Tumor Induction in mice and rats
IACUC Guideline
Effective Date: August 2016

UCSF Office of Ethics and Compliance
Institutional Animal Care and Use Program

General

Tumor induction in research animals is a critically important experimental activity which requires consideration of the effect of the tumor(s) 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 suggested as potentially more objective and reproducible endpoints than clinical signs. These guidelines limit the tumor burden an animal experiences to that which does not cause excessive pain or distress, and apply to spontaneous as well as experimentally induced tumors.

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.
    b. Other sites: Tumor implantation on the face and limbs should be avoided, as there is little space for tumor growth and expansion. Intramuscular implantation should be avoided as distention of muscle is considered to be painful to the animal. Tumor implantation on the ventral surface of the body is discouraged due to the risk of irritation to the tumor site by bedding and the cage floor. Extra attention must be paid if multiple sites are used, and no more than four tumors should be implanted in one animal.
    c. Surgical implantation procedures must be described in the IACUC protocol. Refer to the IACUC Rodent Surgery Guidelines for information about anesthesia, analgesia, and aseptic technique.
    d. De novo and metastatic tumor models: For each tumor model, a PI must evaluate the possible adverse effects, likely incidence of adverse effects, proposed methods of controlling severity (e.g. analgesia, anesthetic, sedation), and the definition and implementation of humane endpoints.

2. Tumor size
    a. One observer should perform all tumor measurements in a given study.
    b. Calipers must be used to measure tumor size in order to avoid discrepancies.
    c. Tumor size must not exceed 20mm (2.0cm) in any direction in an adult mouse and 40 mm (4.0cm) in adult rats. The allowable tumor sizes will be decreased for immature or genetically small animals.
    d. If multiple tumors are present, the combination of the two largest diameters may not exceed 2.0cm for mice and 4.0cm for rats.
    e. 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 $\leq$ 2. Animals displaying such signs must be euthanized even if the maximum tumor size has not been reached.

3. Monitoring: Clinical observations and/or palpation are 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. Frequency

        1. Mice and rats with developing tumors are to be observed at least three times weekly until a palpable tumor nodule is present. Once the tumor is palpable, daily monitoring (including weekends and holidays) is required. Deviations from this monitoring schedule must be justified in the protocol.
        2. If tumors are located in a location that is not palpable, a monitoring schedule should be established based on pilot 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 associated with tumor progression may 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. Depressed or restless activity or abnormal aggression
        6. Vocalizations/ Respiratory difficulty
        7. Cranial deformity/neurological signs
        8. Rough hair coat and/or hunched posture
        9. Skin pathology
        10. Restricted mobility
        11. Changes in feces/urine and/or perianal soiling
        12. Aggression
        13. Eye/nose discharge
        14. Pale ears, nose, feet, tail (in light colored mice)

4. Tumor Ulceration

    a. Definition and Descriptions
        1. Ulceration is typified by necrosis of superficial tissues, and may be dry, suppurating or exudative.
        2. Tumors that show redness on the surface but no open wound or break in the epidermis are not considered ulcerated.
        3. Ulcerated or necrotic tissue may continuously exude body fluids and predisposes to infection.
        4. As soon as a tumor has ulcerated, the growth pattern will alter, which may be sufficient grounds for terminating the experiment. Ulcerated or necrotic tissue may result in a continuous loss of body fluid and/or infection.

b. Justification
    1. Scientific justification in the IACUC protocol is required in order to maintain animals with ulcerated tumors.
    2. Examples of appropriate justification:
        1. Novel treatment is being tested to resolve tumor ulceration and reduce tumor burden.
        2. Tumor ulceration is the area under study.
    3. Some tumors are more likely to ulcerate. However, this alone is not sufficient justification for maintaining animals with tumor ulceration.
    4. When ulceration is characteristic of the tumor line, the aim should be to complete the experiment in the latent period before ulceration.
    5. All animals maintained with ulcerated tumors must be placed in USDA category E.

c. Humane Endpoints, unless otherwise specified in the protocol, should include:
    1. Tumor ulceration shows no stabilization within 7 days of treatment.
    2. Ulcerated tumor is actively bleeding
    3. Ulcerated tumor shows visible signs of infection.
    4. Animals show discomfort associated with tumor ulceration such as biting/scratching.

d. Monitoring
    1. Animals with ulcerated tumors must be monitored daily including weekends and holidays.

5. 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 as to the maximum acceptable size. These animals must be monitored very closely for any severe impairment in physiological or neurological function and be 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
        2. Unable to maintain upright posture or ambulate
        3. Lethargy or failure to respond to gentle stimuli
        4. Hypothermia
        5. Bloodstained or mucopurulent discharge from any orifice
        6. Labored respiration – particularly if accompanied by nasal discharge or cyanosis
        7. Ulcerated tumors
        8. Significant abdominal distension
        9. Incontinence, inappetance, or prolonged diarrhea
        10. Exophthalmos (bulging eye)
        11. Cranial deformity or neurological signs (seizures, circling)

6. Required postings: Expected tumor characteristics/clinical presentations, endpoints specific to the model, and criteria for euthanasia must be posted in the animal housing room.
7. Exceptions:
    a. Dams nursing a litter may be exempt from these guidelines until the litter is weaned. LARC veterinary staff should be contacted for guidance in this situation.
    b. Other exceptions to these guidelines may be approved by the IACUC if scientifically justified. Any requested exception must be described in the IACUC protocol, and should include detailed information about animal health monitoring and humane endpoints.

References

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