AEEC Guidelines

Guidelines for Procedures Involving Tumour Induction in Rodents

Objective	To provide parameters to confine pain and suffering associated with tumour growth in rodents
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Introduction

It is important to consider the effect of tumour on the animal when planning tumour induction studies. Tumour burden can cause excessive pain and distress to animals that compromise animal welfare and data integrity.

All procedures involving tumour production must be approved by the AEEC prior to their implementation and descriptions must include injection site, volume of injection, and frequency of monitoring and humane endpoints. Death as an endpoint for any animal is not accepted by the AEEC unless scientifically justified.

Guidelines

Tumour Implantation or Production

Preparation (including reconstitution, weighing, and diluting) and administration of cytotoxic drugs or chemicals should be carried out in a chemical fume cupboard or Class 2B biosafety cabinet. Double gloves should be worn and the work has to be done over absorbent pads.

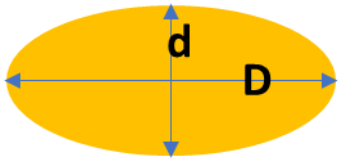
Tumour implantation sites should be chosen to minimize adjacent tissue damage or disruption to normal physiology. It is recommended that tumour(s) should be placed into a site(s) that do not interfere with normal body functions such as ambulation, eating, defecation and urination etc. In general, the **back or flank** are considered most appropriate. If other sites are to be used, the PI must describe and justify in the AEEC application.

Anesthesia is recommended for all tumour implantations and mandatory for intradermal.

For De Novo and metastatic tumour models, PI should evaluate the potential adverse effects from literature and previous data, and propose method to control severity (e.g. analgesia, anesthetic). **Humane endpoints** should be defined in the AEEC application.

1. Tumour size
   a. Calipers (recommend electronic) must be used to measure tumour size to avoid discrepancies.
   b. Single tumour must not exceed 20 mm (2.0 cm) at the largest diameter in an adult mouse and 30 mm (3.0 cm) in adult rats. Tumour volume must not exceed 2000mm^3

c. Multiple tumours may be allowed to grow up to 10% tumour burden of the body weight (measured at the day of injection). The burden is additive and the limitation on any single tumour still applies (2000mm^3).

	**Mass (mg) = Tumour volume (mm^3) = d^{2}x D/2 where d and D are the shortest and longest diameter in mm, respectively** For example: D= 25mm; d = 15mm 15^2 x 25/2 = 2812mm^3 or 2.8g Exceed endpoint of 2000mm^3

d. If possible, one observer should perform all tumour measurements in each study to minimize variability.

	Using a slide caliper to measure a subcutaneous tumour on a nude mouse

2. Monitoring

Clinical observations will be needed for monitoring animal deterioration from tumour progression. Other examination techniques may be required for specific sites (e.g. respiratory rate for lung cancer, neurological disturbance for brain neoplasms, and blood cell counts for leukemias).

Schedule:

a. **Once weekly** as a minimum until a palpable tumour nodule is present (5-7.5mm in diameter),
b. At least **three times weekly** (including weekends and holidays) once tumour is palpable/visible.
c. If tumour growth reached 1000mm^3 in the days before termination, **daily or twice daily** monitoring may be necessary.

If tumours are in a location(s) not palpable or visible, a monitoring schedule should be established based on pilot studies.

Pilot studies can be used to familiarize the animal researcher to possible adverse effects and to define the critical time scale of adverse effects. Consider tumour site, growth rate, invasion, distension, ulceration, metastasis, and production of cachectic factors.

Clinical signs:

- Grimace score
- Dull or closing eyes
- Decreased food/water intake
- Dehydration
- Weight loss/condition score
- Vocalizations
- Respiratory difficulty
- Rough hair coat
- Hunched posture
- Skin pathology
- Restricted mobility
- Changes in feces/urine and/or perianal soiling
- Aggression
- Eye/nose discharge

3. Humane Endpoints:

Animal welfare takes priority over tumour measurements in decisions regarding euthanasia or other interventions. If tumours cannot be measured externally, animals must be monitored closely for any physiological or neurological signs and be euthanized as such signs become apparent. Animals displaying such signs **must be euthanized even if the maximum tumour size/burden has not been reached**.

The following clinical signs are indications of **morbidity**. Tumour-bearing animals exhibiting these signs **must be euthanized** unless otherwise stipulated in the AEEC:

- Exceed maximum tumour size, tumour volume
- Ulcerated/necrotic tumours
- Weight loss >15% from the start of the experiment

- Body condition score (Appendix 1)
- Lethargy, inappetence
- Bloodstained or mucopurulent discharge from any orifice
- Labored respiration
- May occur when tumour is interfering with function of vital organs
- Significant abdominal distension
- Mobility restriction, inability to access food and water

Relevant Information

These guidelines have been developed after discussion between the AEEC, the Animal Welfare Officer and Post-Administrative Monitoring (PAM) team members.

It is the responsibility of the investigator to assure that all individuals performing **procedures involving tumour production** are adequately trained to do so.

Training: LASEC and/or the Animal Welfare Officer are available to provide training as needed and would be delivered as a 'Surgery' module. Ancillary modules available include "Anesthesia', 'Aseptic Techniques 'and 'Tumour Monitoring'.

References

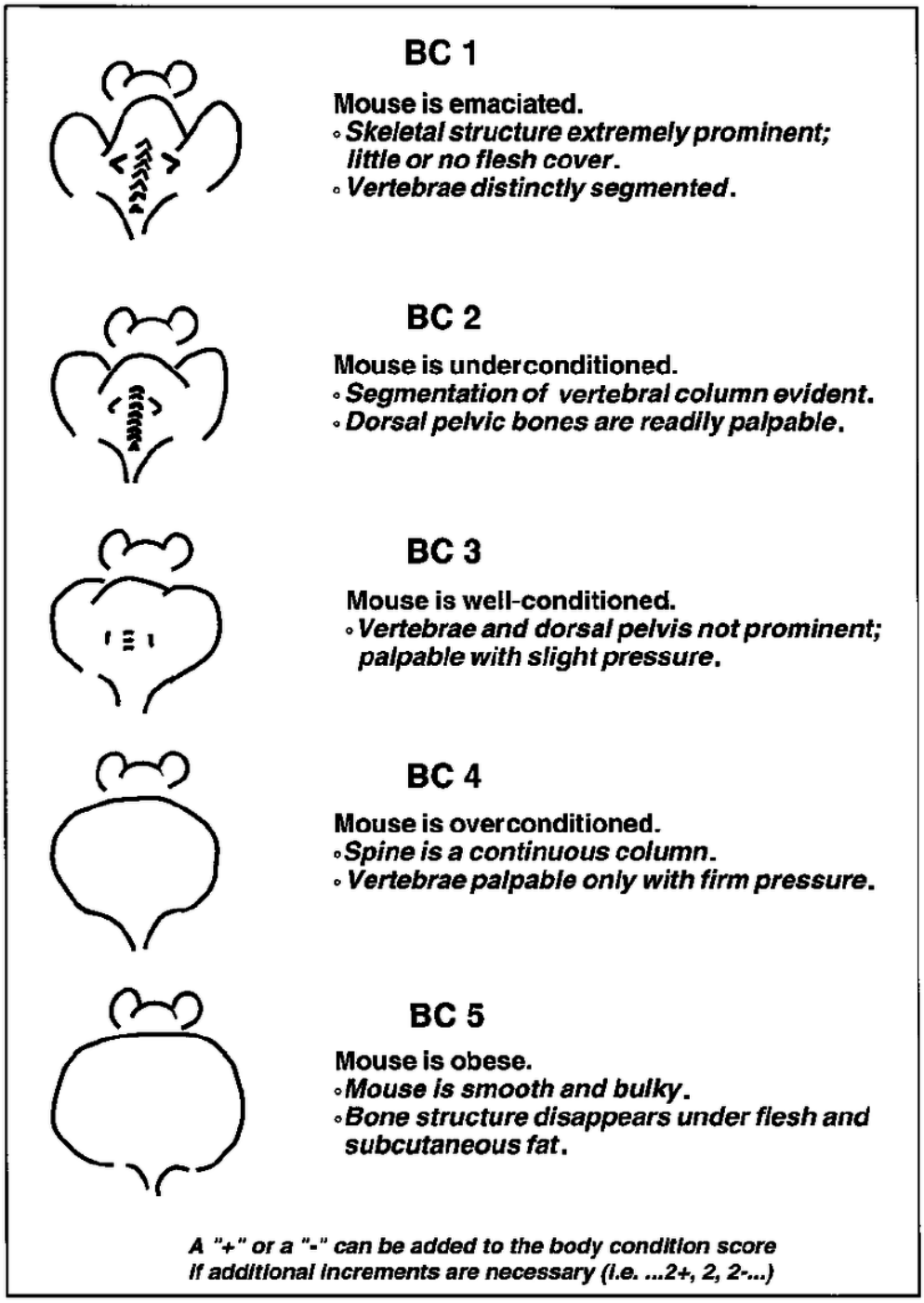
Euhus, et al. (1986) Tumour measurement in the nude mouse. Journal of Surgical Oncology 31:229-234

PHS (Public Health Service) (2002) Public Health Service policy on humane care and use of laboratory animals.

Workman et al. (2010) Guidelines for the welfare and use of animals in cancer research. British Journal of Cancer 102:1555-1577

Wallace J. (2000) Humane endpoints and cancer research. ILAR Journal 41:87-93.

Appendix 1. Body Condition Score



Ullman-Cullere et al. (1999) Body Condition Scoring: A rapid and accurate method for assessing health status in mice. Laboratory Animal Science 49(3):319-23