

Washington State University
Institutional Animal Care and Use Committee

Guideline #8: Tumor Burden Guide

Approval Date: 3/9/2021 (Replacing Version 12/2017)

A. Purpose

The purpose of this document is to establish guidelines that will allow investigators and OCV veterinary staff to objectively evaluate the health and welfare of animals carrying spontaneous or experimentally induced tumors, and to determine when euthanasia might be warranted to alleviate pain and distress associated with these tumors. The recommendations presented in this document are intended to provide guidance, but not supersede the information presented in the [WSU IACUC Policy #8](#) for Humane Endpoints.

B. General

When using animal models for the study of cancer, it is often necessary that the animals utilized experience tumor growth and possibly metastatic disease. The determination of humane endpoints should involve the Principal Investigator (PI) and veterinarian and must be approved by the IACUC prior to the start of the study. To maximize data acquisition and minimize pain and distress, the animal's health and welfare must be regularly and comprehensively assessed and documented as tumors and associated disease progress. The PI and/or research staff are responsible for monitoring the animals for endpoint criteria. Office of the Campus Veterinarian (OCV) personnel can assist in the development and assessment of appropriate endpoints. All unanticipated adverse events must be reported to the IACUC and may require modification of the endpoints.

Please refer to [IACUC Policy #8](#) for additional guidance on the development of humane endpoints and [IACUC Policy #37](#) for instructions on reporting unanticipated adverse events.

When assessing the general health of animals utilized in studies of cancer, the following clinical presentations warrant development of a management plan as they indicate

diminished health status that may be associated with increasing tumor burden and metastasis:

1. Weight loss and/or decreasing body condition score (BCS). Please view the [rodent BCS score chart](#) for reference. Depending on the tumor type and location, weight gain can occur due to tumor growth or ascites while body condition is decreasing.
2. Animal appears dull, depressed, and/or agitated
3. Dehydration; failure to eat or drink for over 24 hours
4. Hunched posture, reduced mobility, lethargy, lack of responsiveness, rough hair coat
5. Change in feces/urine and/or perianal soiling
6. Respiratory-associated symptoms such as increased respiratory rate, coughing, and nasal discharge
7. In rodents, eye and nose porphyrin (red stain) discharge
8. Abdominal distention due to tumor or ascites
9. Icterus/jaundice
10. Neurological signs such as paralysis, seizures, circling, or ataxia
11. Self-trauma
12. Difficulty with ambulation that might interfere with food and water acquisition
13. Ulceration and necrosis of visible tumors

Immediate action would be warranted for these clinical signs:

1. Distention of the abdomen with fluid (ascites) or palpable mass
2. Abnormal vocalization when touched or handled, indicating severe distress
3. Labored breathing/respiratory difficulty
4. Severe anemia (pale mucous membranes or visible skin surfaces, or decreased packed cell volume)
5. Unresponsive to stimuli, weak, comatose, or moribund
6. Tumor significantly interferes with animal's ability to eat, drink, ambulate or maintain normal posture

Criteria for Endpoints of Solid Tumors (each listed as a standalone, not in addition):

1. Tumor burden (combined burden if more than one mass present) is greater than 15% body weight (Figure 1& 2)
 - a. The weight reference is the weight of the animal on the day of tumor implantation
 - b. The mass of the tumor is calculated from the following formula-
 - i. $\text{Mass (mg)} = \text{Tumor volume (mm}^3) = d^2 \times D/2$ where d and D are the shortest and longest diameter in mm, respectively.

- ii. $\text{Mass of tumor (g) / Weight of the animal (g) x 100 = \% of body weight}$ (see chart below)
2. Mean tumor diameter = or > 20mm in adult mice (~25g) or 40mm in adult rats (~300g) where Mean = $(d + D)/2$.
3. Ulceration, infection or necrosis of tumor

Recommended Monitoring Schedule: A precise tumor monitoring schedule should be described in the ASAF based on information gained from literature searches and previous use. A pilot study with a small number of animals may be necessary for novel experiments. Below is a sample schedule.

1. Prior to tumor inoculations, baseline body weight and body condition score are recorded
2. Animals are observed once per week, even when the tumor is not yet detectable.
 - a. External tumor: After a visual or palpable tumor is evident, the animals are visually monitored daily and BCS, weight, and tumor measurements are recorded weekly.
 - b. Internal tumor: For metastatic or other internal tumors, body weight, BCS and anticipated clinical parameters for the tumor type and location are recorded weekly.
3. As tumor growth progresses and the animal's condition deteriorates, more frequent observations will be necessary (twice weekly, every other day, daily)
4. Please contact the Office of the Campus Veterinarian (OCV) at or.ocv.alert@wsu.edu for assistance in creating a monitoring schedule.

Helpful Documents

1. Please view Figure 1 below for examples of solid tumors in rodents.
2. Rodent Health Monitoring Sheet [\[DOC\]](#) [\[PDF\]](#)
3. Representative Scoring System for Determining Humane Endpoints [\[DOC\]](#) [\[PDF\]](#)
4. Rodent Body Condition Score (BCS) Chart [\[DOC\]](#)
5. Indicators of Pain in Laboratory Animals [\[PDF\]](#)

C. References

1. P Workman et al. Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer* (2010)102,1555–1577

Examples of Solid Tumors in Rodents (how to calculate % body weight, mean tumor size, tumor burden)

Figure 1: Mouse Tumors (25g animal)

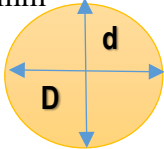
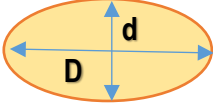
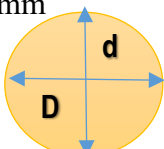
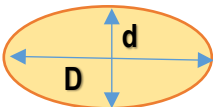
Tumor examples	Mass of Tumor (mg) Tumor volume= $d^2 \times D/2$	Meets Criteria for end point?
d=20mm D=20mm 	$20^2 \times 20/2 = 4000\text{mm}^3$ or 4g Mean tumor size=20mm Tumor burden=4g/25g=16%	Yes-exceeds 15% body weight and mean tumor size 20mm
d=15mm D=25 mm 	$15^2 \times 25/2 = 2812\text{mm}^3$ or 2.8g Mean tumor size=20mm Tumor burden=2.8g/25g=11%	Yes-mean tumor size 20mm

Figure 2: Rat Tumors (300g animal)

Tumor examples	Mass of Tumor (mg) Tumor volume= $d^2 \times D/2$	Meets Criteria for end point?
d=40mm D=40mm 	$40^2 \times 40/2 = 32,000\text{mm}^3$ or 32g Mean tumor size=40mm Tumor burden=32g/300g=11%	Yes-exceeds mean tumor size 40mm
d=35mm D=50 mm 	$30^2 \times 50/2 = 22,500\text{mm}^3$ or 22.5g Mean tumor size=40mm Tumor burden=22.5g/300g=7.5%	Yes-mean tumor size 40mm