



Division of Research

SUBJECT: Tumor Burden in Rodents	Effective Date: 12/17/2021	Policy Number: 10.4.23	
	Supersedes: 12/14/2018 12/18/2015 2/2010	Page 1	Of 7
	Responsible Authorities: Principal Investigator Vice President, Research Institutional Animal Care and Use Committee Assistant Vice President for Research, Research Integrity Assistant Vice President for Research, Comparative Medicine		

- I. Background
Cancer is a serious disease common to humans and many other animal species. The incidence of cancer is increasing worldwide. Hence, research into the biological processes leading to cancer, identifying potential carcinogens, and developing effective preventative measures as well as treatments is a major scientific objective. Experimental animal models of cancer allow for the collection of scientific and preclinical data not available from *in vitro* or human epidemiological studies. Cancer, both spontaneous and induced, can cause profound physiologic and metabolic changes in the research subject. Laboratory animals can experience significant adverse effects not only due to the induction of cancer but also due to investigative or treatment regimen. Therefore, humane endpoints must be developed unique to each cancer study to protect animal welfare while still achieving meaningful experimental outcomes.

- II. Purpose
To establish a policy that will provide guidance to research personnel and animal care staff on acceptable tumor endpoint criteria, to observe animal well-being while achieving meaningful experimental outcomes, and comply with laws and regulations governing animals in research, teaching and testing.

- III. General Statement
Spontaneous tumor models, induction of tumor development and tumor implantation in animals are critically important experiments to study biological processes, potential environmental carcinogens and efficacious preventative and treatment measures. These cancer models have the potential to induce pain and/or distress due to the physiologic and metabolic changes expected. A key aspect of the animal welfare regulations is that pain and distress in laboratory animals be prevented or minimized whenever possible.

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Prevention and lessening of pain/distress is not only imperative for animal well-being but also for the quality of research conducted. Therefore, it is the responsibility of the Principal Investigator in conjunction with the Attending Veterinarian to find humane endpoints that are meaningful. In addition, any cells or biological material injected in an animal must be screened for murine or human pathogens depending on its source.

IV. Policy

- A. All injectable and/or implantable materials used for establishing tumors in animals must be described in the IACUC protocol. Murine derived materials must be tested for murine pathogens to avoid infecting resident rodent colonies if not already tested by the vendor. The AV as required by the IACUC will establish appropriate veterinary care and health surveillance programs including the review of test results of such materials. Tumors or cells derived from humans may potentially carry disease that could infect caretakers or technicians. Human tumor cell lines require IBC review and approval.
- B. The site of tumor implantation should be chosen to minimize damage to adjacent normal structures and allow for tumor enlargement. Sites such as the eye, footpad, brain, muscle or tail restrict tumor expansion and should be avoided if not scientifically justified.
- C. Cancer studies should be designed in a way to be able to complete experiments before tumor development or tumor-associated disease causes death or a significant decline in the animal.
- D. Humane endpoints need to consider the cumulative effect of all the experimental challenges associated with the particular cancer studied on the animals involved.
- E. The maximum tumor size guidelines are intended only to suggest an upper limit on allowable solid tumor size. The overriding consideration for humane endpoints of oncological experiments as well as spontaneous tumors must be the overall health of the animal.
- F. Research personnel must follow humane endpoint criteria outlined in the policy (see procedures). If these endpoint criteria interfere with experimental objectives the investigator must justify and describe it in the IACUC protocol.
- G. If studies require moribundity or mortality as an endpoint, a strong scientific rationale must be provided in the protocol.
 1. All tumor-bearing animals must be observed on a scheduled basis and findings documented to assess the progress of tumor growth and/or metastasis, and the general condition of the animal.
- H. Onset of close monitoring by research personnel, monitoring frequency and pertinent information recorded depends on the study, i.e. determined by expected animals' status and progress of the experimental challenge, and requirements set forth in the IACUC protocol.
- I. Recordkeeping is the responsibility of the Principal Investigator. Records must be kept by the research laboratory and be available to the Attending Veterinarian, IACUC or Research Integrity staff at any time requested.
- J. Cachexia, characterized by an energy imbalance leading to consistent weight loss, wasting of muscles and eventual death, is one of the most serious conditions in cancer bearing animals. Body weight, however, is often unreliable as a humane endpoint due to weight loss being set off by tumor growth/weight gain. Therefore, evaluation of the body score and developing distress scoring systems is often preferable.
- K. Continuous monitoring of genetically modified animals for tumor development or phenotypic abnormalities may be necessary from birth onward.
- L. In circumstances involving declining health status, moribundity, or unrelieved pain and discomfort in a tumor bearing animal, every attempt will be made to contact the PI and

to reach consensus with the PI keeping experimental endpoints in mind. However, the final evaluation, regulatory responsibility and decision rests with the Attending Veterinarian.

- M. Rodent tumor cell lines or cell lines passaged through rodents should be tested for common rodent pathogens prior to their use in animals.

V. Definitions

- A. **Cancer** or **neoplasia** are terms to describe a number of diseases characterized by uncontrolled, abnormal growth of cells in tissues or the blood, which may develop localized in specific tissues or cells or may spread from the primary tumor to other parts of the body (secondary tumor growth or metastases).
- B. The term **Humane Endpoints** describes the setting of clear, predictable and irreversible criteria that allow early termination of the experiments before the animal experiences significant harm while still meeting the experimental objectives. Humane endpoints are chosen to minimize or terminate the pain or distress of the experimental animals via euthanasia rather than waiting for their deaths as the endpoint.
- C. **Death as an Endpoint** refers to projects in which the animals' non-experimentally induced death is required as a measured data point. It does not refer to projects in which the animals will be euthanized prior to non-experimentally induced death for tissue collection or project termination.
- D. **Moribundity** is defined as "a dying state". Animals are considered to be moribund if they are in a debilitating physical state where death is imminent and treatment ineffectual.

VI. Accountability

The Principal Investigator (PI) will be responsible for:

- § Describing all experimental procedures in the animal care and use protocol, considering the impact of the tumor study on the animals including pain and/or distress, measures to alleviate pain and/or distress if possible, monitoring frequency and humane endpoints.
- § Assuring that personnel are appropriately trained to being able to assess tumor bearing animals and recognize pain and/or distress.
- § Assure that research personnel follows the animal care and use protocol, especially in regards to monitoring, assessing described experimental and animal welfare criteria and associated recordkeeping.
- § Involving the AV or his/her designee in the design of the study pertaining to painful/distressful procedures and whenever any pain/distress is recognized that is unexpected or cannot be relieved with the measures described in the animal care and use protocol.

The IACUC will be responsible for:

- § Reviewing and approving, requiring modifications in (to secure approval) or withholding approval of IACUC protocols and/or amendments, assessing the appropriateness of monitoring frequency, the measures to be implemented to assess tumor bearing animals including alleviation of pain/distress as much as possible and proposed humane endpoints.
- § Providing oversight for all animal procedures conducted including painful/distressful conditions as well as provision of pain/distress relief.
- § Develop and direct an appropriate training program.

The Research Integrity office will be responsible for:

- § Administrative support of the IACUC members to facilitate their regulatory function
- § Maintaining policy and assure regular review and update as necessary by the IACUC
- § Keeping relevant training records and provide to the IACUC for review

The Office of Comparative Medicine (CM) will be responsible for:

- § Veterinary review of IACUC protocol(s) and advice to PI on appropriate study design concerning painful/distressful conditions as well as appropriate monitoring, supportive care measures and humane end points.
- § Providing support and training for all personnel including animal care staff regarding recognition of pain/distress in rodent species used in oncological studies and administration of supportive care as applicable.
- § Daily observation of all animals housed in CM managed vivaria and notification of the AV or his/her designee and the research personnel whenever signs of pain/distress or set forth humane endpoints are observed in any of the animals under their care.

VII. Procedures

A. Study Design and IACUC Protocol

1. The animal model must be described in detail to reflect either spontaneous cancer development (e.g. use of particular transgenic lines), the injection/implantation of tumor cell lines, the use of carcinogens or any other form of induction of cancer development.
2. The origin, incidence, and time of onset of spontaneously developing tumors in the host animal must be indicated to allow for tailored monitoring procedures.
3. Tumors might metastasize and produce secondary disease in other organs. The incidence of metastatic disease, the organs/organ systems seeded by metastases and the expected onset of associated disease have to be described.
4. If the tumor recipient's immunologic or physiologic status needs to be changed, e.g. through immune-suppressive agents, to promote engraftment of particular experimental tumor lines, this needs to be indicated in the protocol.
5. Anticipated health concerns and functional deficits must be stated along with what care will be provided. A scoring system might have to be developed.
6. Humane endpoints, i.e. a meaningful endpoint considering both experimental outcome and animal welfare, must be listed and a relevant animal care plan outlined.
7. If below outlined endpoint criteria interfere with experimental objectives the investigator must indicate in the IACUC protocol
 - a. Which humane endpoint criterion interferes with the study
 - b. Justify the need to maintain animals beyond the specific endpoint criteria
 - c. Describe alternate endpoints
 - d. Outline the plan for minimizing pain/distress and associated monitoring
8. If studies require moribundity or death as an endpoint a strong scientific rationale must be provided in the protocol including
 - a. What alternatives were considered and why the alternatives are not possible to be used
 - b. Why measures to relieve pain/distress cannot be provided

- c. Justification for minimum number of animals allowed to reach moribundity/death
 - d. Whether animals will be euthanized when moribund and if not, what information will be gained during the period between moribundity and death
 - e. A plan for animal care and monitoring procedures
- B. Studies evaluating the metastatic tumors often require removal of the primary tumor to allow for continued growth of the metastatic ones. If primary tumors are to be removed, it is the responsibility of the researcher to include endpoints for when the primary tumors are to be removed (size, ulceration, impairment of ambulation, etc.).
- 1. A monitoring and record keeping plan must be developed based on the model chosen, described in the protocol and followed throughout the entire study.

C. Monitoring of Animals

- 1. Monitoring of animals utilized in cancer models is the responsibility of the research personnel.
- 2. Animals that have experimental or spontaneous tumors must be observed regularly but no less than 3 times a week for tumor size, ulceration/necrosis or infection of tumors, and associated signs of pain or distress.
- 3. As the tumor(s) progresses and/or the overall health condition deteriorates animals must be observed at least once daily including weekends and holidays.
- 4. Tumors located in areas with limited tissue mass (e.g., on a muscle) must be watched carefully to assure that the tumor does not interfere with or inhibit movement.
- 5. Weighing the animal on, at least, a weekly basis is required. As the cancer progresses, more often weighing may be required.
- 6. Lymphoid tumors, ascetic tumors, and animal models of tumor metastasis all present special problems with assessment of tumor burden. An animal bearing these kinds of tumors must be monitored very carefully on a protocol by protocol basis for mobility, body condition score, dehydration, overall appearance, signs of pain/distress, or any indication of deteriorating health.
- 7. In studies where early endpoints would contradict study objectives animals might have to be monitored several times a day.
- 8. Studies expecting pain/distress that cannot be alleviated (category E) require written monitoring records, which should be developed specific to the tumor model used.

D. Humane End-Points in Cancer Models

- 1. Ulcerated, necrotic tissue is one of the most common findings in tumor models. Ulcerated or necrotic tissue may result in a continuous seepage of body fluids and predisposes to infection.
- 2. Any tumor-bearing animal must be humanely euthanized if one of the following conditions occur unless this is the intent of the researcher and is justified and approved in the associated IACUC protocol:
 - a. The animal becomes incapacitated and ceases to perform normal functions, such as grooming, eating and drinking, respiration, urination, or defecation
 - b. Self-mutilation
 - c. The tumor load causes an impediment resulting in labored ambulation or inability to move preventing the animal from retrieving food and/or water
 - d. There is skin necrosis and/or ulceration over a tumor
 - e. The animal exhibits marked lethargy, is unresponsive to a mild stimulus

- f. The animal becomes cachectic and loses more than 15-20% of pre-procedural body weight or has a very low body condition score, i.e. <2 (see body condition scoring for mice as per Ulman-Cullere and Foltz and for rats as per Hickman and Swan).
 - g. Subcutaneous tumors exceed the maximum allowable size of 20 mm in any dimension for a mouse or 40 mm in any dimension for a rat.
3. In addition to the above listed conditions the following clinical signs are indications of morbidity. Tumor-bearing animals exhibiting these signs should be euthanized based on severity of clinical signs.
- a. Persistent anorexia or dehydration
 - b. Unable to maintain an upright posture or impaired mobility
 - c. Hunched abnormal posture
 - d. Muscle atrophy or emaciation
 - e. Hypothermia
 - f. Bloodstained or mucopurulent discharge from any orifice
 - g. Labored respiration – particularly if accompanied by nasal discharge or cyanosis
 - h. Enlarged lymph nodes or spleen
 - i. Anemia (pale pinnae {ears}, feet or mucous membranes)
 - j. Significant abdominal distension
 - k. Incontinence with urinary scalding, prolonged diarrhea or constipation
 - l. Exophthalmos (bulging eyes)
 - m. Restlessness

E. Recordkeeping

- 1. Investigators must maintain records on monitoring body weight (e.g., initial baseline and subsequent body weights) or body score (e.g. to allow determinations of tumor vs. body weight and weight loss). A control animal of same strain and age is used for comparison.
- 2. Certain studies might require development of a scoring system for determining humane endpoints, which is study specific and recorded per animal. The scoring system could include evaluation of body weight changes, body condition score, physical appearance, any measurable clinical signs such as body temperature, respiration and cardiac rate, unprovoked behavior and behavioral responses to external stimuli.
- 3. Records should include time and frequency of monitoring sessions, the name/initials of the person monitoring the animals, identification of the animals, protocol number, weight and/or body score, the number of animals displaying signs of illness, types of health concerns, and/or any treatments given to the animals.

VIII. Policy Renewal Date
12/17/2024

IX. References

1. Toth LA. 2000. Defining the moribund condition as an experimental endpoint for animal research. ILAR J 41:72-79.
2. ARAC Guidelines for Endpoints in Animal Study Proposals, http://oacu.od.nih.gov/ARAC/documents/ASP_Endpoints.pdf.
3. Ulman-Cullere M, Foltz C, 1999 Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice, LAS Vol 49 no 3 pg 319-323.
4. Hickman D, Swan M, 2010 Use of a Body Condition Score Technique to Assess Health Status in a Rat Model of Polycystic Kidney Disease, JAALAS Vol 49 No 2 pg 155-159.
5. Wallace J. 2000. Humane endpoints and cancer research. ILAR J 41:87-93.

POLICY APPROVAL

Initiating Authority

Signature: _____

Date: _____

Name: Daniel C. Flynn, Ph.D., Vice President for Research

Executed signature pages are available in the Initiating Authority Office(s)