GUIDELINE FOR HUMANE INTERVENTION POINTS IN RODENT CANCER MODELS

1. PURPOSE

The purpose of this document is to guide scientists performing cancer experiments involving rodents at LAF, HKUST in the humane endpoint of animals.

2. SCOPE

Laboratory Animal Facility (LAF's) staff, Principal Investigator (PI) and Users

3. INTRODUCTION

3.1. The Guide for the Care and Use of Laboratory Animals (the Guide, NRC 2011) states that tumour models require special consideration of humane endpoints (p. 27).

3.2. Humane endpoints and interventions on all experimental animals are definite limits placed on the level of pain and distress they should be allowed to experience. These endpoints are defined with compassion and are based on extensive data derived from research studies and observations.

3.3. The adverse effects on research animals for studies of tumour growth or therapy are usually greater and a specific set of scientific assays and humane endpoints and intervention should be employed.

4. DOCUMENTATION

For all in vivo cancer research, the Animal Experiment Protocol (AEP) should contain:

4.1. Justification of animal numbers based on clear experimental design and a detailed statistical analysis;

4.2. Information on expected tumour kinetic, growth characteristics, biology in proposed model (point 6.2). A pilot study should be included if these factors are unknown;

4.3. Clear experimental endpoints;

4.4. Clear clinical intervention points to minimize pain and distress to the animal (analgesia, anaesthesia). Clear statement of when animals reach clinical intervention points, they should be euthanised humanely unless special justification can be provided.

5. GENERAL GUIDELINES

5.1. TUMOUR TYPES

(a) Subcutaneous Tumours (visually monitored)

Intervention or endpoint should be decided based on the following:

(i) Total tumour burden (point 6.3)

(ii) Body condition

(iii) Interference with locomotion, eating, drinking

(iv) Ulceration (point 6.4)

(b) Internal or Orthotopic Tumours (cannot be visually monitored)

Humane euthanasia should be carried when one or more of the following is observed:

(i) Total tumour burden approaches 5-10% of animal's pre-experimental body weigh

(ii) Lethargy

(iii) Unable to eat, drink or ambulate

(iv) Respiratory distress or abnormal respiratory pattern

(v) Body condition score (BCS) <2/5

5.2. TUMOUR LOAD

Detail description of the expected multicity of tumour growths should be included in the AEP.

5.3. TUMOUR BURDEN

(a) Burden should be limited to the minimal requirement for valid scientific outcome.

(b) Tumour Burden is calculated by adding the volume of each individual tumour and should not exceed 10% of the animal's baseline body weight (original body weight before tumour growth). Total tumour burden should not exceed 6.0cm^3 [1]on the adult mouse. (Reference 7.4, 7.5, 7.7).

[1] 1 cubic centimetre (cm^3) = 1 gram (g)

5.4. METASTASIS

(a) Metastatic models should be clearly described in the AEP.

(b) Consider resecting primary tumours when possible.

(c) Exceptional intervention points for certain experimental animals with tumours (e.g. mammary) that will not develop metastasis should be clearly detailed in the AEP.

(d) Pulmonary metastasis

Clinical presentation:

(i) Can be vague and may include rough coat, hunched posture, anorexia, dehydration, decreased activity, decreased grooming, dyspnea.

(ii) Consider using the Pulmonary Assessment of Advanced Metastasis (PAAM)[2] technique to assess respiratory distress for presence or progression of pulmonary metastatic disease.

5.5. MONITORING:

Monitoring for pain and distress of all animals is the responsibility of the PI and users.

5.6. Scientists should keep detailed monitoring records from when the tumour is palpable (subcutaneous tumours), or when adverse clinical signs likely attributed to the tumour are observed (internal tumours), until euthanasia.

(a) Records should include:

(i) Animal identification

(ii) Tumour measurement

(iii) Monitoring frequency

(iv) General observations (activity level, appetite, mobility, physical appearance)

(b) Records should be attached to the cage.

5.7. The frequency of monitoring should increase during critical phases based on tumour volume and/or burden, and tumour ulceration score (Refer to Annex 2) and when

[2] Restrain mouse using thumb and forefinger of non-dominant hand. Using forefinger of dominant hand, apply gentle to moderate digital pressure just caudal to the xiphoid sternum for 3 seconds. In normal mice, no response or a mild increase in respiratory rate is observed. In mice with advanced pulmonary metastasis, a pronounced increase in chest excursion during respiration, or agonal breathing, is observed. Mice typically develop advanced clinical signs within 1 or 2 days of a positive PAAM assessment.

the humane intervention points are near. Employing a colour-coded system can help identify monitoring frequency.

(a) Yellow: Weekly monitor, no tumour measurement needed - mice with small palpable tumours or with a low tumour burden, ulceration score 0-1

(b) Green: Twice a week monitor with weekly tumour measurements - mice have reached approximately 50% of tumour volume or tumour burden endpoint, with an ulceration score of 2-3.

(c) Red: Daily monitoring with twice-weekly tumour measurements - mice are approaching endpoint, with an ulceration score >3

6. CLINICAL INTERVENTION POINTS

6.1. Refer to Annex 1 for measurable observations and assessment.

6.2. No individual tumour should exceed 2.0cm in any one dimension on an adult mouse, and should not excess 4.0cm in any one dimension on an adult rat.

(a) Tumour volume

(i) 2ml in mice

(ii) 5ml in rats

(iii) $4/3\ \pi\ \mathrm{x}\ [\frac{\mathrm{LxWxH}}{2}]$

6.3. Tumour burden should not exceed 10% of initial body weight.

(a) 10% BL BW mice

(b) >5% BL BW rats

(c) % = cumulative tumour weight/ BLBW x 100

6.4. Tumour ulceration/necrosis (Annex 2)

(a) Presence of ulceration of the tumour generally warrants humane euthanasia.

(b) Special circumstances might be present for maintaining animals past these clinical intervention points based on their ulceration scoring (0-6). Refer to Annex 2 for detailed tumour ulceration scoring.

(i) Low scores (0-2) may require no treatment.

(ii) Scores of 3-4 should require treatment to alleviate pain and discomfort if the animal is not sacrificed at this time.

(iii) Scores 5 relates to any tumour that has ulcerated with a large lesion and significant necrosis and bleeding are observed. Euthanasia should be carried out immediately in these scenarios.

6.5. Animals with tumours that interfere with normal functionality leading to difficulty in prehension or ambulation should be immediately humanely euthanised.

6.6. AEPs utilizing death or moribundity as an endpoint should contain strong scientific justification and approval from the AEC.

7. REFERENCES

7.1. Morton DB, Griffiths PH. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Vet Rec. 1985 Apr 20;116(16):431-6.

7.2. Montgomery CA. (1990) Oncological and toxicological research: Alleviation and control of pain and distress in laboratory animals. Cancer Bulletin. 42:230-237.

7.3. Mendoza A, Gharpure R, Dennis J, Webster JD, Smedley J, Khanna C. A Novel Noninvasive Method for Evaluating Experimental Lung Metastasis in Mice. Journal of the American Association for Laboratory Animal Science : JAALAS. 2013;52(5):584-589.

7.4. Workman P, et al. UKCCR Guidelines for the welfare of animals in experimental neoplasia. Cancer and Metastasis Reviews 8: 82-88, 1989.

7.5. Workman P, Aboagye EO, Balkwill F, et al. Guidelines for the welfare and use of animals in cancer research. British Journal of Cancer. 2010;102(11):1555-1577. doi:10.1038/sj.bjc.6605642.

7.6. Florida International University IACU, Division of Research, SOP – Death as an Endpoint

7.7. Wallace J, Humane endpoints in cancer research. ILAR Journal, Volume 41, Issue 2, 1 January 2000, Pages 87–93, https://doi.org/10.1093/ilar.41.2.87.

Annex 1: Clinical Measurable Observations and Assessment

Parameter		Endpoint assessment
General Appearance	Body condition	Dehydration, decreased body weight, missing anatomy, abnormal posture, hypothermia, swelling, tissue masses
	Eyes	Exophthalmos, microphthalmia, ptosis, reddened eye, lacrimation, discharge, opacity
	Nose, Mouth, and Head	Head tilted, nasal discharge, malocclusion, salivation
Organ impairment	Integument	Skin and fur - Discoloration, urine stain, pallor, redness, cyainosis, icterus, wound, sore, abscess, ulcer, alopecia, ruffled fur
	Alimentary	Rectal prolapse, distend abdomen (ascites, pregnancy, tumour, ileus), Faecal discoloration, blood in the feces, softness/diarrhea
	Neurological	Circling, blindness, dementia, convulsion, loss of consciousness, tremors, ataxia, hyperactivity
	Respiratory	Dyspnea, tachypnea, apnea
	Urogenital	Discoloration of urine, blood in urine, polyuria, anuria, paraphimosis
	Musculoskeletal	Immobility, fracture, atrophy
Body weight	Weight loss over 20% of initial BW	
Tumour Characteristics	Appearance	Ulceration Necrosis Infection - Scabbing, ulceration, exudates, colour (deep red, purple, blue or black), heat, pain upon palpation. Animals should be individually caged and monitored for carnivorism or excessive chewing.
	Functionality	Difficulty with prehension, to drink, to groom or to ambulate
	Invasiveness	Local: Difficulty with prehension, to drink, to groom, or to ambulate. Pain upon palpation. Distant: Specific organ failure assessed by physical examination and clinical symptoms

	Volume	Mice: 2000 mm3 (2.0ml) Rats: 5000 mm3 (5.0ml)
	Burden	Mice: 10% baseline BW Rat: over 5% baseline BW

Annex 2: Tumour Ulcerations and Scoring

Lesion characteristics	**Score**
No lesion	0
Erythmia, hyperaemia locally, intact skin	1
Superficial skin abraisions	2
Small skin ulceration (<3mm) without necrosis	3
Small skin ulceration with necrosis	4
Large skin ulceration (>3mm) with/without necrosis	5