

## Guideline for Endpoint Criteria in Animal Study Proposals

The ACUC requires that animal experimentation protocols include clearly defined experimental and humane endpoints. Well defined endpoints ensure that animals do not experience unnecessary pain or distress in the completion of one's scientific objectives which is consistent with the US Gov't Principles for the Utilization of Animals In Research which state, "Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative."

Experimental endpoints are the time at which the experiment will end due to completion of the scientific objectives. For example: 1) in a pharmacokinetic study the experimental endpoint might be a set time(s) after administration of an experimental agent at which animals will be euthanized to collect tissues for analysis or 2) in a metastasis study the experimental endpoint might be the identification of lung mets on iVIS imaging.

Humane endpoints are the time at which the experiment will be terminated prior to defined experimental endpoints due to the health of the animal. In the examples above: 1) if the agent being tested caused unexpected toxicity the animals might be euthanized early or 2) if animals developed ulcerative tumors or severe abdominal distension before lung metastasis then the study would be terminated early. Please note that the stress and potential for distress increases as the animal reaches these criteria and a pre-moribund state. Animals should be euthanized at the earliest applicable endpoint.

Experimental endpoints are dependent on the goals of the experiment. They should be described in the ASP, justified for experimental reasons, and then adhered to during the course of the study.

Humane endpoint criteria must also be defined in the ASP. The following serves as recommended guidelines for humane endpoints. Criteria that differ from these must be justified in the ASP with a scientific rationale for the change clearly noted and referenced as needed. The general criteria and body weight criteria are applicable to all ASPs. All other categories are relevant only for those ASPs with the respective condition.

Animal Condition	Endpoint	Notes
<b>General signs</b>	<ul style="list-style-type: none"> <li>• Hunched posture, rough hair coat</li> <li>• Dehydration</li> <li>• Rapid or labored breathing, dyspnea, coughing</li> <li>• Reduced/impaired mobility affecting the ability to obtain food or water</li> <li>• Paralysis</li> <li>• Pallor or cyanosis</li> <li>• Hemorrhage</li> <li>• Diarrhea, constipation or markedly reduced food intake</li> <li>• Signs of neurological impairment</li> <li>• Impaired ability to urinate or defecate</li> <li>• Visible jaundice</li> </ul>	Animals are always monitored daily by facility staff but should be monitored at least 2-3x per week by laboratory staff as well.

<b>Body Weight</b>	<ul style="list-style-type: none"> <li>Loss of &gt;15% normal body weight from prestudy baseline<sup>1</sup></li> <li>Body Condition Score &lt;1.5<sup>b</sup></li> </ul>	Animals should be weighed at baseline and then as appropriate. At the time of weighing a body condition score should be estimated using the attached guideline.
<b>Subcutaneous Tumor Burden</b>	<ul style="list-style-type: none"> <li>Single tumor approaching 2 cm (mice) or 3 cm (rat) in any one direction</li> <li>For studies with multiple tumors, any single tumor must not exceed 1cm in any one direction and the combined dimensions should not exceed 2 cm as above</li> <li>Tumor Ulceration/Necrosis<sup>2</sup></li> <li>Tumors interfering with normal movement that impairs their ability to perform bodily functions or obtain food or water</li> <li>Tumors interfering with normal bodily functions such as urination, defecation, breathing, chewing, or swallowing</li> </ul>	Observations by laboratory staff should occur at least weekly until tumors reach 1 cm and then more frequently as needed such that no tumor exceeds 2cm in any one direction unless otherwise justified in ASP
<b>Abdominal Tumor burden</b>	<ul style="list-style-type: none"> <li>Significant abdominal enlargement that interferes with any bodily function or movement such that the ability to obtain food or water is impaired</li> <li>Measurable tumors approaching 2 cm (mice) or 3 cm (rat) in any one direction</li> <li>Any tumor that interferes with normal physiologic function independently of observed abdominal distension</li> </ul>	Observations by laboratory staff should occur 2-3x/week and measurements made as described for subcutaneous tumors above.
<b>Intrathoracic Tumor Burden</b>	<ul style="list-style-type: none"> <li>Moderate dyspnea from diaphragm compression method [index finger over distal sternum (7<sup>th</sup> sternebra) to apply pressure over diaphragm to stop diaphragm respiration forcing inter-coastal respiration].<sup>a</sup></li> </ul>	This test should be performed weekly or more often as needed
<b>Intracranial tumor burden</b>	<ul style="list-style-type: none"> <li>Circling – if animal has weight loss or decreased ability to obtain feed &amp; water</li> <li>Convulsions</li> <li>Limited response to stimuli</li> </ul>	
<b>Intrabone tumor burden</b>	<ul style="list-style-type: none"> <li>Tumors interfering with normal movement that impairs their ability to perform bodily functions or obtain food or water</li> <li>Apparent discomfort or pain</li> <li>Fracture</li> </ul>	
<b>Ascitic Burden</b>	<ul style="list-style-type: none"> <li>Circulatory shock as evidenced by pale eyes, ears, and/or muzzle</li> <li>Dyspnea</li> <li>Significant abdominal enlargement that interferes with any bodily function or</li> </ul>	Animals should be monitored at least once daily 7 days/week. ARAC guidelines must be followed (insert reference). Animals can be tapped to relieve ascitic

	movement such that the ability to obtain food or water is impaired and that cannot be relieved by abdominal tapping as noted in the notes section.	pressure up to 3 times.
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<sup>1</sup> frequency of body weights must be included in the ASP if endpoint includes a change in normal body weight

<sup>2</sup> justification and use of the tumor ulcerated checklist is required if ulcerated tumors are expected

#### References:

- a. [Mendoza, A. et al. A novel noninvasive method for evaluating experimental lung metastasis in mice. J Am Assoc Lab Anim Sci. 2013 Sep; 52\(5\):584-9](#)
- b. Ullman-Cullere, M.H. and Foltz, C.J. Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice. Lab. Animal Science; 49 (3) 319-323, 1999