The University of South Alabama IACUC has developed the following document in collaboration with veterinary staff to provide research investigators parameters/criteria for assessment of tumor burden and welfare of rodents used in cancer experiments. Humane interventions, humane endpoints, and experimental endpoints should be specified in the IACUC protocol.

**KEY POINTS:** This guideline discusses the following topics:

- Monitoring and endpoints
- Implantable/Inducible tumors
- Evaluation of palpable tumors
- Imaging and chemotherapy
- Evaluation of non-palpable tumors and ascites-producing tumors

**MONITORING AND HUMANE ENDPOINTS**

Upon tumor implantation, a post-procedure care record must be maintained for the tumor-bearing animals. This record must contain the following information: tumor cell line, site of implantation, anesthesia used (if any) during cell injection, date of implantation, and the contact information for the person responsible in the laboratory. This record will be used for each anesthetic event (for imaging or other procedures) and humane intervention and must be maintained in the animal room. The PI and research staff should also check this record for notes/comments made by veterinary staff. After a visible or palpable tumor is evident, the animals must be documented by the laboratory group at least weekly, or more frequently if required by veterinary staff. Laboratory staff may need to increase the frequency of observations based on tumor growth rate and/or the general condition of the animal (possibly including weekends and holidays.) The overall wellbeing of the animal takes priority over precise tumor measurements in decisions regarding euthanasia or other interventions. The following should serve as the foundation for determining humane endpoints for rodents used in tumor studies.

In all cases, the veterinarian has the authority to determine that an animal must be euthanized for humane concerns. However, the veterinarian will make every attempt to communicate with the PI and research staff if humane interventions or humane euthanasia is needed.

i. **Body Condition Score (BCS) and/or body weight**

The general physical condition of the animal is an important factor in effectively following the progression of tumors in rodents. Scoring systems from “1” (emaciated/wasted) to “5” (obese) are often used. BCS is a helpful adjunct to assessment of overall health of the animal. It is important to note that treatments designed to affect tumor growth (such as chemotherapeutics) can lead to weight loss and poor body condition. Thus, the BCS becomes an important assessment tool in the tumor
burden experiments. Weight loss is a less desirable criterion because tumor mass may increase more rapidly than cachexia occurs in the patient, leading to a falsely optimistic assessment of the animal’s health.

Rodents must be euthanized if any of the following is observed by veterinary staff:

a. Body condition score is 1/5;

b. Body condition score is 2/5 AND the mouse is lethargic;

c. The tumor affects gait, posture, ability to eat, drink, urinate or defecate, regardless of tumor burden;

d. Weight loss greater than 20% of control animals; or significant weight GAIN in animals with ascites-producing tumors.

ii. Clinical features should be used as humane endpoint criteria in models with non-palpable tumors. These must be included in the IACUC protocol.

iii. General clinical signs should be assessed in each mouse undergoing tumor implantation. Lethargy, change in ambulation, diarrhea, neurological signs, dyspnea, etc. must be recorded in the post-procedure care record and reported to DCM staff.

iv. The known biology and effects of any individual tumor model must be described in the IACUC protocol. Include: expected clinical signs, anticipated moribundity/mortality, planned humane interventions, criteria for the assessment of humane endpoints, and scientific justification if particular humane interventions could interfere with the study and need to be withheld.

v. Moribund animals should be euthanized immediately.

IMPLANTABLE AND INDUCIBLE TUMORS

Rodent Pathogen Testing
All transplantable murine tumors must be assayed for contamination with murine viruses to prevent the possible spread of pathogens into our rodent colonies. IDEXX RADIL http://www.idexxradil.com/ PCR Profile Impact III is required prior to the approval to inject rodent cells or implant rodent cells into recipient rodents. Please contact DCM to schedule to have murine cell lines tested. Human cell lines must also be tested if they have been passaged through rodents.

Implantation Sites
Tumor implantation sites 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 the least
amount of site-related morbidity. If other sites are to be used, they should be described and justified in the IACUC protocol. Appendages, including the paws and tail, should be avoided unless scientifically justified.

- Sites involving the face or perineum should be avoided. They may interfere with eating, drinking, urinating, and defecating.\(^6\)
- Intramuscular implantation should be avoided as this is considered to be painful due to the distension of the muscle by the tumor.
- Tumor implantation on the ventral surface of the body should also be avoided due to the risk of irritation to the tumor site in contact with the bedding and floor of the cage.
- The maximum suspension volume (tumor cells, adjuvant and/or experimental reagents) injected per subcutaneous site should be 20 mL/kg in mice unless scientifically justified in the IACUC-approved protocol. Generally, a total volume for injection of 100 µl is preferred. For orthotopic sites, this volume should be reduced to avoid excessive tissue damage or leakage (e.g., 30 µl into the prostate or 5 µl into the brain).\(^7\)
- The total number of cells injected per subcutaneous site should be no greater than 1 million cells unless otherwise approved in the IACUC protocol.
- Implantation into multiple sites should be justified and described in the IACUC protocol. (Note: when evaluating endpoint criteria, the TOTAL tumor burden will be considered.)

**Induction Agents**

Drugs used to induce tumors are to be listed in the animal use protocol. Non-pharmaceutical grade drugs are to be identified and their use must be justified. Read and follow guidance on signage regarding proper disposal.

**EVALUATION OF VISIBLE OR PALPABLE TUMORS**

Tumor burden should be determined by evaluating the following:

- Body condition score (preferred) or body weight. See previous section on “Monitoring and Humane Endpoints.”
- Objective dimensional criteria (size)
- Anatomical location
- Incidence of multiple tumors
- Tumor ulceration

This guidance assumes that a normally sized adult rodent will be studied (a ~25 g mouse). The allowable sizes for tumors will be decreased if the tumors are injected into immature or genetically small mice.

**Tumor Size and Location**

The concern of size for individual tumors is related to central necrosis, ulceration of skin overlying tumors, and abrasions. When on the dorsum or flank of an adult mouse, tumors may be allowed to grow to a diameter of 1.5 cm at their widest point—as long as the rodent remains otherwise healthy. Any tumor burden larger than 1.5 cm must be scientifically justified and described in the IACUC protocol. This justification must be approved by the IACUC.

**Multiple Tumors**

Multiple tumors that are individually smaller than the single tumor limit may not have the same negative sequelae as a single, large tumor. However, the total tumor burden will be assessed when determining endpoints. Generally, the TOTAL diameter should not exceed 2 times what would be approved as a solitary mass.
Tumor Ulceration
Ulceration of a tumor requires euthanasia, unless approved by the IACUC. For example, certain tumor cell lines are more prone to ulcerate even when tumor burden is low. The characteristics of the line(s) should be detailed in the IACUC protocol (see previous section on “Monitoring and Humane Endpoints”). Some ulcerated tumors may be treated for a short period of time if the animal is healthy at the discretion of veterinary staff in an effort to reduce the need to implant tumors in replacement animals. This determination is solely based on the clinical judgment of the veterinarian.

ASCITES

In cases where tumors are expected to grow with accumulation of ascites, rodents must be weighed prior to inoculation and subsequently be followed by weight measurements at regular intervals — described in the protocol and based on the expected rate of fluid accumulation. When the body weight exceeds 120% of initial weight, the rodents must be euthanized or abdominocentesis (“abdominal tap”) must be performed. In addition to weight measurement, BCS should be part of the evaluation of the animals as described above. Abdominocentesis may only be performed by trained lab members two times per animal before humane euthanasia will be required at the second fluid tap. Ascites pressure should be relieved before abdominal distension is great enough to cause discomfort, increased respiratory rate, or interfere with normal activity. The veterinarian may require euthanasia at any time for humane reasons.

IMAGING

Imaging and study treatments must be detailed in the IACUC protocol and recorded in the post-procedure care record.

CHEMOTHERAPY

Some chemotherapeutic agents require special handling, precautions, and disposal. Cages must be clearly marked. Signs will be posted by DCM regarding PPE and other precautions. Read and follow the instructions.

EVALUATION OF NON-PALPABLE TUMORS

Evaluating liquid tumors (e.g. leukemia) and tumors in central areas of the rodent’s body (e.g. bone, brain, lungs) can be challenging. Tumor size will likely not be useful unless measured by imaging. 1,6,7 For these models, the BCS and clinical evaluation of the animals takes priority regarding decisions on humane endpoints. The expected clinical signs and the humane endpoints regarding those signs must be clearly described in the protocol.

REFERENCES


