University at Buffalo Institutional Animal Care and Use Committee
Policy on Ulcerated Subcutaneous Tumors

Purpose

Ulceration of subcutaneous tumors is generally considered to be a humane endpoint for euthanasia as per the IACUC's Policy on Humane Interventions and Endpoints. The IACUC does recognize, however, that scientific understanding of tumor models has progressed and that there are exceptional circumstances where maintenance of animals with ulcerated subcutaneous tumors may be allowed if scientifically justified.

Effective monitoring systems and endpoints must include limits on the tumor burden and severity of tumor associated disease. This IACUC Policy outlines monitoring and endpoints for animals with ulcerated subcutaneous tumors.

Definitions

- **Ulcerated tumor:** Loss of the epidermis layer and at least the superficial portion of the dermis layer of the skin. With subcutaneous tumors, ulcers typically form from cell death of the epidermal and dermal layers and additional effects may be seen. Ulcerated tumors may continue to exude body fluids (exudate). This predisposes the animal to infection and dehydration. Ulcerated tumors may also bleed.
- **Cavitated tumor**: Following skin ulceration, tumors may develop a sunken appearance and are often described as "cavitated", "pitted" or "cratered." These terms describe a tumor that is invaginated (sunken in the middle) with sharply defined walls. Cavitated tumors may expose deeper tissues, become infected, trap bedding and debris and become infected.
- **Scab/crust**: Material formed by drying of exudate or secretions on the skin surface. For a scab to be present over a tumor, there must be an ulcer. The scab formation is part of the skin's attempt to heal. The clinical terminology for scab is "fibrin crust."
- **Eschar**: Black, necrotic layer of dead tissue that can form on compromised skin over a tumor. Sometimes this is temporary and may be replaced with healed or scarred skin.
- **Necrosis**: Death of living cells. In tumors, cell death may be caused by "oncotic necrosis," which is death following irreversible cell injury by hypoxia, ischemia and membrane injury. This may be visible grossly as black coloration.
- **Potential Causes of Tumor Ulceration**:
  1. Certain types of tumors or cell lines are more prone to ulceration.
  2. Rapid tumor growth that exceeds the skin's ability to stretch, causing skin rupture.
  3. Alterations in blood supply (e.g., the tumor outgrows its blood supply).
  4. Tumors with internal hemorrhagic areas are that are prone to ulceration.
  5. Mechanical trauma resulting from the location of the tumor causing constant friction with bedding or caging. Examples include tumors implanted on the ventral surface of the animal.
  6. Self-induced trauma due the animal scratching or chewing at the tumor.
  7. Technique: The mechanics of the injection process causes tracking of tumor cells into the dermal layer during implantation.

Effective Date: 11/17/2025
Next Review Date: 11/28
First Approved: 11/2025
Revised: NA

8. Experimental therapy (e.g., photodynamic therapy, certain types of immunotherapy).

Policy

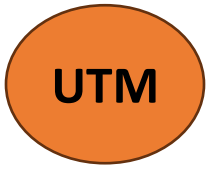
1. **IACUC Justification and Approval:**
   Scientific justification in the approved IACUC protocol is required to maintain mice with ulcerated tumors
   - **a.** Examples of appropriate scientific justification which must be explained within the substance administration procedure that describes the injection of the specific tumor cell line(s):
     - **i.** The treatment under investigation aims to resolve the tumor ulceration and reduce tumor burden and/or tumor ulceration in the tumor model under study.
     - **ii.** The treatment under investigation aims to target cancer in its later stages, which often present with ulcerated tumors.
     - **iii.** The treatment under investigation causes necrosis of the tumor (e.g., photodynamic therapy, certain types of immunotherapy) which may later heal.
   - **b.** Some tumors are more likely to ulcerate. However, this alone is not enough justification for maintaining animals with tumor ulceration!
   - **c.** Justification for keeping animals with ulcerated tumors must be evaluated on a model-dependent basis.
     - **i.** The PI should provide evidence that every effort has been made to choose models that are not prone to ulceration.
     - **ii.** When ulceration is a characteristic of a tumor line, the PI should attempt to complete the experiment in the latent period before ulceration whenever possible.
   - **d.** Some ulcerated tumors may be treated for a short period of time if the animal is healthy *at the discretion of veterinary staff* to reduce the need to implant tumors in replacement animals.
     - **i.** This applies when an approved IACUC protocol does not allow for tumor ulceration. Asking for this veterinary determined allowance should be a rarity not a regular request.
     - **ii.** This determination is solely based on the clinical judgment of an LAF veterinarian.
     - **iii.** Such animals must be placed on medical report and kept under the care of LAF Veterinary Staff.

2. **IACUC Protocol Renewal**:

When IACUC protocols are renewed, information must be provided regarding the welfare of the animals with ulcerated tumors, as well as whether keeping animals with ulcerated tumors was scientifically warranted. The following information must be provided with the renewal:

- **a.** Was success of the study dependent on keeping animals with ulcerated tumors?
- **b.** What percentage of animals with tumors had ulcers?
- **c.** If the experiment studied therapies aimed at healing ulcerations, what percentage of mice with ulcerated tumors experienced healing?
- **d.** What animal welfare issues occurred?

3. **Cage and Mouse Identification**:
   - **a.** Cages containing animals with ulcerated tumors that are approved in the IACUC protocol must be marked "Ulcerated Tumor Monitoring" by placing an orange **"UTM" sticker** on the cage card.



   - **b.** When cages contain multiple animals with ulcerated tumors, the individual animals must be easily identified (e.g., ear punch, ear tag) with a clearly designated system.

4. **Monitoring Schedule (All experimental animals need to be observed daily as per IACUC Policies and Procedures):**

Monitoring frequency will depend on the tumor model, specifically, the characteristics and progression of the ulcerated tumor. The monitoring frequency must be outlined in the IACUC protocol. The principal investigator can justify a monitoring frequency based on their understanding and experience with their specific model. With novel models, data may need to be collected from a pilot group of animals, and extra monitoring may be warranted until the IACUC, LAF Veterinary Staff, and PI Staff are confident that the animals are not at risk of health or welfare concerns.

- **a.** For many models where tumors develop ulcers, animals must be monitored and scored daily once ulcers appear.
- **b.** For tumors where ulcers develop very rapidly, animals may need to be monitored and scored 2 times day once ulcers appear.
- c. All animals with ulcerated tumors must be weighed. The weighing frequency must be specified in the IACUC protocol.

5. **Monitoring Documentation**:
   - **a.** All daily monitoring, scoring, weights, supportive care, and treatment must be recorded in a monitoring log by the responsible laboratory staff.

b. If multiple mice within a cage have tumor ulceration, each mouse must be identified and scored individually.
c. Notations in the log must include the name of the person performing the scoring and the date and time of entry.
d. Monitoring records must be kept in the animal housing room so they can be reviewed by IACUC personnel or LAF Veterinary Staff.

6. **Clinical Care:** Some clinical treatments and husbandry interventions may be implemented to reduce negative impacts on animal welfare once ulcers are observed.
   a. Changing cage bedding from corncob to soft bedding (e.g., alpha-dri) may reduce mechanical trauma to tumors located on the ventral surface of the animal. Tumors located in other areas (dorsum, flank) are not likely to benefit from this practice. All types of bedding may adhere to ulcerated tumors.
   b. If animals show evidence of self-trauma due to scratching, trimming the toenails is warranted.
   c. Topical treatment with antibiotic ointment (e.g., triple antibiotic ointment, Dermagel®, etc.) may help to prevent infection and inflammation. Note that application of ointments may promote adherence of bedding to the ulceration and/or encourage mice to lick the ointment from themselves or cage mates. Tumors with ulcerations that are small, stable, and dry may be better without ointment.
   d. Animals showing dehydration or decreases in body weight on body condition score may benefit from supplemental food (e.g., wet chow and diet gels such as Boost®).
   e. Ulcerated tumors are expected to be painful. Analgesics should be provided if they do not interfere with research objectives and should be described in the IACUC protocol. If scientifically contraindicated, this information must also be included in the approved protocol.
   f. A substance administration procedure should describe the monitoring and provision of clinical care for any ulcerated tumor model. Any topical treatments, diet supplements or analgesics should be included as substances with a description of when each should be given.

7. **Humane Endpoints for Euthanasia:** The following clinical signs are indications of humane endpoints requiring euthanasia. Animals exhibiting any one or more of these signs must be euthanized.
   a. General subcutaneous tumor endpoints:
      i. >20% weight loss from baseline.
      ii. Body condition score (BCS) = 1/5 (emaciated)
      iii. Tumor size ≥ 2 cm in diameter in any direction (mouse) or ≥ 4 cm in diameter in any direction (rat). If multiple tumors are present, the sum of

the diameter of all the tumors should not exceed 2 cm (mouse) or 4 cm (rat).

iv. Tumor that impairs mobility or use of a limb.

v. Unable to maintain upright posture or ambulate.

vi. Lethargy to the extent that the animal fails to respond to gentle stimuli (moribund condition).

vii. Labored respiration.

b. Endpoints relating specifically to tumor ulceration:

i. Tumor ulceration with active bleeding.

ii. Tumor exudate (pus or wet discharge) that does not resolve within 3 days or results in dehydration at any point.

iii. Deeply cavitated tumor that is > 4 mm deep, and/or exposes muscle or bone.

iv. Evidence of severe self-mutilation of the tumor (e.g., actively chewing or scratching tumor resulting in bleeding or trauma).

v. The animal displays signs of pain that cannot be alleviated with analgesic treatment. Pain in mice can be assessed via the facial grimace. Mice in pain will grimace as evidenced by narrow, closed eyes; bulge on top of nose (wrinkled nose); cheek bulge; ears back or flat; and whiskers pointing back or "standing out on end".

vi. Tumor ulceration diameter exceeding 50% of the total diameter of the tumor. For example, if the subcutaneous tumor is 1 cm in diameter, then the ulceration should not exceed 5 mm in diameter. Large ulcers increase the risk of fluid loss, infection, bleeding, and pain.

vii. If multiple tumors are present, then the total diameter of all of the tumors added together cannot exceed 2 cm (mouse), and the total diameter of all of the ulcers added together cannot exceed 50% of the total tumor diameter.

References:

1. National Research Council. 2011. Guide for the Care and Use of Laboratory Animals, 8th Edition. Washington (DC): The National Academies Press. pp. 27, 34. Guide for the Care and Use of Laboratory Animals, 8th edition. National Academies Press (nih.gov)
2. New York State Laboratory Animal Welfare Program Inspection Tool, 2025. Laboratory Animal Welfare Program | New York State Department of Health, Wadsworth Center

Revision #	Description of Change	Effective Date
00	New IACUC Policy currently under review	--/--/--