BACKGROUND

Tumor (cancer) implantation in research animals is a critically important experimental activity which also requires consideration of the effect of the tumor on the animal. The importance of limiting the discomfort, pain and distress animals may experience during the conduct of biomedical research is well-recognized and is the primary force behind the animal welfare regulations that govern the use of animals in research. Outcomes of tumor studies vary depending on the species and strain of animals, the route of injection for transplantable tumors, and the subsequent cancer treatment. Effective monitoring systems and endpoints should include limits on the tumor burden and severity of tumor-associated disease. This policy limits the tumor burden an animal experiences to that which does not cause excessive pain or distress, and are for cumulative tumor burden per animal. At all times, the well-being of the research animals must be balanced against requirements of the study.

POLICY AND PROCEDURE

- **Tumor Type** - Because transplantable tumors, hybridomas, cell lines, and other biologic materials can be sources of murine viruses that can contaminate rodents, all transplantable murine tumors, must be assayed for contamination with adventitious murine viruses to prevent possible spread of pathogens into our rodent colonies. For more information, contact the Attending Veterinarian. All transplantable human tumor lines require Institutional Biosafety Committee (IBC) review and approval.

- **Tumor Location** – The site of the tumor implantation should be chosen to minimize damage to adjacent normal structures. The IACUC recommends implanting tumors on the dorsum or flank of an animal as these areas will likely have minimal or no site-related morbidity.

- **Tumor Size** - The visible size of the tumor is only one of the criteria used for determination of humane endpoint. The overriding consideration for humane endpoints of oncological experiments as well as spontaneous tumors must be the overall health of the animal. For subcutaneous tumors the maximum allowable size is 20 mm in diameter for a mouse or 40 mm diameter for a rat. If the animal is host to more than one tumor, this size is the maximum allowable size for all tumors combined.

- **Monitoring** - Animals that are on a tumor production study must be monitored by the investigators daily during the time when the tumor is not yet detectable, to observe when tumor growth has begun, including weekends and holidays. After a visual or palpable tumor is evident, the animals may require more frequent observations. Monitoring frequency may increase based on tumor growth rate, study parameters, and general condition of the animal. Tumors should be measured with calipers along the longest point and documented once visually observed. Monitoring should involve the overall condition of the animal including appearance, posture, behavior and physiological responses. Food and water intake must be monitored and recorded daily by the investigator.
Body Condition Score - Assessment of the overall health of the animal should take priority over assessment of tumor size or loss of body weight. In some cases, tumor growth may result in an increase in body weight and a decrease in lean body mass (close assessment of tumor size and body weight is especially important for younger, growing animals as failure to maintain weight gain comparable to untreated control animals may indicate adverse tumor effects). The body condition scoring system (BCS), developed by Ullman-Cullere and Foltz (Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice. Lab. Animal Science; 49, 319-323, 1999), has proven to be a reliable indicator of general health and utilizes a scoring system that ranges from “1” (emaciated /wasted) to “5” (obese). The BCS offers a useful, rapid and objective assessment of an animal’s health, especially in cases where treatments designed to affect tumor growth (e.g., radiation, chemotherapy) lead to poor body condition. This system quantitatively assesses a number of indicators of health status, including body weight, physical appearance, measurable clinical signs, unprovoked behavior and response to external stimuli. The use of this method, however, does not preclude other criteria for euthanasia prior to study endpoint, including, but not limited to the ability to eat or drink, labored breathing, ulceration (a breakdown of the skin cells resulting in exposure of the underlying tissue) and necrosis (death of cells in an organ or tissue due to disease, injury, or failure of the blood supply) at the tumor site. See Appendix I: Body Condition Scoring (BCS) Guide for mice and Appendix II: Body Condition Scoring (BCS) Guide for rat.

Humane Endpoints - Experiments should be completed before tumor development or tumor-associated disease causes death or a significant deterioration in the host. The overall well-being of the animal takes priority over precise tumor measurements in decisions regarding euthanasia or other interventions. In circumstances involving declining health status, morbidity, or unrelieved pain and discomfort, every attempt will be made to contact the PI and to reach consensus with the PI bearing experimental endpoints in mind. However, the final analysis of when and if euthanasia is required is the responsibility of the Attending Veterinarian. The following clinical signs are indications of morbidity. The presence of one or more of the criteria below is indication for euthanasia:

- The BCS is < 2
- Impaired mobility (the inability to reach food and water)
- Inability to remain upright
- Interference with a vital physiological function: This includes respiration, mastication, swallowing, urination, defecation or locomotion
- Location of the tumor on the animal’s ventral abdomen causing the tumor to be abraded or medial thigh interfering with locomotion
- Hunched abnormal posture for > 48 hours
- Labored breathing and cyanosis [bluish pinnae (ears), foot pads or mucous membranes]
- Clinical dehydration (≥5%) and/or prolonged (≥24 hours) decrease in food intake
- Muscle atrophy, signs of lethargy and lack of physical activity
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- Diarrhea or constipation for more than 48 hours
- Hematological or biochemical values that indicate organ failure
- Severe anemia [pale pinnae (ears), foot pads or mucous membranes]
- Bloodstained or mucopurulent discharge from any orifice
- Self-mutilation, lack of grooming behavior, or rough/unkempt hair coat for >48 hours
- Enlarged lymph nodes or spleen
- Abdominal distension, ascites, or pleural effusion
- Cranial deformity
- Neurological signs (circling, seizures, weakness, restless/uncomfortable, altered level of consciousness
- Exophthalmos (bulging eye)
- Skin pathology including ulceration or necrosis of tumor for ≥ 48 hours.

TRAINING

Investigators and staff responsible for monitoring the animal on tumor studies should not only be familiar with normal animal health and behavior, but must also be able to observe adverse changes in health, behavior, or tumor burden. Specifically, since there are differences in normal behavior between different mouse and rat strains, the responsible research staff must be familiar with the animal(s) on study **BEFORE** the experiment begins.

EXCEPTIONS

If there is a strong scientific justification for maintaining tumors exceeding any of the guidelines described above, then the investigator must specifically request an exemption from the policy and receive approval from the IACUC.
REFERENCES

Boston University IACUC Tumor Policy for Mice and Rats

Duke University and Duke University Medical Center Animal Care and Use Program Policy on Tumor Burden in Rodents

Guide for the Care and Use of Laboratory Animals, 8th Edition (2011)

University of Pennsylvania IACUC Guideline on Rodent Tumor and Cancer Models
http://www.upenn.edu/regulatoryaffairs/Documents/iacuc/guidelines/iacucguideline-rodenttumorandcancermodels.pdf

The University of North Carolina at Chapel Hill
APPENDIX I:

BC 1
Mouse is emaciated.
- Skeletal structure extremely prominent;
little or no flesh cover.
- Vertebrae distinctly segmented.

BC 2
Mouse is underconditioned.
- Segmentation of vertebral column evident.
- Dorsal pelvic bones are readily palpable.

BC 3
Mouse is well-conditioned.
- Vertebrae and dorsal pelvis not prominent;
palpable with slight pressure.

BC 4
Mouse is overconditioned.
- Spine is a continuous column.
- Vertebrae palpable only with firm pressure.

BC 5
Mouse is obese.
- 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-...)
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APPENDIX II:

BCS 1 - Rat is emaciated
- 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.

BCS 2 - Rat is under-conditioned
- 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.

BCS 3 - Rat is well-conditioned
- 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.

BCS 4 - Rat is overconditioned
- 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.

BCS 5 - Rat is obese
- 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.

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