Guidelines Involving Experimental Neoplasia Proposals in Mice and Rats

1. Proposals utilizing development/induction/injection of tumors in laboratory animals as an experimental procedure are initially approved by the NCI-Frederick ACUC. To avoid subjecting animals to unnecessary pain or distress, these proposals require periodic follow-up regarding the condition and health of the animals (see Paragraph 2).

2. All scientific and support staff should not only be familiar with normal animal health and behavior, but should also be able to observe adverse changes in health, behavior, or tumor burden. When these adverse cases are identified and the animal is not immediately euthanized, it is very important that several individuals be notified. They include the principal investigator, investigator support personnel, animal care personnel, manager/supervisor, and the attending veterinarian. The *Animal Health Evaluation SOP* which guides the communication of animal health concerns to the veterinary staff is attached.

3. In circumstances involving declining health status, morbundity, or unrelieved pain and discomfort, every attempt will be made to reach consensus with the principal investigator bearing experimental endpoints in mind. However, the final analysis and discharging of the NCI-Frederick's animal care and use regulatory responsibility rests with the attending veterinarian. All animal study proposals must have an *Animal Disposition Authorization Form* attached to proposal at the time of submission (effective July 2003).

4. Some tumors can cause significant changes in animal health. In particular, animals should be **observed daily** for any indication of the following:

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

5. Particular attention must be paid to the body system most likely to be affected by the tumor type (e.g. solid, ascitic, lymphoid, etc.) and organ system (e.g., skin, peritoneum, spleen, lymph node, etc.).

6. The site for injection of solid tumors should be carefully chosen to permit room for tumor growth and to avoid unnecessary distress whenever possible (e.g., subcutaneous flank or back are considered to cause the least distress). Note:

some tumor cells injected IP, grow SQ, and animals should be monitored for skin ulceration or other problems, such as impaired mobility.

7. For tumor regression studies, careful attention should be paid to any animal exhibiting an ulcerated and/or necrotic tumor. To deter cannibalization, any animal exhibiting an ulcerated or necrotic tumor should be separated immediately and singly housed until tumor regression is

complete. A watch card should be placed on each individual cage containing a mouse with an open tumor, recording the date of the tumor opening on the card. Personnel are responsible for ensuring adherence to (a) ACUC approved regression timelines; (b) endpoints as described in the animal study proposal; (c) the ACUC Guidelines for Experimental Neoplasia; and (d) the ACUC Guidelines for Endpoints in animal study proposals (i.e., euthanizing the mouse if the tumor becomes infected, interferes with ambulation/eating/ drinking, or the mouse becomes otherwise debilitated).

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

- Primary tumor size (e.g., for subcutaneous tumors the maximum size is 20 mm in diameter for a mouse and 40 mm in diameter for a rat. Please note that alternate tumor growth sites require the provision of clinical endpoints to alleviate adverse health conditions) or metastatic growth interferes with normal behavior and condition of the animal (e.g., locomotion, exploration, grooming). Justification to exceed this size restriction must be approved by the NCI-Frederick ACUC in advance.
- Tumor interferes with the animals ability to eat and/or drink
- 20% weight loss *(emaciated appearance; rapid weight loss over two to four days; or progressive weight loss over a few weeks)*
- Tumor becomes ulcerated, infected, or necrotic with break of overlying skin
- Palpation of tumor elicits a pain response
- Animals become moribund, weak, comatose, unresponsive, or death appears imminent
- Animal showing signs of respiratory difficulty
- Animal showing signs of hypothermia (i.e., cold to the touch, pale extremities)

9. **Death as an endpoint is not sanctioned by the ACUC.** Extenuating circumstances for which death as an endpoint is expected requires scientific justification.

10. The **duration** of the experiment should be as short as possible and the number of animals required for scientific and statistical evaluation of results should be kept to a minimum.

11. **Monitoring** procedures must be closely adhered to. Animals must be observed daily by the animal care staff. 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 Study Proposal (ASP) and must provide guidance to the technical staff on all study matters. Failure to adequately

monitor animals or to abide by the conditions stated in the ASP will result in disciplinary action by the ACUC or LASP.

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