UNIVERSITY OF NORTH CAROLINA
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Animal Care and Use Program

Guidelines for Tumor Production in Rats and Mice

Objective:	To provide tumor growth parameters which limit rodent pain and distress
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General

Tumor implantation in research animals is a critically important experimental activity which requires consideration of the effect of the tumor or tumors on the animal. 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 more objective and reproducible experimental endpoints than clinical signs. Humane endpoints are differentiated from experimental endpoints as those at which pain/distress can be relieved or prevented.

These guidelines limit the tumor burden an animal experiences to that which does not cause excessive pain or distress, and are for cumulative tumor burden per animal. Consultation with the Attending Veterinarian must occur prior to the start of any tumor-related studies.

When the activity of the tumor in a specific animal model is unknown, either through previous experience or publications, a small pilot study may be recommended to determine the optimal experimental and humane endpoints.

Guidelines

1. Tumor Implantation or Production
    - **a.** Subcutaneous or intradermal: The tumor(s) should be placed into a site or sites that will not interfere with normal body functions such as ambulation, eating, drinking, defecation and/or urination. Subcutaneous or intradermal sites on the back or in the flank are considered to cause the least distress. Sites involving the special senses should be avoided, and intramuscular implantation should be avoided as distention of muscle is considered to be painful to the animal. Extra attention must be paid if multiple sites are used, and no more than four tumors should be implanted in one animal. Sites other than those suggested may be used but must be scientifically justified.
    - **b.** De novo and metastatic tumor models: For each tumor model, a PI should evaluate the possible adverse effects, likely incidence of adverse effects, proposed methods of controlling severity (e.g. analgesia, anesthetic, sedation, euthanasia), and the definition and implementation of humane endpoints.

2. Tumor size
    - **a.** Optimally, one observer should perform all tumor measurements in a given study.
    - **b.** Calipers or imaging (e.g., in vivo, 3D scanners) should be used to measure tumor size. The same instrument should be used throughout the study to avoid discrepancies.
    - **c.** Tumor size must not exceed 20 mm (2.0 cm) at the largest diameter in an adult mouse and 40 mm (4.0 cm) in adult rats. Larger tumors must be specifically justified in the IACUC protocol.

If multiple subcutaneous tumors are implanted, smaller maximum tumor sizes must be described in the IACUC protocol.

d. Health limitations may be evident before the tumor reaches the maximum standards above. Some limitations may include mobility restriction, the inability to access food and water, pressure on internal organs or sensitive regions of the body, or body condition score (BCS) of < 2. Unless scientifically justified, animals displaying such signs must be euthanized, even if the maximum tumor size has not been reached.

3. Monitoring: 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) [3].

a. Schedule

1. Mice and rats with developing tumors are to be observed *no less than* three times weekly until a palpable tumor nodule is present (5-7.5 mm in diameter), followed by daily monitoring (including weekends and holidays). Deviations from this monitoring schedule must be discussed and justified in the protocol.

2. If tumors are in a location that is not palpable, a monitoring schedule should be established based on pilot studies or published studies. Pilot studies can be used to familiarize the animal researcher to possible adverse effects and to define the critical time scale of adverse effects. Features to consider include tumor site, growth rate, invasion, distension, ulceration, metastasis, and production of cachectic factors.

3. If tumor growth is rapid in the days before termination, twice daily monitoring may be necessary.

b. Variables: Clinical signs (general or specific) which may be associated with tumor progression include

1. General appearance; including dull or closing eyes
2. Decreased food/water intake
3. Dehydration
4. Weight loss (assess by weighing) and/or Body Condition Score (BCS)
5. Lethargy, depression, restless activity, scratching at tumor site
6. Vocalizations/respiratory difficulty
7. Cranial deformity/neurological signs
8. Rough hair coat and/or hunched posture
9. Skin pathology (e.g., ulceration, purulent discharge from infection)
10. Restricted mobility
11. Changes in feces/urine and/or perianal soiling
12. Eye/nose discharge

4. Humane endpoints: The overall well-being of the animal takes priority over precise tumor measurements in decisions regarding euthanasia or other interventions. Tumors induced in body cavities (cranium, orbit, abdomen, or thorax) may have additional limitations that are not associated with an acceptable size. These animals must be monitored very closely for any severe impairment in physiological or neurological function and euthanized as soon as such signs become apparent.

a. The following clinical signs are indications of morbidity. Tumor-bearing animals exhibiting these signs should be euthanized based on severity of clinical signs:

1. BCS $\leq$ 2; Muscle atrophy or emaciation (or accepted weight loss percentage)
2. Hypothermia
3. Bloodstained or mucopurulent discharge from any orifice
4. Labored respiration – particularly if accompanied by nasal discharge or cyanosis

5. Ulcerated tumors
    - Note: Ulceration may be acceptable for certain skin tumors. This condition and the treatment must be discussed in the protocol.
6. Significant abdominal distension
7. Incontinence, inappetence, or protracted diarrhea
8. Lethargy, inability to move when stimulated, or moribund

5. IACUC Protocol: In the protocol form, PIs must discuss the nature of the tumor study being proposed in the experimental design section. PIs should also complete Appendix E – Tumor Studies, where more detailed information on tumor production should be provided. If expecting to produce tumors in mice and/or rats, PIs should follow this guidance document ("Guidelines for Tumor Production in Rats and Mice"). However, if a PI is requesting IACUC consideration of departures from this guidance document, departures must be described in Appendix A – Requests for Departures / Deviations. Possible departures that must be described may include:
    a. Tumor size larger than 20 mm (2.0 cm) for mice or 40 mm (4.0 cm) for rats
    b. Maximum tumor size of multiple implanted subcutaneous tumors
    c. Justification of tumor ulceration
    d. Endpoints particular to your model not described above.

References

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Revision History

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