The University of Pennsylvania Institutional Animal Care and Use Committee (IACUC) has developed the following guideline to help research investigators develop criteria for assessment of tumor burden on the welfare of rodents used in cancer experiments. This guideline is relevant to all investigators using models of neoplasia, including all subcutaneous, ascites-producing, liquid, or non-palpable tumors, particularly in rodent species. Humane interventions and endpoints should be determined and specified for all animals that will undergo tumor development as an expected part of the experimental protocol.

Please see Assigning Pain or Distress Categories to IACUC Protocols to determine how the tumor production or cancer model procedure should be categorized.

This guideline discusses the following topics:
- Monitoring and endpoints
- Implantable/Inducible tumors
- Evaluation of visible or palpable tumors
- Ascites produced by tumors
- Non-palpable or “liquid” tumors

MONITORING AND ENDPOINTS

Animals that are on a tumor production study must be monitored by the laboratory at least once per week during the time when the tumor is not yet detectable, in order to observe when tumor growth has begun. After a visible or palpable tumor is evident, the animals must be monitored by the laboratory group at least twice weekly. More frequent observations may be necessary as determined by the ULAR veterinarian, based on tumor growth rate, study parameters, and general condition of the animal (possibly including weekends and holidays.) The overall wellbeing of the animal should take priority over precise tumor measurements in decisions regarding euthanasia or other interventions.

i. **Body Condition Score (BCS)**

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) which are often part of tumor load studies, can lead to weight loss and poor body condition. Thus, the BCS becomes an important assessment tool in the tumor load experiments.

Rodents must be euthanized if:
- The body condition score is 1/5
- The body condition score is 2/5 and the mouse has decreased activity/responsiveness
- The tumor affects the rodent’s gait or normal posture, ability to eat, urinate, or defecate independent of the size of the tumor
- A ULAR veterinarian determines that the animal should be euthanized for humane concerns
ii. An activity or adverse behavioral scoring system may also be effective in placing objective measures for determination of humane endpoints in models with non-palpable tumors.\(^5,6\) If used, this should be discussed with a ULAR veterinarian and included in the ARIES animal use protocol.

iii. General clinical signs should be assessed.\(^7\) Any evidence of lethargy, change in ambulation, diarrhea, neurological signs (e.g. circling, head tilt) or increased respiratory effort should be reported to the ULAR veterinary staff immediately.

iv. The known biology and effects of any individual tumor model should be described in the ARIES animal use protocol, including expected clinical signs, anticipated moribundity/mortality, interventions for the relief of pain and suffering, and objective criteria for the assessment of humane endpoints.

v. Moribund animals should be euthanized immediately.

### IMPLANTABLE AND INDUCIBLE TUMORS

**Rodent Pathogen Testing**
Because transplantable tumors, hybridomas, cell lines, and other biologic materials can be sources of murine viruses that can contaminate rodents (Guide), all transplantable murine tumors must be assayed for contamination with adventitious murine viruses to prevent the possible spread of pathogens into our rodent colonies.

IDEXX RADIL [http://www.idexxradil.com/] PCR Profile Impact II (mice) or Impact VI (rats) is required prior to the approval to inject rodent cells or implant rodent cells into recipient rodents. Please submit materials as part of the ARIES protocol application or directly to ULAR Diagnostic Services to be reviewed prior to final approval by IACUC.

**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...
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described and justified in the ARIES protocol.
- Sites involving the face, limbs or perineum should be avoided as there is little to no space for
tumor growth and expansion, and they may interfere with eating and drinking.\textsuperscript{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.

Induction Agents
Drugs used to induce tumors (for example, doxycycline in drinking water) are to be listed in the animal
use protocol. Non-pharmaceutical grade drugs are to be identified (e.g. tamoxifen) and their use must
be justified.

EVALUATION OF VISIBLE OR PALPABLE TUMORS

Evaluating tumor burden based only on a percentage of body weight is generally not accurate—while
the growing tumor(s) may cause an increase in body weight, the general condition of the rodent may
be decreased (loss of lean body mass), resulting in a relatively stable body weight but an unhealthy
animal.

Tumor burden should be determined by evaluating the following:
- Body condition score (BCS). See previous section on “Monitoring and Endpoints.”
- Objective dimensional criteria (size)
- Anatomical location
- Incidence of multiple tumors
- Tumor ulceration

The guidance below assumes that a normally sized adult rodent will be studied (a ~25 g mouse or a 250+
g rat). The allowable sizes of 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 adult rodent, tumors may be allowed to grow
to a diameter of 2.0 cm (or 4.2cm\textsuperscript{3}) in mice and 4.0 cm (33.5cm\textsuperscript{3}) in rats (NIH ARAC) at their widest
point—as long as the rodent remains otherwise healthy.

Multiple Tumors
Multiple tumors that are individually smaller than the single tumor limit may not have the same
negative sequelae as a single tumor. Multiple tumors may be allowed to grow up 150\% (or 6.3cm\textsuperscript{3}) of
the volume compared with the volume of a single tumor. Please note that the limitation on any single
tumor (2.0 cm diameter in mice) will still be valid.

Tumor Ulceration
Ulceration (overt open lesion or scabbed area) of a tumor does not necessarily require euthanasia, but it
does require more frequent monitoring and potentially treatment, as defined below. The level of follow-
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up care for ulcerated tumors is based on both the size of the ulceration and the clinical judgment of the veterinarian.
  - **Pinpoint (≤ 1mm) ulcerations** at the site of tumor injection must be monitored at least 2 times per week for worsening of the ulceration site.
  - **Ulcerations (> 1mm)** of the surface area of the tumor shall be monitored at least 3 times per week and must be reported to the ULAR veterinary staff for evaluation and potential treatment.

ASCITES PRODUCED BY TUMORS

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. Juvenile animals that are maturing (those ≤ 8 weeks of age) that develop ascites must be monitored based upon the above expectations; however, their growing rate must be compared to age-matched control animals or published growth curves for the background strain (see www.jax.org for more information).

Ascites pressure should be relieved before abdominal distension is great enough to cause discomfort, increase respiratory rate, or interfere with normal activity. The abdominal “tap” should be performed by trained personnel using proper aseptic technique, with manual restraint or anesthesia, and by using the smallest needle possible (e.g. 22 gauge) that allows for adequate flow (NIH ARAC). In addition to weight measurement, BCS needs to be part of the evaluation of the animals as described above.

NON-PALPABLE OR LIQUID 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 due to inability to measure size or because of the sensitivity of areas to compressive lesions.¹,⁶,⁷ For these models, the BCS and clinical evaluation of the animals take priority regarding decisions on humane endpoints. The expected clinical signs and the humane endpoints of those signs must be clearly described in the protocol. A scoring system (as mentioned above in this document) may be most helpful in this scenario. The evaluation of clinical signs in an animal with a tumor burden of this type should include consultation with a ULAR veterinarian.
REFERENCES


