NU–IACUC POLICY

Northeastern University Institutional Animal Care and Use Committee

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| Tumor Implantation, Use, and Clinical Endpoints |

*Approved: May 10, 2022*

Rodents are commonly used to study tumor biology and the development of new therapeutic approaches to cancer. Enhanced monitoring and appropriate supportive care are necessary to prevent and minimize animal pain and distress in experimental oncology studies consistent with the scientific requirements of the study. The Principal Investigator should state the degree of tumor development required to meet the scientific objectives of the study. For all cancer research models, endpoints should be established in advance and approved by the Institutional Animal Care and Use Committee (IACUC) to determine when animals should be euthanized. The standard guidelines recommended below are common in experimental oncology and should be used whenever possible. Extended humane endpoints require specific scientific justification as well as enhanced monitoring and supportive care. Veterinary staff and the IACUC can request records of the clinical assessments of these animals (i.e. notations on health check cards, cage-side monitoring logs, and personal laboratory notebooks). Thus, it is imperative to properly maintain complete and contemporaneous records for these animals in a readily accessible location and format.

Prior to Starting Tumor Work

1. All animals to be implanted with tumors must be identified at the time of ordering on the Animal Requisition Form.
2. All tumor and cell lines must be tested for adventitious rodent pathogens prior to use in the animals via PCR testing. Documentation of test results must be submitted to the DLAM Director prior to tumor use. For information on the PCR testing, contact the DLAM Director at x3958.
3. All rodent tumors or cells obtained from the ATCC (American Type Culture Collection), which are not tested for adventitious rodent pathogens, must also be tested. Human tumors or cells obtained from the ATCC (American Type Culture Collection) do not need to be tested.
4. Tumor bearing animals must be observed frequently by the investigator/staff to assess the progress of the tumor growth (measure size) and/or metastasis, the progress of therapeutic regiments, and the general condition of the animal. Additionally, once a tumor is close to the endpoint, the animal must be observed daily or more frequently by the investigator and/or their staff.

Standard Humane Endpoints for Tumors in Mice and Rats

The following are the standard humane endpoints for tumor studies:

1. Cumulative tumor burden/animal (see formulas for calculating volume below)
   1. Mice: 1 cm3 and/or no greater than 1.5 cm in diameter in any one direction
   2. Rats: 4.5 cm3 and/or no greater than 4 cm in diameter in any one direction
2. Body Condition Score (BCS) of <2/5 and/or weight loss >15% from baseline body weight (excluding tumor weight)
3. Presence of necrosis or ulceration
4. Prolonged labored breathing or respiratory distress
5. Significant abdominal distention (i.e., ascites)
6. Compromised ability to eat or drink
7. Absence of or abnormal fecal or urine output
8. Lethargy or reluctance to move
9. Abnormal gait, paresis or paralysis
10. Other clinical signs of pain, such as vocalizations or chewing of the lesion, that are nonresponsive to analgesic therapy

Animals should be assessed at least twice a week for the above endpoints.

Extended Humane Endpoints for Subcutaneous Tumors in Mice

Under special circumstances some of the currently accepted tumor humane endpoints do not align with scientific goals in the study of tumor biology (i.e. study of metastasis, tumor therapeutics, etc.) In such cases and with scientific justification, the following tumor sizes and development of necrosis/ulcerations may be allowed:

1. Tumor size no greater than 1.5 cm3 and/or no greater than 2 cm in diameter in any one direction.
2. Persistence of visible ulceration or necrosis (no greater than 5 mm in diameter in any one direction) at the primary tumor site may be permissible provided that:
   1. Animals in pain will receive analgesia, unless the protocol includes justification for no analgesics, in consultation with veterinary staff. Euthanasia is required for painful animals which do not respond to analgesia.
      1. Buprenorphine or buprenorphine SR should be considered as a first choice analgesic.
      2. Acetaminophen (Tylenol®) may be used at a dose of 300 mg/kg added to water and changed every 48 hours. Acetaminophen is not preferred as a sole analgesic for severe pain.
      3. Scientific justification must be given if no analgesics are being given.

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| Species | | Drug | Route | **Dose (mg/kg) or (mg/ml or L)** | **Drug Concentration (mg/ml)** | **ml to add to a full water bottle (473ml)** | **Final Concentration in Water (mg/ml)** | |
| Mouse | | Acetaminophen | In Water | 300 | 50 or 32 | 14.0 or 22.0 | 1.500 | |
|  | |  |  |  |  |  |  | |
| Rat | | Acetaminophen | In Water | 300 | 50 or 32 | 24.0 or 37.0 | 2.500 | |
| **Drug dosages are based on:** | | | | | | |
| 30 g Mouse drinking 6 ml/day (15 ml per 100 g BW/day) | | | | | | |
| 350 g Rat drinking 42 ml/day (12 ml per 100 g BW/day) | | | | | | |
| **Note:** A full CMS water bottle = 16 oz. (473 ml) | | | | | | |

* 1. Animals with infection will receive topical or systemic antibiotic treatment. Euthanasia is required for animals which do not respond to antibiotic therapy.
  2. Animals with weakness or painful conditions of the feet will receive supportive care on the cage floor, i.e. Hydrogel and/or Dietgel.

The other standard humane endpoints (#’s 4-10 as listed above) still apply to these studies.

## Monitoring for Mice with Extended Tumor Endpoints

Increased monitoring should begin when tumors reach 1 cm3, are greater than 1.5 cm in diameter in one direction, and/or when necrosis and/or ulceration develops. Recommended monitoring includes:

1. Daily assessment, including weekends/holidays
2. Weighing at least 3x per week.
3. Tumor measurement at least 3x per week.

## Formulas for the Determination of Tumor Size

Tumor volume is calculated using tumor measurements, utilizing one to three measured dimensions. Any of these formulas can be used. Other formulas require specific description in the protocol.

* Tumor volume = ½ (length x width x height)
* Tumor volume = ½ (length × width2)
* Tumor volume = ½ (diameter3)

The humane endpoints for tumor burden recommended below are *cumulative*. For example, if a mouse has two tumors with diameters of 0.75 cm and 1 cm respectively:

* Tumor 1: = ½ (diameter3) = ½ (0.75cm3) = 0.21 cm3
* Tumor 2: = ½ (diameter3) = ½ (1.0cm3) = 0.5 cm3

Cumulative tumor burden = 0.21 cm3 + 0.5 cm3 = 0.71 cm3

## Formulas for the Determination of Body Weight Loss Excluding Tumor Weight

Body weight loss is determined by using the current body weight and the baseline body weight. Given that tumors will increase body weight of the animal, the weight of the tumor should be excluded from this calculation.

Tumor weight is determined by assuming each 1 cm3 of tumor weighs 1g. For example, a 0.75cm3 tumor weighs 0.75g.

The formula for body weight loss excluding tumor weight is as follows:

Text, letter

Description automatically generated

## Special Consideration for Euthanasia Endpoints of Pulmonary Metastasis Studies

Tumor burden in the lungs is difficult to assess without advanced imaging or molecular assays. Euthanasia endpoints of studies expecting pulmonary metastasis should include the endpoints previously described AND the non-invasive Pulmonary Assessment of Advance Metastasis (PAAM) test. Significant pulmonary tumor burden will reduce respiratory capacity and results in a positive PAAM test. (Mendoza et al., 2013). (See Figure 1 below)

Graphical user interface, application

Description automatically generated

Figure 1. (A) Mouse is restrained by using the thumb and forefinger of the nondominant hand, and the ring finger is used to secure tail. The mouse is aligned parallel to the forearm of the evaluator, with the head pulled back slightly. (B) Digital pressure is applied gently just caudal to the xiphoid sternum by using the forefinger of dominant hand. (C) Gentle to moderate pressure is applied for 3 s. In normal mice or mice without advanced pulmonary metastasis, this digital pressure results in either no response or only a mild increase in respiratory rate (negative PAAM). In mice with advanced pulmonary metastasis, the described 3 s of digital pressure results in a pronounced increase in chest excursion during respiration or agonal breathing (that is, open-mouth breathing, gasping; positive PAAM).

The euthanasia endpoint for these studies would include positive PAAM test.

Mouse Body Condition Score Guidelines

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| **Mouse Body Condition Score (BCS)** | **Diagram** | **Photo** | **Description** |
| **1** | bcs | BCS-1(2).JPG | **Mouse is emaciated.** Skeletal structure is extremely prominent. Little or no flesh cover. Vertebrae are distinctly segmented. |
| **2** | bcs | BCS-2.JPG | **Mouse is under-conditioned.** Segmentation of vertebral column is evident. Dorsal pelvic bones are readily palpable. |
| **3** | bcs | BCS-3.JPG | **Mouse is well-conditioned.** Vertebrae and dorsal pelvis are not prominent but palpable with slight pressure. |
| **4** | bcs | BCS-4(2).JPG | **Mouse is over-conditioned.** The spine is a continuous column. Vertebrae are palpable only with firm pressure. |
| **5** | bcs | BCS-5.JPG | **Mouse is obese,** smooth and bulky. Bone structure disappears under flesh and subcutaneous fat. |

Ullman-Cullere MH, Foltz CJ (1999) Body condition scoring: a rapid and accurate method for assessing health status in mice. *Lab Anim Sci* **49**: 319–323

# Regulations and Reference Documents:

(Currie, Sena, Fallon, Macleod, & Colvin, 2014; Mendoza et al., 2013; Paster, Villines, & Hickman, 2009; Van Loo et al., 1997; Wallace, 2000; Workman et al., 2010)

1. Currie, G. L., Sena, E. S., Fallon, M. T., Macleod, M. R., & Colvin, L. A. (2014). Using animal models to understand cancer pain in humans. *Current Pain and Headache Reports*, *18*(6). https://doi.org/10.1007/s11916-014-0423-6
2. Mendoza, A., Gharpure, R., Dennis, J., Webster, J. D., Smedley, J., & Khanna, C. (2013). A novel noninvasive method for evaluating experimental lung metastasis in mice. *Journal of the American Association for Laboratory Animal Science*, *52*(5), 584–589.
3. Paster, E. V., Villines, K. A., & Hickman, D. L. (2009). Endpoints for mouse abdominal tumor models: Refinement of current criteria. *Comparative Medicine*, *59*(3), 234–241.
4. Van Loo, P. L. P., Everse, L. A., Bernsen, M. R., Baumans, V., Hellebrekers, L. J., Kruitwagen, C. L. J. J., & Den Otter, W. (1997). Analgesics in mice used in cancer research: Reduction of discomfort? *Laboratory Animals*, *31*(4), 318–325. https://doi.org/10.1258/002367797780596211
5. Wallace, J. (2000). Humane endpoints and cancer research. *ILAR Journal*, *41*(2), 87–93. https://doi.org/10.1093/ilar.41.2.87
6. Workman, P., Aboagye, E. O., Balkwill, F., Balmain, A., Bruder, G., Chaplin, D. J., … Ryder, S. (2010). Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer*, *102*(11), 1555–1577. https://doi.org/10.1038/sj.bjc.6605642