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 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.

**KEY POINTS:** 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 HUMANE ENDPOINTS

Animals that are currently being used in a tumor production study must be documented 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 documented by the laboratory group **at least twice weekly**. Laboratory staff may need to increase the frequency of observations at the request of 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. The following should serve as the foundation for determining humane endpoints for rodents used in tumor studies.

### 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.[1] Scoring systems from "1" (emaciated/wasted) to "5" (obese) are often used.[2-4] 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:

- The body condition score is 1/5
- The body condition score is 2/5 and the mouse is profoundly lethargic (significantly 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

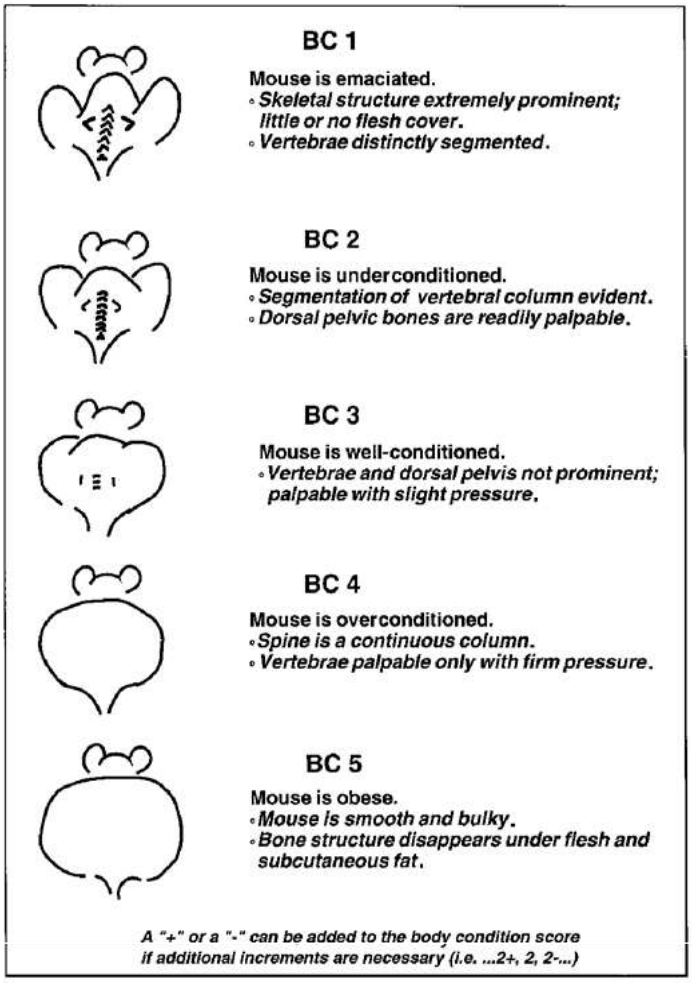
- A ULAR veterinarian determines that the animal should be euthanized for humane concerns

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 in each mouse undergoing tumor implantation.[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 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.[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 and 10 mL/kg in rats unless otherwise justified in the IACUC-approved protocol.[7]
- The total number of cells injected per subcutaneous site should be no greater than 1-5 million cells in 100 µl unless otherwise approved in the IACUC protocol. For orthotopic sites, this volume should be reduced to avoid excessive tissue damage or leakage (e.g., 50,000 cells in 30 µl into the prostate, or 10-50 000 cells in 5 µl into the brain).[7]

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

As mentioned above, 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 overall unhealthy animal.

Tumor burden should be determined by evaluating the following:

- Body condition score (BCS). See previous section on "Monitoring and Humane 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 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 adult rodent, tumors may be allowed to grow

to a diameter of 2.0 cm (or 4.2 $cm^3$) in mice and 4.0 cm (33.5 $cm^3$) 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 to a total diameter of 3.0 cm in mice and 6.0 cm in rats. For example, a mouse may have two tumors each of 1.5 cm diameter, three tumors of 1 cm diameter, etc. Please note that the limitation on any single tumor (2.0 cm diameter in mice and 4.0 cm in rats) 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-up care for ulcerated tumors is based on both the size of the ulceration and the clinical judgment of the veterinarian.

- **Ulcerations ≤ 4mm** at the site of tumor injection must be monitored and documented at least 2 times per week for worsening of the ulceration site.
- **Ulcerations > 4mm** of the surface area of the tumor shall be monitored at least 3 times per week by the laboratory staff, and must be reported to the ULAR veterinary staff for evaluation and potential treatment, unless otherwise approved by the IACUC in the protocol. Euthanasia may be requested for humane reasons at the veterinarian's discretion. Documentation of laboratory staff monitoring of mice with ulcerated tumors is required, and this documentation would be reviewed during post-approval monitoring or as directed by the IACUC.

## 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). In addition to weight measurement, BCS needs to be part of the evaluation of the animals as described above. Abdominocentesis may only be performed by trained lab members three times per animal before humane euthanasia will be required at the third fluid tap. The veterinarian may request or require euthanasia at any time for humane reasons.

Ascites pressure should be relieved before abdominal distension is great enough to cause discomfort, increased 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).

## 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 regarding those signs must be clearly described in the protocol. A scoring system 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.

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