Johns Hopkins University
Animal Care and Use Committee

Tumor Study Guidelines in Mice and Rats[1]

PURPOSE:
This document was developed to assist researchers in establishing criteria to ensure the welfare of mice and rats involved in **solid** tumor studies.

GUIDELINES:

1. **General**

This guideline provides investigators with the requirements and recommended humane endpoints for conducting spontaneous and experimentally-induced solid tumor studies in mice and rats. Investigators producing or passaging solid tumors in mice or rats should use the information contained in this document as a reference when preparing their IACUC protocol. These details must also be included for solid tumors that are predicted to occur spontaneously or are induced in specific strains of rodents or genetically engineered rodents.

Careful consideration should be given to the following items:

- methods of reducing pain and distress
- tumor size, to include multiple metastatic and internal tumors
- clinical signs associated with tumor development
- frequency and method(s) of monitoring
- potential clinical complications of tumor burden
- criteria for intervention, humane endpoints, and the overall health of the animal

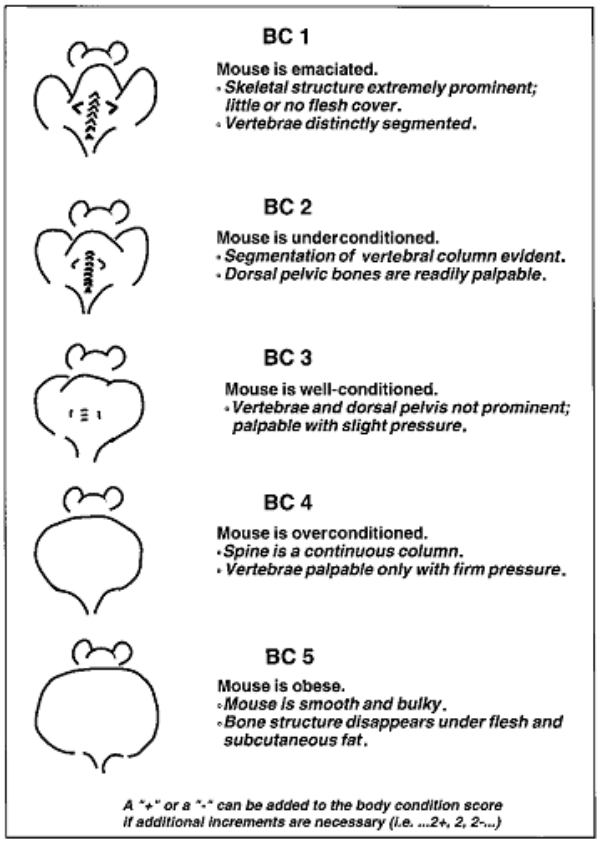
2. **Definitions**

   a. **Moribund:** Clinically irreversible condition evident in a live animal where death is inevitable.
   b. **Ulceration:** Subcutaneous tumors may devitalize the overlying skin, causing necrosis and a surface wound. Ulceration is a lesion typified by necrosis of superficial tissues, which may be dry, suppurating, or exudative.

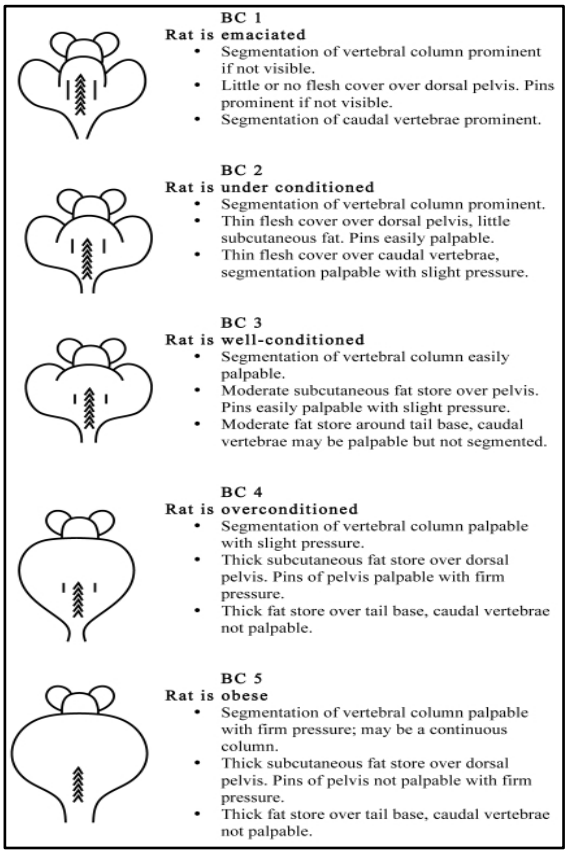
[1] Approved by the JHU Animal Care and Use Committee 1/17/02; revised 10/05, 10/18/, 5/24

c. **Body Condition Score (BCS):** An objective scale to help assess the general physical condition and overall health of the animal. Physical condition is the most important factor in monitoring the progression of tumors in rodents.

Body Condition Scores (BCS) of Mice



Body Condition Scores (BCS) of Rats



d. **Humane Endpoint**: the earliest point at which pain or distress in an animal is prevented, terminated, or relieved via euthanasia. Monitoring for humane endpoints in an approved protocol as well as an animal's general well-being is especially important with metastatic or internal tumors that cannot be easily measured externally.

3. <u>Tumor Implantation or Production and Location</u>

a. **REQUIRED** Injection implantation procedures (e.g., subcutaneous or intradermal): The tumor(s) should be placed into a site(s) that will not interfere with normal body functions (e.g., ambulation, eating, drinking, defecation, urination). Subcutaneous or intradermal sites on the back or in the flank are considered to cause the least distress**. If locations other than the back or flank are selected (e.g., mammary fat pad), they should be scientifically justified in the protocol and the humane endpoints clearly**

defined. Extra attention must be paid if more than one (1) site is used. Two (2) tumor sites are the maximum to be implanted into one animal.

b. **REQUIRED** Surgical implantation procedures (e.g., orthotopic): These procedures **must** be described in the IACUC protocol. Refer to the ACUC Rodent Survival Surgery, Analgesia for Rodents, and Anesthesia Gas Guidelines for information on anesthesia, analgesia, and aseptic technique.

c. **REQUIRED** *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 monitoring, controlling severity (e.g., analgesia, anesthetic, sedation), and the definition and implementation of humane endpoints.

4. Tumor Size/Volume and Number

a. Size/Volume:

REQUIRED The humane endpoint (euthanasia required) for a single solid tumor is 2.0 cm (mouse) and 4.0 cm (rat) in any direction. The tumor size may not exceed these dimensions without an IACUC-approved exception based on scientific justification.

i. **REQUIRED** If multiple tumors are present, the combined or cumulative burden may not exceed the maximum allowed burden of a single tumor (e.g., the longest dimension of each tumor in a mouse having multiple tumors are added together, and the **sum must remain ≤ 2.0 cm**.), unless otherwise approved by the ACUC.

ii. **OPTIONAL** Other methods of measuring tumors (such as the weight of the tumor relative to the weight of the animal or a calculated volume) may be used, but need to be described in the protocol.

iii. **REQUIRED A maximum linear measurement (as provided below) MUST also be included in the protocol**, to animal care/husbandry personnel with a standard monitoring capability.

REQUIRED Internal tumors (e.g., within the cranium, thoracic cavity, mouth, behind the eyes, or within the pelvis) and metastatic tumors may interfere with vital functions and result in morbidity or mortality. **In these studies, tumor size may not be a useful indicator and the primary assessment should be overall health status**. As the use of imaging or biomarkers may detect tumors before they are visible or can be palpated, consideration should also be given to the use of imaging in studies where the tumors are not readily observable.

OPTIONAL For tumors internal to a body cavity, volume can be measured using 3D imaging (CT, MRI, ultrasound, etc.). Alternatively, a common method of estimating volume is the formula $V = d^2 \times D/2$, where 'd' is the shortest diameter and 'D' is the longest diameter. If volume is used, the humane endpoint (maximum volume allowed) for mice is 4,000 mm^3 and for rats is 32,000 mm^3. **Multiple tumors should have a combined total**

volume that is under the limit of a single tumor. In such cases, **volume endpoints should be clearly defined in the protocol.** See example calculations in **Table 1**. If tumor volume is used as the primary endpoint, this does not supersede the previously stated maximum (cumulative) linear measurement of 2 cm in any direction.

TABLE 1. Sample volume calculations in determining humane endpoints in mice

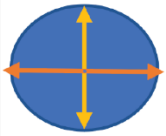
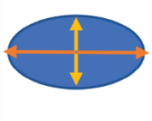
Sample tumor dimensions	**Volume of tumor = d^2 x D/2**	**Humane endpoint?**
 d=20mm D=20mm	= 20^2 x 20/2 = 400 x 10 = 4000mm^3	Yes • Volume = 4 000mm^3 • But, d, D = 2cm
 d=15mm D=20mm	= 15^2 x 20/2 = 225 x 10 = 2250mm^3	Yes • Volume < 4000mm^3 • But, D = 2cm
 d=15mm D=15mm	= 15^2 x 15/2 = 225 x 7.5 = 1687.5mm^3	No • Volume < 4000mm^3 • d, D < 2cm
 Primary d=15mm D=15mm *** AND ***	<u>Volumes</u> Primary = 15^2 x 15/2 = 225 x 7.5 = 1687.5mm^3	Yes • Volume < 4000mm^3 • Sum of longest dimensions > 2cm

TABLE 1. Sample volume calculations in determining humane endpoints in mice

Sample tumor dimensions	Volume of tumor = d^2 x D/2	Humane endpoint?
 Secondary d=10mm D=10mm	Secondary = 10^2 x 10/2 = 100 x 5 = 500mm^3 *Sum of Primary and Secondary Volumes* =1687.5mm^3 + 500mm^3 Volume =2187.5mm^3 <u>Linear Dimensions</u> Primary D = 15mm Secondary D = 10mm *Sum of longest dimensions of Primary and Secondary* = 15mm + 10mm = 25mm	

b. Number:

REQUIRED In **mice**, the humane endpoint (euthanasia required) is when one or more tumors reach a combined size of 2.0 cm in any direction. In **rats**, the humane endpoint is when the combined size reaches 4.0 cm in any direction. No single or combined tumor dimension may exceed 2.0 cm (mouse) or 4.0 cm (rat) without an IACUC-approved scientific justification. Individual tumors that are smaller than the single tumor maximum size may not have the same adverse health consequences as a single, large tumor. Nevertheless, even some relatively small tumors can interfere with basic bodily functions, especially when located in the face or perineum**. When multiple tumors are present, the assessment of overall health status should take priority**.

5. **Monitoring and Recordkeeping**

a. Frequency of monitoring

REQUIRED All animals involved in tumor studies should be monitored for tumor size, health status, metastatic or internal tumors, and pain/distress by qualified laboratory personnel. The exact monitoring and documentation frequency is based on the IACUC-approved protocol. **The IACUC protocol must include the frequency of monitoring, what will be monitored, and any change in monitoring as the tumor(s) develops.** The guidelines listed below apply to weekdays, weekends, and holidays.

REQUIRED After implantation of tumor cells, or in animals that develop spontaneous tumors, animals need to be monitored regularly with intervals of no greater than 4 days apart.

RECOMMENDED More frequent observations may be necessary depending on the rate of tumor growth and when the general health of the animal shows signs of deterioration. Monitoring as frequently as twice daily may be appropriate depending on the progression of disease. Animals displaying general health concerns (e.g., not eating, drinking, reduced mobility), or approaching 50% of the maximum tumor size, should be monitored at least daily for humane endpoints.

b. Types of monitoring

REQUIRED All animals shall be monitored via physical examination for clinical complications, body condition score, and humane endpoints.

RECOMMENDED Monitoring of subcutaneous tumors can be monitored via physical examination and the use of calipers to measure the size of the tumor in at least two of the three dimensions. Internal or metastatic tumor size can be measured via non-invasive imaging or palpation.

c. Records

RECOMMENDED Records should be kept and be available upon request with all pertinent information including time and frequency of monitoring sessions, the name of the person monitoring the animals, identification of the animals, protocol number, the number of animals displaying symptoms, types of symptoms, and any treatments given to the animals.

6. Tumors with Clinical Complications, Ulceration, Ascites

a. Clinical Complications

REQUIRED Cases involving single or multiple tumors that alter the health status of the animal, its ability to ambulate normally, or obtain food and water should be euthanized.

Table 2. Selected Clinical Observations

Parameter	What to look for
General appearance	Dehydration, weight loss ≥ 20% or body condition score ≤ 2, abnormal posture, hypothermia, abnormal appearance of limbs, abdominal distension, swelling, tissue masses, vocalization
Skin and fur	Discoloration, urine stain, pallor, redness, blueness, jaundice, wound, abscess, ulceration, bald spot (alopecia), ruffled fur, bloody or purulent discharge
Eyes	Enlarged eyes, small eyes, droopy lids, red-eye, tears, discharge, opacity
Nose, mouth, and head	Head tilted, nasal discharge, malocclusion, drooling
Respiration	Abnormal or labored breathing
Urine	Discoloration of back/ventral fur, blood in urine, excessive or no urination

Table 2. Selected Clinical Observations

Parameter	What to look for
Feces	Discoloration of back/ventral fur, blood in the feces, softness/diarrhea
Genital area	Prolapses, paraphimosis (constriction of penis)
Locomotion	Hyperactivity, hypoactivity, impaired movement, poor coordination, circling, tremors

b. Ulceration

RECOMMENDED Ulceration in a tumor that is smaller in size than 2.0 cm does not necessarily require euthanasia if the animal is healthy, but it will require more frequent monitoring and potential treatment (e.g., antibacterial ointment). Some mice with ulcerated tumors, particularly those with scabbed/dry ulcerations, may be permitted to continue as part of the experiment in an effort to reduce the need to transplant tumors into replacement animals. Ulceration may also reflect a positive, anti-tumorigenic response to therapy as responsive tissue becomes necrotic. Seepage of blood or body fluids from ulcerated tumors can predispose the animal to infection and will require treatment or euthanasia as per veterinary discretion. If ulceration is expected, this should be described in the protocol.

c. Ascites

Refer to the ACUC Guideline for using the ascites method for Monoclonal Antibody Production for additional information on ascitic tumors.

7. Humane Endpoints and Assignment to Pain Categories

a. Humane endpoints: Common signs of pain and distress in rodents include those listed in Table 2. Furthermore, animals in pain or distress may not interact with their cage mates, or cage mates may become aggressive towards them. The affected rodent may become uncharacteristically aggressive toward a familiar human handling it. Animals may squeal when picked up or when an affected area is touched. Persistent vocalization indicates substantial pain or distress.

Metastatic spread of a tumor may be difficult to assess grossly. Signs of metastasis may include the signs of distress listed above, as well as limb paralysis, difficulty walking, climbing, or breathing. Moreover, metastatic tumor cell lines may form primary tumors at a slower rate than their non-metastatic counter parts. In some cases, a decrease in the size of a transplanted primary tumor can signal that the transplanted tumor cells are moving or metastasizing to other parts of the body.

Because the growing tumor and potential buildup of ascites fluid contributes to overall body weight at a time when the host may actually be losing body mass, emaciation is a more reliable indicator of a serious condition than loss of body weight. Thus, in evaluating the physical condition of animals during tumor progression, it is useful to utilize a scoring system such as **the Body Condition Score (BCS)** to assess overall health. The scoring systems for mice and rats are shown in the chart above.

REQUIRED Animals that exhibit signs described above in the presence of a tumor burden and have a body condition score of 2 or less must be euthanized. If the skeletal structure is not easily visualized in a mouse with ascites, it may be necessary to pick up the mouse and palpate the vertebral column to assess emaciation.

Regardless of the type of tumor, the overall health and condition of the animal must take priority over precise tumor measurements in decisions regarding euthanasia or other interventions.

b. Assignment to pain categories

Category C:

i. Most subcutaneous, implanted tumors are thought to cause no pain or discomfort and thus should be classified as USDA Pain Category C. If animals have reached humane endpoints without experiencing more than momentary pain or distress and are euthanized at that time, they shall also be classified as USDA Pain Category C. Other types of tumors (e.g., spontaneously developing, virus-induced, metastatic) may have a different pain profile and thus alternate pain categories should be considered.

ii. The process of injecting tumor cells using a syringe and needle is a Category C procedure if surgery (i.e., incision) is not required.

Category D:

i. The animals should be classified as USDA Pain Category D when the tumor burden is expected to cause pain/distress or disruption of normal activity. Examples include situations where palliative treatments are used to relieve pain and discomfort [e.g., anesthetics, analgesics].

ii. The process of making an incision in the skin as part of the implantation of tumor cells is a Category D procedure.

Category E: In cases where the tumor burden is expected to cause pain/distress or disruption of normal activity; yet, scientific justification is provided for withholding palliative treatments (e.g., anesthetics, analgesics) or exceeding humane endpoints, the animals should be classified as USDA Pain Category E.

SUMMARY CHECKLIST OF MANDATORY ITEMS TO INCLUDE IN ANIMAL USE PROTOCOL

	Location in this Guideline	**Mandatory Items to include in Protocol**	**Location in JHU ACUC Protocol Format to place information**
☐	3a	Location of implanted tumor and expected location of tumor growth	Question 14a
☐	3b	Description of surgical implantation procedures	Question 15a
☐	4a	Expected tumor size and declaration that tumor will not exceed limit in this Guideline	Question 14a
☐	4ai	If multiple tumors are expected, include a declaration that the sum of the longest tumor dimensions will not exceed 2.0 cm (in mice) or 4.0 cm (in rats)	Question 14a
☐	4aii	A description of linear measurement of tumor, and how it will be assessed	Question 14a

☐	6a, Table 2	Declaration that overall health assessment takes priority over size of tumor and what clinical signs will be assessed	Question 14a
☐	6b	Description of actions if tumor is ulcerated	Question 14a- if monitoring and treating Question 16c- if euthanasia
☐	6c	Description of actions if ascites may be expected to develop	Question 14a- if removing excess fluid Question 16c- if euthanasia
☐	5a	Frequency and method of monitoring	Question 14a
☐	2c, 7a	Description of humane endpoints and Body Condition Score that will be observed	Question 16c
☐	7b	Assignment of animals to USDA Pain Categories	Question 17 a-c

If you have any questions or need assistance, please consult ACUC Office staff (443-287-3738) or an RAR veterinarian (410-955-3273). For evenings, weekends, and holidays, the veterinarian on clinical call can be paged (410-283-0929).

Revised 4/24