GUIDELINES FOR THE USE OF RODENTS IN EXPERIMENTAL NEOPLASIA

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Background

Tumor development in rodents evokes a range of effects that depend on the experiment, tumor line, and the response of the individual animal. This document provides guidelines for designing and conducting procedures that will accomplish the experimental objectives while ensuring the welfare of animals used in tumor development studies and of animals with spontaneous tumors.

Guidelines

- Experimental neoplasia procedures and associated details (e.g., injection site location, tumor cell source, monitoring, and endpoints) must be described and justified in the Animal Use Protocol (AUP) and approved by the Animal Care and Use Committee (ACUC).
- The Office of Laboratory Animal Care (OLAC) and the Office of Environment, Health and Safety (EH&S) must be consulted when developing protocols involving inoculation with cells as all implants may harbor viruses or transmissible agents. Both primary and cultured human cell lines may harbor human pathogens that may proliferate in permissive or immunodeficient rodents. Appropriate testing of cell lines and biohazard containment procedures are required for rodents when implanted with human cells. Tumors from rodent cell lines or any cell lines that have been passaged through rodents must be tested prior to use *in vivo* to avoid inadvertent introduction of rodent pathogens into vivaria. OLAC must be contacted prior to use of all human or rodent-derived biological materials in live rodents. Please refer to the Animal Care and Use Committee (ACUC)'s Testing Biologicals Used in Laboratory Rodents Policy.
- Tumor implantation sites should be chosen to minimize interference with normal body functions. Subcutaneous implantation is considered the least disruptive with the flank as the preferred location. All tumor injection sites must be justified and approved in the AUP.
- Per the *Guide for the Care and Use of Laboratory Animals* (*Guide*), criteria for experimental and humane endpoints (i.e., endpoints of experimental neoplasia) must be determined and justified in the AUP. These criteria must include:

  - Ulcerated or necrotic tumors: any animal with an ulcerated or visibly necrotic tumor must be euthanized as soon as the ulcer is identified
  - Tumors that interfere with normal activity (e.g., ambulation, urination, defection, or ability to reach food/water), regardless of size
  - Weight loss greater than 10% of baseline weight, or a body condition score of two or less on a scale of one to five(see Appendix A and B)
  - Clinical signs of illness such as hunched posture, respiratory difficulties, or reticence to move
  - Tumors exceeding 1.5 cm (mice) or 2.5 cm (rats), unless justified in the AUP
  - Multiple tumors – The diameters of all tumors totaled must not exceed 1.5 cm (mice) or 2.5 cm (rats). These should be considered maximum size; earlier size end-points should be used if possible.
- These guidelines apply to experimental tumors and cell lines as well as tumors which arise spontaneously. The development of spontaneous tumors is considered a humane endpoint unless otherwise specifically approved in the AUP.
- Laboratory staff must label all cages that contain rodents undergoing experimental neoplasia with:
  - The date and site of injection
  - The cell line identity
  - Monitoring dates and corresponding tumor measurements
  - The name and phone number of the individual primarily responsible for monitoring the animals
  - See Appendix C for examples of Monitoring Card and log
- Animals must be observed with sufficient frequency to ensure that they are euthanized according to established endpoints. Once tumor masses begin to develop, a monitoring card must be placed, and animals must be inspected daily by the lab. All animals should be inspected at a frequency sufficient to identify endpoints prior to OLAC staff (i.e., rapidly growing tumors may require greater than once daily monitoring). Measurements must occur daily once tumors are evident.

References

- Hickman DL, Swan M. Use of a Body Condition Score Technique to Assess Health Status in a Rat Model of Polycystic Kidney Disease. Journal of the American Association for Laboratory Animal Science : JAALAS. 2010;49(2):155-159.

- Ullman-Culleré MH, Foltz CJ. Body condition scoring: a rapid and accurate method for assessing health status in mice. Lab Animal Science. 1999: 49(3): 319-23.

- Institute of Laboratory Animal Research (ILAR). National Research Council (2011). *Guide for the Care and Use of Laboratory Animals* (8th edition). Washington, D.C.: The National Academies Press.

- UC Berkeley ACUC Guidelines for Humane Endpoints in Animal Studies

- UC Berkeley ACUC Policy on Testing Biologicals Used in Laboratory Rodents

Appendix A
Mouse Body Condition Scoring

BC 1

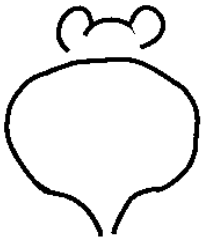
Mouse is emaciated.
- ***Skeletal structure extremely prominent; little or no flesh cover.***
- ***Vertebrae distinctly segmented.***

BC 2

Mouse is underconditioned.
- ***Segmentation of vertebral column evident.***
- ***Dorsal pelvic bones are readily palpable.***

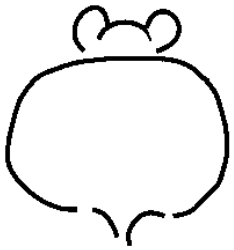
BC 3

Mouse is well-conditioned.
- ***Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.***



BC 4

Mouse is overconditioned.
- ***Spine is a continuous column.***
- ***Vertebrae palpable only with firm pressure.***



BC 5

Mouse is obese.
- ***Mouse is smooth and bulky.***
- ***Bone structure disappears under flesh and subcutaneous fat.***

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

Appendix B
Rat Body Condition Scoring

BC 1
Rat is emaciated
- Segmentation of vertebral column prominent if not visible.
- Little or no flesh cover over dorsal pelvis. Pins prominent if not visible.
- Segmentation of caudal vertebrae prominent.

BC 2
Rat is under conditioned
- Segmentation of vertebral column prominent.
- Thin flesh cover over dorsal pelvis, little subcutaneous fat. Pins easily palpable.
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure.

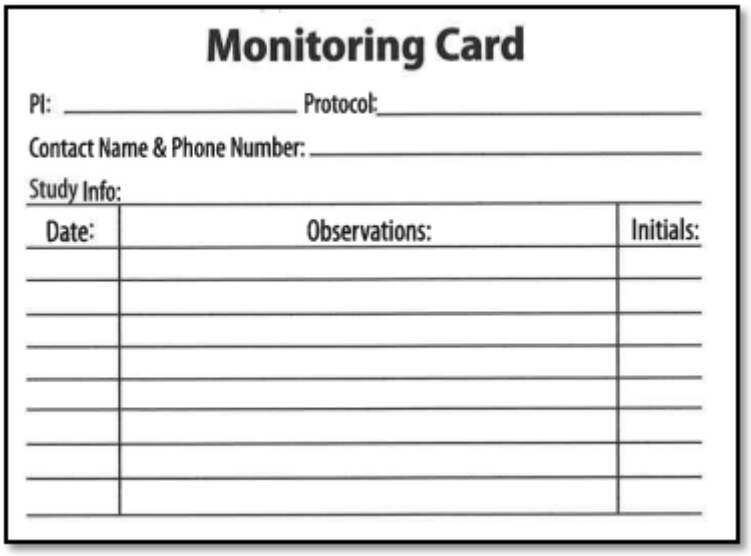
BC 3
Rat is well-conditioned
- Segmentation of vertebral column easily palpable.
- Moderate subcutaneous fat store over pelvis. Pins easily palpable with slight pressure.
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.

BC 4
Rat is overconditioned
- Segmentation of vertebral column palpable with slight pressure.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.

BC 5
Rat is obese
- Segmentation of vertebral column palpable with firm pressure; may be a continuous column.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis not palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.

Appendix C

Monitoring Cage Card Example



Monitoring Card

PI: _____ Protocol: _____

Contact Name & Phone Number: _____

Study Info: _____

Date:	Observations:	Initials:

Appendix D
Tumor Monitoring Log Example

MONTHLY TUMOR MONITORING LOG

Investigator: _____ Campus Phone: _____ Emergency Phone: _____
A.U.P. #: _____
Responsible Personnel: _____ Campus Phone: _____ Emergency Phone: _____
Additional Contact: _____ Campus Phone: _____ Emergency Phone: _____
Additional Contact: _____ Campus Phone: _____ Emergency Phone: _____

Facility: _____ Room: _____ Procedure Info: _____
Rack: _____

Starting Date: _____ Ending Date: _____

All cages/animals must be initialed every 24-hours including weekends and holidays once tumors are evident.

Month: _____ **Year:** _____ **Animal/Cage #:**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**

Responsible person must **initial each date for each cage/animal checked**.