IACUC Solid Tumor Production* and Cancer Research Policy
Institutional Animal Care and Use Committee

POLICY STATEMENT

Consideration should be given to the known biology of the tumor and the judicious choice of end points for tumor growth taking into account predictable indications of pain, distress or significant deviation from normal behavior. The method and the site for implantation of transplantable or induced solid tumors** requires considerable care to minimize trauma to the host animal.

Attention should be given to avoid sites where tumor growth would cause obvious pain or distress, or where it might limit mobility. Subcutaneous or intradermal growth on the back or in the flank are considered to cause the least distress. Distension of musculature is generally painful and should be considered with intramuscular implants. Implantation of tumors in the footpad, tail, brain and eye are discouraged and will require special justification (note: metastatic tumor cells may be administered by IV injection into the tail).

For spontaneous and transplanted tumors, important features will include growth rate, invasion, distension, ulceration, metastases, and production of cachectic factors. The technical staff must be aware of the parameters of the study, such as tumor growth potential and whether a tumor is likely to become ulcerated. The Investigator must clearly define study parameters and endpoints in their Animal Use Protocol (AUP).

In the case of leukemias, internal, disseminated, metastatic or other occult tumors, determination of the tumor burden may be difficult. The development and/or use of appropriate biochemical and pathological laboratory methods to determine the onset of these tumors may be required.

The IACUC emphasizes the need for frequent monitoring during tumor development to allow for appropriate intervention before significant deterioration of animal health or death occurs. Effective monitoring systems and endpoints should include limits on the tumor burden and severity of tumor-associated disease. The use of altered physiological, biochemical and other biomarkers are suggested as potentially more objective and reproducible endpoints than clinical signs.

* For information regarding monoclonal studies and ascites production, refer to the IACUC Ascites Fluid Collection Policy.

** Contamination of tumor cell lines with human and/or rodent viruses and other microorganisms may compromise experimental results, as well as cause an outbreak of disease among laboratory animals. Screening of all cell lines to be administered to live animals for rodent pathogens is required at least once every 3 years at AU. Studies involving the use of primary human tumors or tissues with uncharacterized blood-borne pathogens must first be reviewed and approved by the Institutional Biosafety Committee (IBC). Screening may be requested using the Cell Line Screening Request form.

REASON FOR POLICY

The AU IACUC is responsible for overseeing humane care and use of animals used in research and teaching and for ensuring adherence to regulatory expectations in the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, and the Animal Welfare Act as well as standards contained in the Guide for the Care and Use of Laboratory Animals. The purpose of this policy is to educate personnel in proper technique to ensure humane treatment of animals involved in tumor and cancer research.

AFFECTED STAKEHOLDER AND ORGANIZATION(S)

Any employee, student, contractor, or external consultant involved with tumor and cancer research at AU.

DEFINITIONS

- Applicable Species
  - Rats
  - Mice

- ascites fluid - a fluid collection in the peritoneal cavity

- anorectic – lacking appetite

- body condition score – an assessment of the animal's weight for age and weight for height ratios, and its relative proportions of muscle and fat

PROCESS & PROCEDURES

Guidelines for Monitoring

All tumor-bearing animals must be observed at least twice weekly to assess their physical condition, tumor growth and/or metastasis. Records must be kept with all pertinent information: protocol number, time and frequency of monitoring, the name of the person monitoring the animal, identification of the animal, animal weight, type of clinical signs, and any treatments given to the animal. Records maintained by the laboratory personnel should be available to the veterinary staff and/or the IACUC upon request.
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- The maximum size for tumors (i.e. size in any one dimension) is 20 mm for a mouse or 40 mm for a rat. In cases of multiple tumors, the maximum size is 10 mm (20 mm total combined tumor size) for mice and 20 mm (40 mm total combined tumor size) for rats. Justification to exceed the size restriction must be approved by the IACUC. Once a tumor has reached 50% of the maximum allowable size (10 mm for mice and 20 mm for rats) the animal must be observed daily including weekends and holidays. Records of observations must be kept.

- Clinical observations and/or palpation will be necessary to monitor for deterioration of clinical condition. Special examination techniques may be required for specific sites (e.g. respiratory rate for lung involvement, neurological disturbance for brain neoplasms, and blood cell counts for leukemias).

**Clinical signs which may be associated with tumor progression:**

- Decreased food/water intake
- Lethargic/depressed activity
- Vocalization
- Cranial deformity/neurological signs
- Rough hair coat
- Skin pathology
- Jaw deformity/malocclusion
- Visual weight loss
- Restlessness
- Respiratory difficulty
- Perianal soiling
- Hunched posture
- Restricted mobility
- Changes in feces/urine

Measurement of body weight changes (both positive and negative changes compared to controls) can be used to assess tumor burden. Baseline body weights must be recorded for each animal at the start of the study and periodically through completion of the study. The period should be stated in the protocol. Considering both weight loss and weight gain from growth, **tumor burden** should not exceed 10% of the animal's normal body weight. **Weight loss** should not exceed 15% of the animal's body weight at the start of the experiment. For younger animals, **failure to maintain weight gain** to within 15% of untreated control animals should be considered as an indication of significant health deterioration.

**Guidelines and Humane Considerations in Experimental Design**

1. Particular attention must be paid to the body system and/or organ system (e.g., skin, peritoneum, spleen, lymph node, etc.) most likely to be affected by the tumor type. The site for injection should be carefully chosen to permit room for tumor growth and to avoid unnecessary distress (e.g. subcutaneous flank or back are considered to cause the least distress).

2. Endpoints must be established to minimize the potential for pain and/or distress. The investigator must have a plan for pre-emptive euthanasia based on clearly defined endpoints in the protocol. **NOTE:** The “intentional death” end point will not be allowed unless it is scientifically justified to the IACUC. Animals expected to become moribund should be euthanized prior to reaching this state.

3. Tissue necrosis or ulceration of the skin overlying the developing tumor may occur. Ulceration or necrotic tissue may result in a continuous loss of body fluids and/or infection, and should require euthanasia of the affected animal unless approved by the IACUC. When it is necessary to maintain an animal with an ulcerated tumor, the status of the ulcer and the animal's overall condition must be assessed daily and in consultation with the attending veterinarian. Laboratory personnel shall maintain the “daily record of observations” concerning animals with ulcerated tumors within the animal room.

4. Animals with tumors that restrict mobility and/or interfere with their ability to acquire food or water may require euthanasia.

5. **All animal experiments must provide for a humane endpoint.** As a general guideline, animals used in experimental procedures involving tumor development will be considered for euthanasia if the following conditions occur:

   - Tumors exceed maximum allowable size, unless approved by the IACUC
     (The maximum total size for tumors is 20 mm for a mouse or 40 mm for a rat. In cases of multiple tumors, the sum of all tumor sizes must not exceed the maximum size of 20 mm for a mouse and 40 mm for a rat).
   - Tumor size or metastatic growth interferes with normal behavior and condition of the animal (e.g. ambulation, eating, drinking, grooming) or causes pain or distress due to its location
   - Weight loss exceeding 15% of the body weight of a conspecific normal animal (taking into account the tumor mass)
   - Tumor becomes ulcerated (break in overlying skin), infected, or necrotic
   - Palpation of tumor elicits a pain response
   - Persistent self-induced trauma
   - Animal appears weak with “hunched posture”, unresponsive, or moribund
   - Animal becomes anorectic
   - Animal appears dehydrated
   - Animal shows respiratory difficulty
   - Body condition score < 2.0
BODY CONDITION SCORE IN MICE

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

BC 2 Mouse is under-conditioned
- 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 over-conditioned
- Spine is a continuous column.
- Vertebrae palpable only with firm pressure.

BC 5 Mouse is obese
- Bone structure disappears under flesh and subcutaneous fat.

FORMS AND RELATED DOCUMENTS

- Animal Welfare Act
- Public Health Service Policy on Humane Care and Use of Laboratory Animals
  http://grants.nih.gov/grants/olaw/references/phspol.htm
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- Toth LA. Moribund condition as an endpoint for animals used in research and testing. ILAR J 41:72-79, 2000.

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(Updated AWA, clarified total combined size for multiple tumors, and added rat body conditions diagram.)