

**University of Cincinnati
Animal Care and Use Program**

Tumor Monitoring

The *Guide* states that “while all studies should employ endpoints that are humane, studies that commonly require special consideration include those that involve tumor models.”

Questions - send an e-mail to lams-veterinary@uc.edu

Requirements for Using Tumor Cell Lines and Primary Tissue

Rodent cell lines, tumor tissue samples, and hybridomas obtained from other institutions (not LAMS colony or reputable commercial sources) should be screened for rodent viruses and mycoplasma (human cell lines if not passed through rodents) contamination prior to inoculation into animals to ensure that they are free from pathogens that UC excludes from our rodent colonies. Please see companion document **Guidelines for use of Biological Substances in Rodents** for additional information. The Institutional Biosafety Committee (IBC) must approve use of human cells/materials or any genetically modified cells with viral vectors in animals.

Tumor Study Considerations

1. Pilot studies using small number of animals should be considered when working with unfamiliar or novel tumor models (e.g. cells, cell number, route, site of growth), investigational anti-tumor drugs, or if the approximate time for induced tumor growth or tumor profile is unknown.
2. For spontaneously arising tumors (e.g. induced by carcinogens, genetic manipulation), researchers should pay particular attention to the time of onset, tumor progression, and any unexpected tumor development sites.
3. Where possible, researchers should use biochemical and pathological indicators, bioluminescent imaging, or other imaging techniques (e.g. CT, PET) to monitor non-superficial tumor burden.
4. Sites of solid tumors will influence the maximum acceptable tumor load, as well as the humane endpoints.
5. Tumors located within the brain, thoracic cavity, footpad, bone, mouth, or behind the eyes may interfere with vital functions, though maximum size is considerably smaller. An assessment of overall health status may take priority.
6. Tumor burden should be limited to the minimum required to establish a valid scientific outcome.
7. When extended survival data is needed from a tumor model, it is necessary to try to determine a specific point or parameter at which to record the animal as “moribund” or as “dead” without jeopardizing the humane treatment of the animals and will not necessitate the use of death as an endpoint.

Selected Criteria for Euthanasia for Tumor Studies

The criteria below are clinical signs of illness or disease that constitute a tumor study humane endpoint and include, but are not limited to:

1. The maximum allowable tumor size for a single spontaneous or implanted tumor on the flank or dorsum is determined by calculating tumor burden and mean tumor size:
 - a. Tumor burden is calculated using the formula: $d^2 \times D/2$, where d is the length in one direction and D is the length in the other direction (90 degrees apart).
 - i. Endpoint Criteria: Tumor burden >15% of body weight
 1. Example (Mouse):
 - a. $d=20\text{mm}$ (20 mm in one direction) and $D=20\text{mm}$ (20 mm in the other direction)
 - b. $20^2 \times 20/2 = 4000\text{mm}^3 = 4 \text{ gm}$
 - c. Mouse weighs 25 gm
 - d. $4 \text{ gm tumor}/25 \text{ gm mouse} = 16\%$ (endpoint criteria met)
 - b. Mean tumor size is calculated using the formula: $(d+D)/2$
 - i. Endpoint Criteria: Mean tumor size of 20 mm (mouse) and 40 mm (rat) or greater, if it remains otherwise healthy.
 1. Example (Rat):
 - a. $(d+D)/2 = (45+35)/2 = 40 \text{ mm}$ (endpoint criteria met)
2. For bilateral tumors, either tumor reaches 20 mm or 40 mm (mouse or rat respectively) or greater in size (longest diameter) or the summation of the longest diameter of first tumor and the longest diameter of second tumor exceeds 3 cm or 6 cm in size for adult mouse and rat, respectively.
3. For multiple spontaneous tumors (e.g. MMTV-PyMT mouse model), tumors may reach more than maximum diameter measurements for bilateral tumors (see #2 above). Total tumor load volume will be calculated using the following equation: $\text{Volume} = (1/2) \times L \times W^2$, and is not to exceed 3cm^3 . LAMS involvement may be required as animals approach endpoints ($2\text{-}3\text{cm}^3$) for animal welfare observations. It may also be recommended to calculate tumor burden with greater than 15% of body weight being considered as endpoint criteria (refer to #1a above).
4. If tumors are expected to grow with accumulation of fluid in the peritoneal cavity (ascites), the animal should be euthanized, or LAMS vet staff contacted for ascites management.
5. For non-palpable tumors, body condition score and overall condition of the animal are typically used to evaluate the state of the animal.
6. The tumor interferes with normal body functions, including but not limited to ambulation, eating, drinking, defecation, or urination.
7. Tumors become ulcerated, remain vascular and are infected.
8. Additional criteria for euthanasia can be found in [ACUP Humane Endpoints Guideline](#).

Monitoring and Recordkeeping

The Animal Care and Use Program (ACUP) requires proper documentation of animal care and use in order to assess compliance with research protocols and clinical care procedures.

1. Research staff should monitor animals at a frequency determined by the known biology of the tumor, ensuring that all relevant staff are able to recognize tumor-specific clinical signs and are able to readily apply humane endpoints.
2. Animals inoculated with tumor cells or that develop spontaneous tumors (e.g. tumorigenic strain) should be observed at least once weekly by research staff in addition to the regular daily husbandry staff observations.
3. Monitoring should increase upon tumor detection to at least two times a week (e.g. Monday and Friday). As tumors near their endpoint (generally 75% of the allowed tumor burden humane endpoint), observations should increase to daily, including weekends and holidays.
4. The frequency of monitoring should increase as dictated by experimental and/or approaching humane endpoints, at the request of the veterinary staff, or if the tumor kinetics are unknown.
5. The size of the tumor burden should be measured by caliper or other mechanisms for palpable/superficial tumors; biochemical and pathological indicators, bioluminescent imaging or other imaging techniques should be used for non-palpable tumors.
6. Body Condition Score (BCS) and clinical evaluations of the animals may take priority over the measured burden of the tumor.
7. Cage cards should indicate the specific date of inoculation as well as the nature of the injected substance (e.g. cells).
8. Each instance of observation should be documented, including a minimum recording of associated information: the date, observation (tumor present/absent, change in tumor, score), and initials of the observer.
9. Records of observations, including weights and/or measurements, as appropriate, should be maintained and made available.