

Institutional Animal Care & Use Program - UTEP	
Title: Scoring & Endpoints in Tumor Studies in Rats & Mice	
Policy#: 012	Date in Effect: 11 December 2014
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In Effect <input checked="" type="checkbox"/> Rescinded <input type="checkbox"/>	Date Rescinded:

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 UTEP 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 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 and Griffiths (1985), *Veterinary Record* 116:431-43
- 2) Montgomery (1990), *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) Workman P, et al, UKCCR Guidelines for the welfare of animals in experimental neoplasia. *Cancer and Metastasis Reviews* 8: 82-88, 1989.
- 5) Wallace J, Humane endpoints in cancer research. *ILAR J.* 41: 79-84, 2000.
- 6) Montgomery, CA, Oncologic and toxicologic research: alleviation and control of pain and distress in laboratory animals. *Cancer Bulletin* 42: 230-7, 1990.
- 7) Morton DB, Griffiths PHM, Guidelines on the recognition of pain, distress, and discomfort in experimental animals and a hypothesis for assessment. *Veterinary Record* 116: 431-6, 1985.
- 8) University of Michigan Tumor Scoring Policy.
- 9) Emory Tumor Scoring Policy.

- 10) Yasui, Nobutaka, et al. Tumor growth and metastasis of human colorectal cancer cell lines in SCID mice resemble clinical metastatic behaviors. *Invasion Metastasis* 17: 259-69, 1997.
- 11) Corbett TH et al. In vivo methods for screening and preclinical testing. In: Teicher B, ed., *Anticancer Drug Development Guide*. Totowa, NJ: Humana. 2004: 99-123. Goldin A, et al. Current results of the screening program at the Division of Cancer Treatment, National Cancer Institute. *Eur J Cancer* 17: 129-42, 1981.
- 12) Goldin A, et al. Current results of the screening program at the Division of Cancer Treatment, National Cancer Institute. *Eur J Cancer* 17: 129-42, 1981.
- 13) Ullman-Culleré M and Foltz C. Body condition scoring: a rapid and accurate method for assessing health status in mice. *Lab Ani Sci* 49(3): 319-23.

D) DEFINITIONS

- 1) BCS: Body Condition Score on a scale of 1-5. See Appendix 1.
- 2) Moribund: Clinically irreversible condition leading inevitably to imminent 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 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 that 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 are an

important tool for 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 as well as other endpoints that more closely meet their scientifically justified and IACUC-approved research objectives.

F) PROCEDURES

- 1) Criteria that establish when an endpoint has been reached:
 - a) Evaluation of five (5) aspects of an animal's condition include:
 - (1) Body weight
 - (2) Physical appearance
 - (3) Measurable clinical signs
 - (4) Unprovoked behavior and response to external stimuli
 - b) Clinical observations may include:
 - (1) Changes in general appearance in skin and hair, eyes, nose, mouth and head, respiration, urine, feces, and locomotion.
 - c) Clinical signs, depending on severity and duration, that may constitute an endpoint include:
 - (1) Rapid weight loss.
 - (2) Diarrhea, if debilitating/causing significant dehydration.
 - (3) Progressive dermatitis.
 - (4) Rough hair coat, hunched posture, lethargy or persistent recumbency.
 - (5) Coughing, labored breathing, nasal discharge.
 - (6) Jaundice and/or anemia.
 - (7) Neurological signs.
 - (8) Bleeding from any orifice.
 - (9) Self-induced trauma.
 - (10) Any condition interfering with eating, drinking, defecation, micturition or ambulation.

- d) Additional signs in neoplastic studies that may constitute an endpoint include, but are not limited to:
 - (1) A tumor burden greater than 10% of the body weight.
 - (2) Visible tumors that ulcerate and/or 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 and adopted as a Standard Operating Procedure (SOP) by both the Unit for Laboratory Animal Medicine (ULAM) at the University of Michigan and Emory University’s IACUC 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; morbidity; weight loss or gain and overall appearance. ***NOTE: All animals should have body weights 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.

	Score
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

	Score
Tumor very small (≤ 5 mm length in mice, ≤ 8 mm length in rats at its longest diameter)	0
Tumor small WITHOUT necrosis/ulceration (≤ 7 mm length, ≤ 10 mm length in rats at its longest diameter)	4
Tumor intermediate in size (7-17 mm, mice; 10-26 mm, rats (longest diameter) but WITHOUT ulceration or necrosis OR small in size (≤ 7 mm length, ≤ 10 mm length in rats at its longest diameter) WITH necrosis/ulceration	9
Tumor intermediate in size with necrosis/ulceration (7-17 mm, mice; 10-26 mm, rats (longest diameter)	12
Tumor large (≥ 18 mm in mice, ≥ 27 mm in rats at its longest diameter) with or without ulceration/necrosis, or severe abdominal distension ($\geq 10\%$ increase over original body weight)	18

c) Body weight change






	Score
No weight loss or gain from pre-inoculation weight	0
BCS ≤ 2 or weight loss of 5-10% OR weight gain of 1-5% from tumor burden or ascites	9
BCS ≤ 2 with weight loss of 10-15% OR weight gain of 6-9% from tumor burden or ascites	12
Continued declining weight loss $\geq 20-24\%$ versus initial measurement for up to 2 days (tumor-bearing animals that have NOT undergone chemotherapeutic treatment) or 4 days (tumor-bearing animals that have undergone chemotherapeutic treatment). Weight loss $>25\%$ will result in immediate euthanasia. Clarification on weight losses: 1. 20-24% weight loss for up to 2 days is used to give a	18

score of 18 to determine the endpoints	
2. 25% weight loss: If an animal has lost >25% of its baseline weight, it will be immediately euthanized.	

3) Summation of scores and course of action

Score	Action
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 in a container. Examples of suitable supplementation:</p> <ol style="list-style-type: none"> 1. Water source alone such as Napa nectar, SC or IP saline or Lactated Ringer's Solution 2. Energy dense such as Bacon Softies (http://www.bio-serv.com/product/Bacon_Softies.html) 3. Energy dense/water such as a DietGel (www.clearh2o.com). <p>Separating animals with higher scores from those with lower scores should be considered.</p>
14-18	Consult veterinary staff or euthanize.
>18	Euthanize immediately.
Ulceration or impaired mobility	Cage isolation to avoid possible aggressive cage mates and allow more equal competition for resources.
>25% Wt loss	Euthanize immediately.

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

	<p style="text-align: center;">BCS 1</p> <p>Animal is emaciated</p> <ul style="list-style-type: none">• Skeletal structure extremely prominent; little or no flesh cover• Vertebrae distinctly segmented
	<p style="text-align: center;">BCS 2</p> <p>Animal is under conditioned</p> <ul style="list-style-type: none">• Segmentation of vertebral column evident• Dorsal pelvic bones are readily palpable
	<p style="text-align: center;">BCS 3</p> <p>Animal is well conditioned</p> <ul style="list-style-type: none">• Vertebrae and dorsal pelvis not prominent; palpable with slight pressure
	<p style="text-align: center;">BCS 4</p> <p>Animal is well over conditioned</p> <ul style="list-style-type: none">• Spine is a continuous column• Vertebrae palpable only with firm pressure
	<p style="text-align: center;">BCS 5</p> <p>Animal is obese</p> <ul style="list-style-type: none">• Animal is smooth and bulky• Bone structure disappears under flesh & SC fat

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 mouse is presented with the following conditions:

1. Normal (BCS = 3).
2. A 5 mm tumor at its longest diameter without ulceration.
3. Weight gain from tumor burden 8%.

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

	Score
Normal (BCS = 3)	0
A 5mm tumor at its longest diameter without ulceration	4
Weight gain from tumor burden 8%	12
Total score	16

ACTION REQUIRED: Consult veterinary staff or euthanize.

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

1. Normal (BCS = 3).
2. A 5mm tumor at its longest diameter with ulceration.
3. Weight gain from tumor burden 5%.

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

	Score
Normal (BCS = 3)	0
A 5mm tumor at its longest diameter WITH ulceration	9
Weight loss of 5%	9
Total score	18

ACTION REQUIRED: Consult veterinary staff or euthanize; this animal has reached the maximal score and must be euthanized with any additional increase in score.

Example #3: A mouse is presented with the following conditions:

1. Underconditioned (BCS = 2).
2. A 5mm tumor at its longest diameter without ulceration.

- Weight loss from tumor burden 5%.

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

	Score
Underconditioned (BCS = 2)	3
A 5mm tumor at its longest diameter without ulceration	4
Weight loss from tumor burden 5%	9
Total score	16

ACTION REQUIRED: Consult veterinary staff or euthanize; this animal is approaching the maximal score and careful monitoring must be done to ensure it does not exceed the maximal score of 18.

Example #4: A mouse is presented with the following conditions:

- Skin or coat in poor condition, dehydration (decreased skin elasticity), emaciated, inactive (BCS = 1).
- A 10 mm necrotic tumor at its longest diameter.
- 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	12
Weight loss 12%	12
Total score	30

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.