (initials) of the person monitoring the animal, identification of the animal, animal weight, type of clinical signs, tumor size (if appropriate), and any treatments given to the animal, in accordance with the approved IACUC protocol. Records maintained by the laboratory personnel should be available to the veterinary staff and/or the IACUC upon request.

- The maximum size for tumors (i.e. size in any one dimension) is 20 mm for a mouse or 40 mm for a rat. In cases of multiple tumors, the maximum size for any one tumor is 10 mm for mice and 20 mm for rats. The size of superficial tumors should be assessed using calipers. Scientific justification to exceed this size restriction must be approved by the IACUC.
- Clinical observations and/or palpation will be necessary to monitor for deterioration of clinical condition. Special examination techniques may be required for specific sites (e.g. respiratory rate for lung involvement, neurological disturbance for brain neoplasms, and blood cell counts for leukemias).

Adverse clinical signs which may be associated with tumor progression:

Decreased food/water intake	Loss of body condition ⁺
Lethargic/depressed activity	Restlessness
Vocalization	Respiratory difficulty
Cranial deformity/neurological	Perianal soiling

signs	
Rough haircoat	Hunched posture
Skin pathology	Restricted mobility
Jaw deformity/malocclusion	Changes in feces/urine

+ See Appendix A for a Body Condition Scoring System for Mice

- Measurement of body weight changes (both positive and negative changes compared to controls) can be used to assess tumor burden. Baseline body weights must be recorded for each animal at the start of the study and periodically through completion of the study. The period should be stated in the IACUC approved protocol. Considering both weight loss and weight gain from growth, tumor burden should not exceed 10% of the animal's normal body weight. Weight loss should not exceed 20% of the animal's body weight at the start of the experiment. 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.

PROTOCOL EXPERIMENTAL DESIGN CONSIDERATIONS

Endpoints must be established to minimize the potential for pain and/or distress. The investigator must have a plan for pre-emptive euthanasia based on clearly defined endpoints in the protocol. Investigators should describe in their research protocols the behavioral observations and clinical indicators of pain and distress that will be used as criteria for euthanasia. **NOTE: The "intentional death" end point will not be allowed unless it is scientifically justified to the IACUC. Animals expected to become moribund should be euthanized prior to reaching this state.**

- Particular attention must be paid to the body system and/or organ system (e.g., skin, peritoneum, spleen, lymph node, etc.) most likely to be affected by the tumor type. The site for injection should be carefully chosen to permit room for tumor growth and to avoid unnecessary distress. Subcutaneous or intradermal growth on the back or flank is considered to cause the least distress.
- Some tumors may result in ascites, leading to severe abdominal distention. Distention interferes with a number of physiological systems including but not limited to the respiratory and gastrointestinal systems. Ascitic tumors producing large volumes of fluid can rapidly deplete the animal of essential nutrients such as protein and hasten cachexia.
- Tissue necrosis or ulceration of the skin overlying the developing tumor may occur. Ulceration or necrotic tissue may result in a continuous loss of body fluids and/or infection, and should require euthanasia of the affected animal unless approved by the IACUC. When it is necessary to maintain an animal with an ulcerated tumor, the status of the ulcer and the animal's overall condition must be assessed in consultation with the veterinary staff.
- Animals with tumors that impact mobility and/or interfere with the animal's ability to acquire food or water, may require supportive care or euthanasia.

HUMANE ENDPOINTS

All animal experiments must provide for a humane endpoint. As a general guideline, animals used in experimental procedures involving tumor development will be considered for euthanasia if the following conditions occur:

- Tumors exceed maximum allowable size unless approved by the IACUC. (The maximum size for tumors is 20 mm for a mouse or 40 mm for a rat. In cases of multiple tumors, the maximum size for any one tumor is 10 mm for mice and 20 mm for rats).
- Tumor size or metastatic growth interferes with normal behavior and condition of the animal (e.g. ambulation, eating, drinking, grooming) or causes pain or distress due to its location.
- Weight loss exceeding 20% of the body weight of a conspecific normal animal (taking into account the tumor mass).
- Body Condition Score (BC) = 1
- Tumor becomes ulcerated (break in overlying skin), infected, or necrotic.
- Palpation of tumor elicits a pain response.
- Self-induced trauma associated with tumor location.
- Animal appears weak with “hunched posture”, is unresponsive, or moribund
- Animal becomes anorectic.
- Animal appears dehydrated.
- Animal shows respiratory difficulty.
- Ascites production due to tumor progression and which results in an increase in body weight of 20% due to ascitic fluid.

Note: Following veterinary assessment of animals exhibiting the above adverse clinical signs, euthanasia may be performed at the discretion of a CCM veterinarian.

Related Policies:

Policy on Animal Observation and Record Keeping

[Most recently revised October 2016]

REFERENCES

1. Guide for the Care and Use of Laboratory Animals, 8th edition, NRC, 2011.
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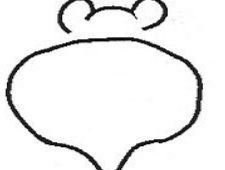

ADDITIONAL READING RESOURCES

1. Carstens E, Moberg GP. Recognizing pain and distress in laboratory animals. *ILAR J* 41:62-71, 2000.
2. Dennis M. Humane endpoints for genetically engineered animal models. *ILAR J* 41:94-98, 2000.
3. Hendriksen CFM, Morton DB, (eds.). Humane Endpoints in Animal Experiments for Biomedical Research. In: Proceedings of the International Conference, November 22-25, 1998, Zeist, The Netherlands. London: Royal Society of Medicine Press Ltd, 1999.
4. Hendriksen CFM, Steen B. Refinement of vaccine potency testing with the use of humane endpoints. *ILAR J* 41:105-113, 2000.
5. JCAHO [Joint Commission on the Accreditation of Healthcare Organizations]. Pain Assessment and Management Standard, Comprehensive Accreditation Manual for Hospitals: The Official Handbook (CAMH), Standard RI.1.2.8, 1999. www.jcaho.org/standard/pm_hap.html#ri12.
6. Morton DB. A systematic approach for establishing humane endpoints. *ILAR J* 41:80-86, 2000.
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9. PHS [Public Health Service]. Public health service policy on humane care and use of laboratory animals. Washington, DC: US Department of Health and Human Services, 1996.
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11. Sass N. Humane endpoints and acute toxicity testing. *ILAR J* 41:114-123, 2000.
12. Stokes WS. Humane Endpoints for Laboratory Animals Used in Toxicity Testing. In: Proceedings of the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, Bologna, Italy, August 31-September 2, 1999. New York: Elsevier Sciences, 2000.
13. Stokes WS, Hill RN. The Role of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in the Evaluation of New Toxicological Testing Methods. In: Proceedings of the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, Bologna, Italy, August 31-September 2, 1999. New York: Elsevier Sciences, 2000.
14. Tannenbaum J. Ethics and pain research in animals. *ILAR J* 40:97-110, 1999.

15. Toth LA. Defining the moribund condition as an endpoint for animals used in research and testing. *ILAR J* 41:72-79, 2000.
16. USC [US Code]. National Institutes of Health Revitalization Act. PL 103-43. 42 USC. Washington DC: US Gov't. Printing Office, 1993.
17. USDA [US Department of Agriculture]. Animal Welfare Report. Fiscal Year 1998. Animal and Plant Health Inspection Service. APHIS 41-35-059. Washington, DC: USDA, 1998.
18. Wallace, J. Humane Endpoints in Cancer Research. *ILAR Journal* 41:87-93, 2000.

APPENDIX A

Body Condition Scoring System for Mice

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

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)

Ullman-Cullere MH, Foltz CJ. Body condition scoring: a rapid and accurate method for assessing health status in mice. *Lab Anim Sci.* 1999 Jun;49(3):319-23.