

Animals Used in Cancer Research and Procedures Involving Tumor Production

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Introduction

Cancer is the general term used to describe a number of diseases that are characterized by the abnormal, uncontrolled growth of cell populations within tissues or the blood (4). Experimental animal models of cancer are often used to study the mechanisms of cancer growth, spread (metastasis), and therapy from the whole animal down to the molecular and cellular level. In vivo models of cancer may involve the induction of solid tumors, ascitic tumors or leukemias. Induction of cancer in experimental animals may involve exposure to carcinogenic substances, inoculation with viruses or malignant cells, local or whole-body irradiation, or genetic manipulation (4). The potential for tumor growth, metastasis, illness and pain in animal models makes determination of endpoints and monitoring critical. Prior to the initiation of these types of studies, investigators should provide detailed information including: the tumor type; expected growth, metastasis, and adverse effects on the proposed animal model; and the degree of tumor development required to meet the scientific objectives. This information will aid in the establishment of scientific and humane endpoints (3,4). Where outcomes are unknown, pilot studies using small numbers of animals may be necessary to better characterize the disease (3). "Protocols should include criteria for initiating euthanasia, such as degree of physical or behavioral deficit or tumor size, that will enable a prompt decision to be made by the veterinarian and the investigator to ensure that the endpoint is humane, and the objective of the protocol is achieved" (2).

Purpose

Ultimately, the purpose of these guidelines is to promote good science, maximize the data, promote animal well-being and minimize the potential for animal suffering (5). Minimum standards have been developed for procedures involving induced or spontaneous tumors in rodents. Careful monitoring, use of appropriate analgesics and supportive care, and well-defined endpoints can help to reduce potential loss of data. Animals that have developed severe physiologic derangements that may be exacerbated by pain, inadequate intake, dehydration, or metastatic disease may yield unusable or misleading data, tissues may be lost for accurate post-

mortem evaluation if animals are not preemptively euthanized, and animal losses may compromise statistical power if the endpoints are poorly defined. It is the responsibility of the investigator to ensure use of techniques and procedures which result in the least pain and distress to the animal, while adequately addressing the needs of the experimental design.

Responsibility

The principal investigator is responsible for ensuring that these guidelines are followed by all laboratory personnel and that individuals are appropriately trained in the performance of the procedures described here and in the IACUC approved APF. It is the responsibility of the investigator to ensure use of techniques and procedures which result in the least pain and distress to the animal, while adequately addressing the needs of the experimental design. For each model, the investigator must evaluate the possible adverse effects, likely incidence of adverse effects, likely sites of metastasis, proposed methods of controlling severity (e.g., analgesia, anesthetic, sedation), and the definition and implementation of humane endpoints. All procedures must be reviewed and approved by the IACUC prior to their implementation. Any exceptions to these guidelines must be reviewed and approved by the IACUC.

Anesthesia/Analgesia

Where animals must be used, the degree of pain and distress must be minimized by judicious use of anesthetics and analgesics (3). Anesthesia is recommended for tumor implantations and may be required depending on specific implantation site and procedure. The investigator is encouraged to make provisions for pain relief in the protocol or provide justification as to why pain medication is withheld. The use of analgesics does not preclude the establishment of defined endpoints or appropriate monitoring of animals.

Humane Endpoints

Euthanasia is indicated for the following conditions:

1. Tumor size is greater than 2cm in diameter in mice or 4cm in diameter in rats.
2. The tumor is necrotic and/or ulcerated.
3. The tumor interferes with the animal's locomotion and daily functions.
4. The animal is exhibiting one or a combination of the following clinical signs (see Clinical Signs under MONITORING/PARAMETERS):
 - a. Inability to move or difficulty in moving
 - b. Neurological signs
 - c. Respiratory compromise
 - d. Bleeding
 - e. Repeated self trauma
 - f. Weight loss*/Loss of Condition*
 - g. Moderate to severe abdominal distension

***WEIGHT LOSS/LOSS OF CONDITION:**

Weight loss in animals may be difficult to determine. An animal may gain weight in the initial phases of tumor growth, or the weight loss may be offset by tumor growth. It is recommended that a combination of weight loss and condition scoring be used to assess animals. Body condition scoring (BCS) of mice has been described by Foltz et al (1) and involves using a scale

of 1-5 with 1=emaciated, 2=thin, 3=optimum, 4=well-fleshed, and 5=obese. A BCS of 2 or 1 suggests a decline in overall condition and euthanasia is recommended (1).

Monitoring/Parameters

Frequency of monitoring may depend on the growth characteristics of the tumor and the onset of critical phases in the development of the tumor and the experimental process. Animals should be monitored more frequently during phases of high growth, the later stages of tumor-induced disease, or if animals are bearing potentially lethal tumors (4).

1. Tumor size

- a. Optimally, one observer should perform all tumor measurements in a given study in order to minimize variables.
- b. External - Use calipers to measure.
- c. Internal – Measure abdominal distension
- d. Leukemic – monitor lymph node enlargement
- e. Tumor size limits (exceptions must be justified in the APF)
 - i. Single tumor: must not exceed 2.0 cm at the largest diameter in an adult mouse and 4.0 cm in adult rats.
 - ii. Multiple tumors: If multiple subcutaneous/ID tumors are implanted, or the model includes spontaneous tumors, smaller maximum tumor sizes must be described in the APF, e.g., Maximum tumor burden 10% of body weight at day of injection, calculated using the formula: $\text{Mass (mg)} = \text{Tumor volume (mm}^3) = d^2 \times D/2$ where d and D are the shortest and longest diameter in mm, respectively. Length X Width X Depth can also be used.

2. Tumor Appearance

- a. Ulceration - When a tumor becomes ulcerated the animal must be euthanized, unless otherwise described in the APF. If ulceration is an expected outcome, acceptable parameters and methods to reduce pain, distress & infection must be defined in the APF.
- b. Necrosis - When a tumor becomes necrotic the animal must be euthanized, unless otherwise described in the APF. If necrosis is an expected outcome, acceptable parameters and methods to reduce pain, distress & infection must be defined in the APF.
- c. Where the tumor is interfering with movement or function of vital organs or when an animal is seen to be in distress, such as labored breathing, it must be euthanized regardless of tumor size.

Solid Tumors

Solid tumors may be localized or metastasizing and may develop in the superficial tissues or internally. Due to the variability in tumor studies, it is difficult to establish specific guidelines that address every situation. Variables include the animal strain, sex, how the tumor is induced, the type of tumor and the activity of the tumor in the host. However, certain criteria can be assessed to aid in the determination of humane endpoints: tumor size, tumor appearance and clinical signs associated with tumor growth, metastatic disease or cachexia. Uncontrolled or

unexpected tumor growth can result in unnecessary animal pain and distress (4). A large tumor that has become ulcerated or necrotic and has outgrown the vascular supply may introduce unwanted variables to the experiment. For external implanted tumors, the tumor should be placed in a location that does not impede the animal's locomotion or daily functions as it enlarges. It is recommended that the tumor be placed on the back, flank or hip areas. A ventrally placed tumor may become ulcerated due to repeated contact with bedding/caging materials. The implantation of tumors into the tail, distal limbs, eye and brain are discouraged (3) as tumor growth may be restricted and severely damage surrounding tissues. The potential for pain with tumors implanted within muscle should be considered with regard to effects on the animal and the use of analgesics. The implantation of multiple tumors must be carefully assessed as to the effect of tumor burden on the animal (impaired function, clinical signs). Also, tumors may coalesce resulting in more significant compromise to the animal (3).

Internal Tumors

Tumors growing internally or tumor-associated metastatic disease may be difficult to monitor. Uncontrolled development of tumors in internal organs may cause distress or death (4). Removal of a primary tumor may stimulate the development of latent metastatic disease elsewhere. Profiling of internal tumors may include serial termination of animals, investigative surgery, medical diagnostic imaging techniques, or palpation for organ enlargement or tumor development. Tumor evaluation criteria in conjunction with clinical signs may aid in the determination of humane endpoints.

Leukemias

Cancer of the blood may be evaluated by examining blood samples for the presence of circulating cancer cells or changes in the cellular constituents of the blood. Changes in these parameters can be used to forecast the onset of clinical symptoms (4). Diagnostic imaging may be used to evaluate for splenomegaly or lymph node enlargement. Palpation may also be helpful in detecting organ enlargement.

3. Clinical Observations and/or Palpation.

a. Schedule

1. Mice and rats with developing tumors are to be observed *no less than* once weekly until a palpable tumor nodule is present (5-7.5mm in diameter), followed by monitoring at least three times weekly (including weekends and holidays). If tumor growth is rapid in the days before termination, daily or twice daily monitoring may be necessary.
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.

b. Variables: Clinical signs 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) 2 or less
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. Eye/nose discharge

*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].

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.

In vivo imaging, IVIS

The in vivo imaging system, IVIS, can be used to examine cellular processes in live mice using fluorescent or bioluminescent cells, microbes, proteins or chemicals depending on investigator needs. This system is a powerful tool that can be used in cancer research to monitor tumor growth or remission. The IVIS can image whole mice, under anesthesia, and can detect fluorescent or bioluminescent signals within the intact animal. Software associated with the IVIS can be used to construct tomographic reconstructions of the whole animal. Fluorescent signals are captured using matched excitation and emission filters. Luciferase signals are measured following i.p. injection of luciferase.

Currently, in order to study changes in animal models, it is necessary to sacrifice the mouse and harvest tissues. The use of the IVIS to perform biophotonic imaging, BPI, would allow sequential imaging of the same animal to follow changes over time for longitudinal comparisons. BPI allows for sequential imaging of the same animals thereby reducing the number of animals needed for studies. Imaging of live anesthetized animals will allow for the use of fewer animals by obtaining quantitative data from an individual animal at multiple time points throughout the study. This will eliminate the need to repeatedly sacrifice groups of animals for data collection at each time point.

References

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