

<b>Institutional Animal Care Program (IACP)</b>	
<b>Title: Scoring and Endpoints in Tumor Studies in Rats and Mice</b>	
<b>Policy number IACP 012</b>	<b>Date in Effect:</b> 08/12/08
<b>Revision 2, 02/07/19</b>	<b>Re-reviewed Date:</b> 02/14/25, no changes
<b>In Effect</b> <input checked="" type="checkbox"/> <b>Rescinded</b> <input type="checkbox"/>	<b>Date Rescinded:</b>

A) RESPONSIBILITIES

- 1) It is the responsibility of all Principal Investigators and their laboratory personnel who inoculate animals with neoplastic cells and/or toxic/chemotherapeutic agents using animals in research at UT San Antonio to abide by this policy.
- 2) Deviations or the use of less stringent endpoints from this policy must be justified to and approved by the Institutional Animal Care and Use Committee (IACUC).

B) APPLICATION

This policy establishes guidelines for a tumor burden scoring system to be used for mice and rats inoculated with neoplastic cells or toxic agents.

C) REFERENCES

- 1) Morton DB, Griffiths PHM, (1985) Guidelines on the recognition of pain, distress, and discomfort in experimental animals and a hypothesis for assessment. *Veterinary Record* 116: 431-6.
- 2) Montgomery CA (1990), Oncological and toxicological research: Alleviation and control of pain and distress in laboratory animals. *Cancer Bulletin* 42:230-237.
- 3) Dr. Liang Xu and Dr. Marc Lippman permitted the use of their previously described tumor burden scoring system standard operating procedure.



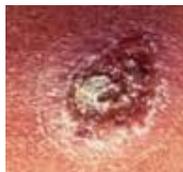
- 4) United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) Guidelines for the Welfare of Animals in Experimental Neoplasia (Second Edition). (1998). *British Journal of Cancer*, 77(1), 1–10.
- 5) Wallace J. (2000), Humane endpoints in cancer research. *ILAR J.* 41: 79-84.
- 6) Unit for Laboratory Animal Medicine, University of Michigan (2014, November 15). Guidelines and SOP on Tumor Monitoring Policy. Retrieved from: <https://az.research.umich.edu/animalcare/guidelines/guidelines-and-sop-tumor-monitoring>
- 7) Emory University Tumor Burden Scoring Guidelines (2013, April 3). Retrieved from: [http://www.iacuc.emory.edu/documents/tumor\\_burden\\_scoring.pdf](http://www.iacuc.emory.edu/documents/tumor_burden_scoring.pdf)
- 8) Yasui, N., Sakamoto, M., Ochiai, A., Ino, Y., Akimoto, S., Orikasa, A., ... Yasui, N. (1997). Tumor growth and metastasis of human colorectal cancer cell lines in SCID mice resemble clinical metastatic behaviors. *Invasion & Metastasis*, 17(5), 259–269.
- 9) Corbett TH et al. (2004) In vivo methods for screening and preclinical testing in Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval (eds. Teicher, B. & Andrews, P.). Totowa, NJ: Humana Press.
- 10) Goldin, A., Venditti, J., Macdonald, J., Muggia, F., Henney, J., & Devita, V. (1981). Current results of the screening program at the division of cancer treatment, national cancer institute. *European Journal of Cancer* (1965), 17(2), 129–142.
- 11) Ullman-Culleré MH and Foltz CJ (1999), Body condition scoring: a rapid and accurate method for assessing health status of mice. *Lab Anim Sci* 49:319-323.

#### D) DEFINITIONS

- 1) BCS: Body Condition Score on a scale of 1-5. See Appendix 1.



- 2) Moribund: Clinically irreversible condition leading inevitably to death.
- 3) Cachexia: General physical wasting, weakness and malnutrition usually associated with chronic disease.
- 4) Metastasis: Spread of cancer from its site of origin.
- 5) Skin ulceration: A breakdown of the skin cells resulting in exposure of underlying tissue and discharge of fluid. Redness or scabbing in the area of the tumor does not necessarily constitute ulceration. The following pictures illustrate typical skin ulcers:



#### E) BACKGROUND INFORMATION

- 1) Optimally, *in vivo* studies are concluded once animals begin to exhibit clinical signs of disease, a practice which maximizes animal welfare and minimizes pain and distress. However, this endpoint is not always compatible with necessary research objectives. Efforts should be made to minimize pain and distress experienced by animals used in research. Checklists and/or scoring sheets may be helpful in ensuring appropriate observations are made, consistently interpreted, and properly documented.
- 2) The checklists provided below provide a humane approach to end pain and distress while maintaining research integrity. Investigators may propose more stringent and/or earlier endpoints in their protocols.

#### F) PROCEDURES

- 1) Criteria that establish when the endpoint has been reached.



- a. Evaluation of five aspects of an animal's condition include: body weight, physical appearance, measurable clinical signs, unprovoked behavior and response to external stimuli (1).
- b. Clinical observations, including: changes in general appearance, skin and hair, eyes, nose, mouth and head, respiration, urine, feces, and locomotion (2).
- c. Clinical signs, depending on severity and duration, that may constitute an endpoint include:
  - i. Rapid weight loss.
  - ii. Diarrhea, if debilitating.
  - iii. Progressive dermatitis.
  - iv. Rough hair coat, hunched posture, lethargy or persistent recumbency.
  - v. Coughing, labored breathing, nasal discharge.
  - vi. Jaundice and/or anemia.
  - vii. Neurological signs.
  - viii. Bleeding from any orifice.
  - ix. Self-induced trauma.
  - x. Any condition interfering with eating or drinking (e.g. difficulty with ambulation).
- d. Additional signs in neoplastic studies that may constitute an endpoint include, but are not limited to:
  - i. A tumor burden greater than 10% of the body weight.
  - ii. Visible tumors that ulcerate and become necrotic or infected.
- e. Any animal found to be moribund, cachectic, or unable to obtain food or water.



2) Scoring System

To better assess tumor burden, a scoring system developed by Xu and Lippman (3) and adopted as a Standard Operating Procedure (SOP) by both the Unit for Laboratory Animal Medicine (ULAM) at the University of Michigan (6) and Emory University’s IACUC (7) will be utilized to maximize study data acquisition while minimizing animal distress and discomfort. Animals are graded on tumor size (mm); degree of necrosis; changes in eating or drinking; mobility; weight loss or gain; and overall appearance. ***NOTE: All animals should have bodyweights recorded at the initiation of any study involving tumor growth and metastatic disease. Normograms can be used for calculating normal mouse body weight for animals treated as neonates or as juveniles.*** Actions to be taken for a range of scores are as follows:

- a. **General Appearance (includes skin, orifices, and posture)** – please see Appendix I at the end of this policy for graphics to be used for the BCS and Appendix II for examples of how the scoring system should work.

	<b>Score</b>
Normal (BCS = 3)	0
Not well groomed, less active, normal posture and gait	2
Skin or hair coat in poor condition (scruffy), less active, decreased signs of grooming (BCS = 2)	3
Skin or coat in poor condition, dehydration (decreased skin elasticity), emaciated, inactive (BCS = 1)	6
Moribund or severely cachexic	18

- b. Tumor size/Degree of Necrosis



	<b>Score</b>
Tumor very small ( $\leq 7$ mm length in mice, $\leq 10$ mm length in rats at its longest diameter)	0
Tumor small with some necrosis/ulceration ( $\leq 7$ mm length, $\leq 10$ mm length in rats at its longest diameter)	4
Tumor necrotic (7-17 mm, mice; 10-26 mm, rats (long diameter))	9
Tumor large ( $\geq 18$ mm in mice, $\geq 27$ mm in rats at its longest diameter), or severe abdominal distension ( $\geq 10\%$ increase over original body weight)	12

Note: for more than one tumor, the added tumor lengths of all tumors will be used to score the tumor. For example, if an animal has three tumors, each measuring 3, 5 and 6 mm on the longest diameter, the total length of the tumors for scoring purposes would be 14 mm.

c. Body weight change

	<b>Score</b>
No weight loss or gain from pre-inoculation weight	0
Weight loss 5-10% or weight gain from tumor burden 0-5%	9
Weight loss 10-15% or weight gain from tumor burden 6-9%	12
Continued declining wt loss $\geq 20\%$ versus initial measurement for up to 2 days (tumored animals that have NOT undergone chemotherapeutic treatment) or 4 days (tumored animals that have undergone chemotherapeutic	18



<p>treatment). Weight loss &gt;30% will result in immediate euthanasia.</p> <p>Clarification on weight losses:</p> <p>20% weight loss for up to 2 days is used to give a score of 18 to determine the endpoints</p> <p>30% weight loss: If an animal has lost &gt;30% of its baseline weight, it will be immediately euthanized.</p>	
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3) Summation of scores and course of action

<b>Score</b>	<b>Action</b>
0-7	Routine daily monitoring.
8-13	<p>Close daily monitoring, which may include twice a day monitoring and daily weighing. May supplement on cage bottom. Examples of suitable supplementation:</p> <ol style="list-style-type: none"> <li>1. Water source alone such as Napa nectar, SC or IP saline or Lactated Ringer’s Solution</li> <li>2. Energy dense such as Bacon Softies (<a href="http://www.bioserv.com/product/Bacon_Softies.html">http://www.bioserv.com/product/Bacon_Softies.html</a>)</li> <li>3. Energy dense/water such as a DietGel (<a href="http://www.clearh2o.com">www.clearh2o.com</a>)</li> </ol>
14-18	Consult veterinary staff or consider euthanasia.
>18	Euthanize immediately.
Ulceration	Cage isolation to avoid possible aggressive cage mates.
>30% Wt loss	Euthanize immediately.



**Animal Study Proposals utilizing death or moribundity as an endpoint:  
Refer to IACP 003.**



**Appendix I: Body Condition Scoring (BCS) Guide (13).**

	<b>BCS 1</b>  Animal is emaciated
	<b>BCS 2</b>  Animal is under conditioned
	<b>BCS 3</b>  Animal is well conditioned
	<b>BCS 4</b>  Animal is well over conditioned
	<b>BCS 5</b>  Animal is obese

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-)

**Appendix II:** Example scoring scenarios

Example #1: A nude mouse is presented with the following conditions:

1. Normal (BCS = 3).
2. A 6 mm tumor at its longest diameter with some ulceration.
3. Weight loss from tumor burden of 8%.

The following table is used to summarize and score the above scenario:

	Score
Normal (BCS = 3)	0
A 6 mm tumor at its longest diameter with some ulceration	4
Weight loss from tumor burden 8%	9
Total score	13

**ACTION REQUIRED:** Consult veterinary staff or consider euthanasia.

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Example #2: A nude mouse is presented with the following conditions:

1. Skin or coat in poor condition, dehydration (decreased skin elasticity), emaciated, inactive (BCS = 1).
2. A 10 mm necrotic tumor at its longest diameter.
3. Weight loss 10-15% or weight gain from tumor burden 6-9%.

The following table is used to summarize and score the above scenario:

	Score
Skin or coat in poor condition, dehydration (decreased skin elasticity), emaciated, inactive (BCS = 1)	6
A 10 mm necrotic tumor at its longest diameter	9
Weight loss 12%	12
Total score	27

**ACTION REQUIRED:** Immediate euthanasia as this animal has exceeded the max score of 18.

**NOTE:** Earlier observations along with a more aggressive course of action could have minimized this animal's pain and distress.