

RODENTS WITH NEOPLASIA

1 Policy statement

Neoplasia is the process of abnormal and uncontrolled cell growth. These cells grow to form masses, also known as neoplasms or tumours. Some tumours are benign, meaning they do not spread to other parts of the body. Others are malignant or cancerous, which refer to their potential to invade locally and/or spread to distal parts of the body. The latter process is referred to as metastasis. Rodent tumours can be experimentally induced; rodents can also be genetically modified to develop a variety of neoplasms. Spontaneous and unexpected tumours can also arise in any animal, and the incidence of this increases with age.

Adequate veterinary medical care is an essential component of the NUS animal care and use programme. This encompasses the oversight of the development of experimental tumour models, the monitoring and care of animals with tumours, and the recommendations of humane endpoints.

2 Definitions

Animal	All live fish, amphibians, reptiles, birds, non-human mammals and cephalopods.
Institutional Animal Care and Use Committee (IACUC)	The Committee, constituted by an institution, responsible for the oversight and evaluation of all aspects of the institution's animal care and use programme.
Transplantable tumours	Tumour cells or tissues that can be harvested from a donor animal and implanted into a recipient animal.
Protocol	A written description of a project put forward by a PI for review by the IACUC.
Engraftment	Successful establishment of transplanted tumor cells within a new host animal.
Hybridoma	A hybrid cell produced by the fusion of an antibody-producing lymphocyte with a tumor cell and used to culture continuously a specific monoclonal antibody.

3 Scope

This policy applies to all animals covered under NUS Animal Care and Use Programme.

4 Guidelines

4.1 Experimental Tumour Models

- 4.1.1 All rodent tumour lines and/or non-rodent tumour lines that have been passaged in rodents must be tested and certified free of adventitious rodent pathogens. Please refer to [CM SOP 605 Biological Material Screening for Rodent Pathogens](#) for further information.

- 4.1.2 When human tissues or cells are used, it is necessary to [assess whether Institutional Review Board \(IRB\) review is required](#). If IRB oversight applies, [an online application must be submitted](#) and approved by IRB before the study begins. A full IRB review is mandatory if the animal research falls within the scope of [restricted human biomedical research](#) (rHBR) as defined under the [Human Biomedical Research Act \(HBRA\)](#).
- 4.1.3 It is also recommended that all cell lines are checked for their provenance and genetic identity prior to use, and characterisation of the tumours be performed periodically to ensure the desired properties are maintained.
- 4.1.4 Transplantable tumours may be induced orthotopically (in the tissue or site of origin), or ectopically (in the subcutaneous spaces e.g. dorsum or flank, or intravenous inoculation). Keep in mind that changing the inoculation site may affect the characteristics of the tumours. Knowledge of the origin, incidence and tumour development pattern is necessary.
- 4.1.5 Pilot tumour growth studies using small numbers of animals (5-10) are strongly recommended to establish growth patterns and ensure the model is reproducible. This will also identify any adverse effects associated with tumour progression and enable humane endpoints to be defined.
- 4.1.6 It must be assumed, unless there is contrary evidence, that the attendant pain and distress of the human or animal disease will also occur in the animal. The PI must therefore take special care to ensure that any pain or distress is minimised and the IACUC is informed of the potential effects of the disease on the animals.
- 4.1.7 The site for induction of tumours should be chosen carefully. Subcutaneous, intradermal and flank sites should be chosen wherever possible. In general, sites around the face and those involving the special senses, the limbs (including the footpad), perineum and abdomen should be avoided as tumours in these locations can significantly affect the welfare of the animal.
- 4.1.8 Prior to the use of footpad, brain, eye sites, perineum and abdomen, specific scientific justification should be provided as to the lack of a comparable alternative. IACUC approval must be obtained prior to initiating such studies. Earlier endpoints may also be required.
- 4.1.9 To promote engraftment of some tumour lines, some animals may need to undergo irradiation or be provided immunosuppressive agents. These animals may require additional care - plans for monitoring and care must be addressed in the relevant IACUC protocol and discussions can be held with the CM veterinarians and staff as necessary.
- 4.1.10 Remember that genetically modified animals can be more prone to developing tumours in both expected and unexpected locations and spontaneous tumours can arise in any animal.

4.2 Monitoring and Care of Rodents with Tumours

- 4.2.1 Animals must be evaluated for clinical signs specific to the type of neoplasia as well as for their overall health and wellbeing. Particular attention should be paid to the monitoring of tumour growth and the impact on the welfare of the individual animal.
- 4.2.2 In genetically modified animals, special care is needed to ensure the detection of unexpected sites of tumour development.

- 4.2.3 Effective monitoring systems and endpoints should include limits on tumour size and severity of tumour-associated disease – for example, interference with locomotion or feeding/drinking or an ulcerated tumour.
- 4.2.4 Regular examination and assessment of all these rodents is warranted. The frequency and parameter(s) of monitoring must be specific to the project, and addressed and approved in the IACUC protocol.
- 4.2.5 Depending on the type of tumour, some recommended monitoring parameters are listed below:
- For solid tumours developing in superficial tissues, measurements can be taken to track progression. The most common methods include taking the diameter of the tumour (determined by the longest length obtainable) or calculating the tumour volume (mm^3) using the formula for prolate ellipsoids:
$$\text{tumour volume} = (\text{length} \times \text{width}^2) / 2$$
 - where length is the longest measurement taken and the width perpendicular to the length. Tumour weight and tumour volume are considered equivalent based on a presumed tissue density of 1 i.e. $1 \text{ mm}^3 = 1 \text{ mg}$.
 - Internal tumours can be evaluated by palpation, biomarkers and diagnostic imaging techniques such as bioluminescence, fluorescence, ultrasonography, magnetic resonance imaging (MRI), micro-CT, micro-PET and micro-SPECT.
 - Cancers of the blood and haemopoietic and lymphatic systems can be identified through the presence of circulating cancer cells or changes in the cellular constituents of the blood.
 - Ascitic tumours, or tumours known to cause fluid accumulation in the peritoneal cavity, are best monitored by performing a clinical assessment of the animal's body condition, appearance and behaviour. With ascitic tumours, including hybridomas, the volume of ascitic fluid should not cause gross abdominal distension.
 - In addition to imaging and clinical examination, pulmonary metastatic burden can be evaluated using the pulmonary assessment of advanced metastasis (PAAM) technique, where an animal is manually restrained and gentle digital pressure is applied just caudal to the xyphoid sternum for 3 seconds. This will result in a pronounced increase in respiratory depth or open-mouth breathing for animals with advanced pulmonary metastasis, and no or mild increase in respiratory rate for normal rodents or rodents without advanced pulmonary metastasis.
 - Bearing in mind that tumour mass and ascitic fluid can significantly affect overall body weight, body condition scoring (BCS) is recommended as a more accurate method for assessing the animal's health status and should be monitored closely. Please refer to [CM SOP 407 Humane Intervention Points](#) for more details on BCS.

4.3 Humane Endpoints for Rodents with Tumours

- 4.3.1 For all animals expected to develop neoplasia, humane endpoints must also be clearly stated in the protocol. These can be defined by tumour characteristics and/or by taking into account the clinical condition of the animal.
- 4.3.2 Maximum allowable sizes and volumes for tumours must also be determined and approved by the IACUC prior to the commencement of the study. Remember that these endpoints should be subjected to continuous adjustment and review. Any deviations and exemptions must be approved by the IACUC.

4.3.3 A scoring system may be useful in reducing inter-observer variability and standardizing endpoints.

Some recommended endpoints based on tumour burden are:

1. Measurements

- The maximum allowable size for solid tumours is 15 mm in mice and 25 mm in rats. For intramuscular tumours in mice, the maximum size is 5 mm.
- The maximum allowable volume for solid tumours is 2000 mm³ in mice and 4000 mm³ in rats. Alternatively, solid tumours must not be more than 10% of the host's body weight.
- For animals with multiple tumours, the total volume of the tumours cannot exceed 150% of a single tumour e.g. 3000 mm³ in mice, 6000 mm³ in rats. Note that the limitation for any single tumour will still be valid.

2. Characteristics and location

- An ulcerated tumour may appear as an open, moist lesion or as a scab, which is indicative of a break in the underlying epithelium. Once ulceration occurs, the growth pattern of the tumour is altered which may interfere with experimental goals. Ulceration and necrotic tissue can also result in a continuous loss of body fluid and/or infection, both of which may have serious implications on the animal's overall condition and the experimental goals. Euthanasia is recommended for animals with such tumours, unless a strong scientific justification is provided and approved by IACUC to support continued monitoring or treatment.
- Some sites cannot bear the maximum allowable size as the tumour may impede the normal function and activity of the animal such as ambulation, eating and/or drinking e.g. the cranium, the thoracic cavity, behind the eye, on the limbs. Similarly, tumours that metastasise to sensitive sites need to be paid close attention. Euthanasia is recommended for animals with tumours that interfere with locomotion or cause abnormal behaviour and/or body function.

3. Clinical condition

- For animals with tumours that are not easily measurable, the clinical condition of the animal is critical for the determination of humane endpoints. These include assessing the animal for general signs of ill health, pain and distress as well as clinical signs specific to the tumour type and site. Please refer to CM SOP 407 Humane Intervention Points for details on general humane endpoints.

4. Experimental endpoints

- In tumour therapy experiments, the endpoints chosen should be as early as possible and be compatible with reliable assessment of the therapy. Death from the tumour should not be chosen as an experimental end-point.

5 References

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6 Related documents/SOPs

1. [IACUC Policy P108 Incidents Reportable to IACUC](#)
2. [IACUC Policy P209 Policy on Use of Death as an Endpoint in Animal Experiments](#)
3. [IACUC Policy P103 Notification to Principal Investigators \(PIs\) with Sick and Injured Animals](#)
4. [CM SOP 605 Biological Material Screening for Rodent Pathogens](#)
5. [CM SOP 407 Humane Intervention Points](#)
6. [CM SOP 403 Recommended Volume for Administered Substances](#)
7. [CM SOP 410 Post-Irradiation Monitoring and Care for Mice](#)
8. [CM SOP 507 Animal Health Reporting](#)
9. [CM 612 Attending Clinical Cases and Follow-up](#)

Revision	Author	IACUC approval date
P216.01 Last reviewed	Dr Foo Tet Wei, John	20 Jun 2016
P216.02. Reviewed to:	Dr Chew Li Li	3 January 2023
<ol style="list-style-type: none"> 1. ensure policies are in compliance with the NACLAR Guidelines 2022 and the updated Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004; and 2. up-to-date with current practices Changes made to: <ol style="list-style-type: none"> 1. "Experimental Tumour Models" to add "It must be assumed, unless there is contrary evidence.... and the IACUC is informed of the potential effects of the disease on the animals"; and 2. "Experimental Tumour Models" to update to "The site for induction of tumours should be chosen carefully. Subcutaneous, intradermal and flank sites should be chosen wherever possible." and "Prior to the use of footpad, brain and eye sites, specific scientific justification should be provided as to the lack of a comparable alternative."; and 3. "Monitoring and Care of Rodents with Tumours" to add "Effective monitoring systems and endpoints should include or feeding/drinking or an ulcerated tumour.", "With ascitic tumours, including hybridomas, the volume of ascitic fluid should not cause gross abdominal distension." and "Bearing in mind that tumour mass increases over time compared to age-matched controls, should be monitored closely."; and 4. add the section for "Experimental endpoints". 	IACUC	
P216.03. Revisions to:	Dr Chew Li Li	19 January 2026
<ol style="list-style-type: none"> 1. update policy format. 2. change the policy title from 'Guidelines for Rodents with Neoplasia' to 'Rodents with Neoplasia'. 3. update title for CM SOP 605 in Section 4.1.1. 4. update requirements for the use of human tissues in Section 4.1.2. 5. include perineum and abdomen in Section 4.1.8. 	Ms Keh Lee Hwei IACUC Designated Reviewers	

6. change to Body Condition Scoring (BCS) instead of body weight for assessing the animal's health status in Section 4.2.5 last point.
7. update Section 4.3.3 Characteristics and location point 1.

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Approved