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 UTSA 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


3) Dr. Liang Xu and Dr. Marc Lippman permitted the use of their previously described tumor burden scoring system standard operating procedure.


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


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.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal (BCS = 3)</td>
</tr>
<tr>
<td>2</td>
<td>Not well groomed, less active, normal posture and gait</td>
</tr>
<tr>
<td>3</td>
<td>Skin or hair coat in poor condition (scruffy), less active, decreased signs</td>
</tr>
<tr>
<td></td>
<td>of grooming (BCS = 2)</td>
</tr>
<tr>
<td>6</td>
<td>Skin or coat in poor condition, dehydration (decreased skin elasticity),</td>
</tr>
<tr>
<td></td>
<td>emaciated, inactive (BCS = 1)</td>
</tr>
<tr>
<td>18</td>
<td>Moribund or severely cachexic</td>
</tr>
</tbody>
</table>
b. Tumor size/Degree of Necrosis

<table>
<thead>
<tr>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor very small (≤7 mm length in mice, ≤10 mm length in rats at its longest diameter)</td>
<td>0</td>
</tr>
<tr>
<td>Tumor small with some necrosis/ulceration (≤7 mm length, ≤10 mm length in rats at its longest diameter)</td>
<td>4</td>
</tr>
<tr>
<td>Tumor necrotic (7-17 mm, mice; 10-26 mm, rats (long diameter)</td>
<td>9</td>
</tr>
<tr>
<td>Tumor large (≥18 mm in mice, ≥27 mm in rats at its longest diameter), or severe abdominal distension (≥10% increase over original body weight)</td>
<td>12</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No weight loss or gain from pre-inoculation weight</td>
<td>0</td>
</tr>
<tr>
<td>Weight loss 5-10% or weight gain from tumor burden 0-5%</td>
<td>9</td>
</tr>
<tr>
<td>Weight loss 10-15% or weight gain from tumor burden 6-9%</td>
<td>12</td>
</tr>
<tr>
<td>Continued declining wt loss ≥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 treatment). Weight loss &gt;30% will result in immediate euthanasia.</td>
<td>18</td>
</tr>
</tbody>
</table>

Clarification on weight losses:
20% weight loss for up to 2 days is used to give a score of 18 to determine the endpoints
30% weight loss: If an animal has lost >30% of its baseline
weight, it will be immediately euthanized.

3) Summation of scores and course of action

<table>
<thead>
<tr>
<th>Score</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7</td>
<td>Routine daily monitoring.</td>
</tr>
</tbody>
</table>
| 8-13  | Close daily monitoring, which may include twice a day monitoring and daily weighing. May supplement on cage bottom. Examples of suitable supplementation:  
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.bioserv.com/product/Bacon_Softies.html](http://www.bioserv.com/product/Bacon_Softies.html))  
3. Energy dense/water such as a DietGel ([www.clearh2o.com](http://www.clearh2o.com)) |
| 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 moribundidity as an endpoint:**
Refer to IACP 003.
Appendix I: Body Condition Scoring (BCS) Guide (13).

BCS 1
Animal is emaciated
- Skeletal structure extremely prominent; little or no flesh cover
- Vertebrae distinctly segmented

BCS 2
Animal is under conditioned
- Segmentation of vertebral column evident
- Dorsal pelvic bones are readily palpable

BCS 3
Animal is well conditioned
- Vertebrate and dorsal pelvis not prominent; palpable with slight pressure

BCS 4
Animal is well over conditioned
- Spine is a continuous column
- Vertebrae palpable only with firm pressure

BCS 5
Animal is obese
- 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 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:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (BCS = 3)</td>
<td>0</td>
</tr>
<tr>
<td>A 6 mm tumor at its longest diameter with some ulceration</td>
<td>4</td>
</tr>
<tr>
<td>Weight loss from tumor burden 8%</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total score</strong></td>
<td><strong>13</strong></td>
</tr>
</tbody>
</table>

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

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:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin or coat in poor condition, dehydration (decreased skin elasticity), emaciated, inactive (BCS = 1)</td>
<td>6</td>
</tr>
<tr>
<td>A 10 mm necrotic tumor at its longest diameter</td>
<td>9</td>
</tr>
<tr>
<td>Weight loss 12%</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total score</strong></td>
<td><strong>27</strong></td>
</tr>
</tbody>
</table>

**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.