

Washington State University
Institutional Animal Care and Use Committee

Guideline #8: Tumor Burden in Rodents

Approval Date: 5/21/2024 (Replacing Version 3/9/2021)

A. Purpose

The purpose of this document is to provide guidelines and expectations for monitoring animals carrying spontaneous or experimentally induced tumors, and to determine 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. Appropriate monitoring criteria, observation frequency and humane endpoints should be determined in consultation with an OCV Veterinarian. The plan must be approved by the IACUC prior to the start of the study. The IACUC expects investigators to follow the monitoring and endpoint criteria described in this guideline unless an alternative plan is approved by IACUC.

In addition to the tumor monitoring plan, a tumor monitoring sheet must be included with the ASAF. This sheet will be used to document the animal's condition, tumor growth and to assist in determining when endpoints have been reached. The PI and/or research staff are responsible for the monitoring and documentation. The tumor monitoring sheet(s) should be present in the animal room or data readily accessible by OCV staff. All unanticipated adverse events must be reported to OCV and the IACUC in accordance with [IACUC Policy 37](#) and may require modifications of the endpoints.

Please contact the OCV at or.ocv.alert@wsu.edu for assistance in creating a monitoring schedule. Please refer to [IACUC Policy #8](#) for additional guidance on the development of humane endpoints.

C. Monitoring & Endpoint Guidelines:

Prior to tumor inoculations, baseline body weight and body condition score must be recorded on the tumor monitoring sheet.

1. Weight loss and/or decreasing body condition score (BCS).

Depending on the tumor type and location, weight gain can occur due to tumor growth or ascites while body condition is decreasing so both need to be monitored. Weight and BCS should be monitored and recorded once per week and at each tumor measurement. Please view the [rodent BCS score chart](#) below for reference.

Animals with a BCS <2 or weight loss \geq 20% should be euthanized. For younger animals (depending on species and strain), failure to maintain weight gain to within 15% of untreated control animals should be considered as an indication of significant health deterioration.

2. Tumor Monitoring & Endpoints

Subcutaneously injected

- Animals should be observed once per week, for initial tumor development.
 - After a visual or palpable tumor is evident, the tumor should be measured using calipers at least twice weekly.
 - Once the tumor's largest diameter measurement reaches 10 mm in mice and 20 mm in rats, the tumor should be measured 3x/week (every 2-3 days). If a rapid growing tumor line is being evaluated, then the frequency should be increased to 4-5 times/week (every 1-2 days).
 - A single tumor with a mean diameter = or > 20mm in adult mice or 40mm in adult rats would meet tumor burden endpoint.
 - Mean = $(d + D)/2$, where d and D are the shortest and longest diameter in mm, respectively.
- OR
- Tumor burden (combined burden if more than one mass present) is greater than 15% body weight (Figure 1 & 2)
 - The weight reference is the weight of the animal on the day of tumor implantation or, if using younger animals still undergoing

development, the mean of the control animals' weight. The mass of the tumor is calculated from the following 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.
 - $\text{Mass of tumor (g)} / \text{Weight of the animal (g)} \times 100 = \% \text{ of body weight (see chart below)}$
- Ulceration of skin overlying tumors and abrasions (overt open lesion or scabbed area):
 - Some solid tumor types are more prone to ulceration than others because of their aggressive and inflammatory nature. Ulceration in a tumor does not necessarily require euthanasia if the animal is healthy, but it will require more frequent monitoring and topical treatment daily until the ulcer has closed. Some mice with ulcerated tumors, particularly those with dry scabbed ulcerations, and not wet seeping ulcers, may be permitted to continue as part of the experiment in an effort to reduce the need to use additional replacement animals.

Systemically (intraperitoneal, intravenous, etc.) injected:

- Body weight, BCS and anticipated clinical parameters for the tumor type and location are to be recorded weekly.
- As tumor growth progresses and the animal's condition deteriorates, more frequent observations will be necessary (twice weekly, every other day, daily)

Animals that have reached the weight loss, BCS or tumor burden endpoints described above should be euthanized within 24-48hrs. If animals show any severe clinical symptoms, they should be euthanized immediately.

If animals are observed with any of the following clinical symptoms, they should be euthanized immediately.

- Abdominal distention due to tumor or ascites
- Difficulty with ambulation that interferes with food and water acquisition.
- Labored breathing/respiratory difficulty
- Severe anemia (pale mucous membranes or visible skin surfaces, or decreased packed cell volume)
- Unresponsive to stimuli, weak, comatose, or moribund

- Or other signs that indicate the animal is in severe distress (e.g., abnormal vocalization when touched).

Helpful Documents

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

D. 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 Tumor Burden Calculation

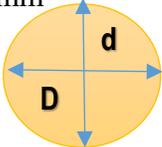
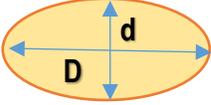
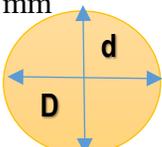
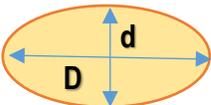
Tumor examples	Mass of Tumor (mg) Tumor volume= $d^2 \times D/2$	Meets Criteria for end point?
d=19mm D=20mm 	$19^2 \times 20/2 = 3610 \text{ mm}^3$ or 3.6g Mean tumor size=19.5mm Tumor burden= 3.6g/23g=15.6%	Yes-exceeds 15% body weight
d=15mm D=25 mm 	$15^2 \times 25/2 = 2813 \text{ mm}^3$ or 2.8g Mean tumor size=20mm Tumor burden=2.8g/25g=11%	Yes-mean tumor size 20mm

Figure 2: Rat Tumor Burden Calculation

Tumor examples	Mass of Tumor (mg) Tumor volume= $d^2 \times D/2$	Meets Criteria for end point?
d=39 mm D=40 mm 	$39^2 \times 40/2 = 30,420 \text{ mm}^3$ or 30.4 g Mean tumor size= 39.5 mm Tumor burden=30.4g/200g=15.2%	Yes- exceeds 15% body weight
d=30mm 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