



condition. Some criteria for establishing a humane endpoint include: tumour size/volume, weight loss, ulceration of tumours, impairment of normal physiological functions (eg eating and drinking), behavioural changes, impact on mobility and severity of clinical signs associated with tumour progression or metastasis. The humane endpoint must be clearly stated in the approved animal ethics protocol with a scoresheet clearly defining the criteria for the humane endpoint.

The endpoints should be designed to be as early as possible to reduce non-specific systemic effects and an increase in the validity and precision of the results obtained, with these varying depending on the type of tumour model studied. Factors that allow scientific decisions to be made at the earliest stage possible should be identified either through pilot studies or consultation of the literature and should take into account the total burden on animal welfare.

Tumour size

If possible, tumour size and volume and therefore progression should be measured. While it should form part of the criteria for scoring it should not be the sole method used to determine the humane endpoint in a study, and should be assessed taking into consideration the overall health of the animal as the criteria for the endpoint. In an adult mouse with a single tumour, the mean diameter of the tumour (measured by $(L \times W)/2$) must not exceed 15mm, while for rats the size must not exceed 28mm. For studies where multiple tumours are grown on one animal, for example two tumours on contralateral flanks, the size of these should be less and should not exceed the maximum burden of a single tumour. These guidelines may vary depending on the type and location of the tumour and therefore method and threshold of measurement must be clearly outlined in the ethics protocol. It may be that animals reach a humane endpoint before these measurements are met, or these measurements are not suitable depending on the location of the tumour. Exact endpoints must be detailed in the approved animal ethics protocol. Precise forms of measurement such as imaging and the use of callipers must be used to avoid discrepancies. Health limitations such as mobility restrictions, access to food and water, pressure on internal organs or body condition may prevent growth to these maximum values and must be taken into account and represented on the appropriate scoresheet.

Reproducibility

There are many variables in tumour models that can make reproducibility difficult. Models, techniques and processes can vary greatly between labs, with the same cell lines producing different characteristics such as immunogenicity and mutations. Where possible, investigators should use models that are well validated at each lab and should consider the variables that drive reproducibility and how study conditions can be optimised. One example could be running the experiment more than once to increase confidence but the benefit of this must be weighed against the potential impact on the animal. The ARRIVE 2.0 Guidelines provide important data points that should be published in tumour model studies to allow for reproducibility by other laboratories.



Blood and other liquid cancers

Due to differences in disease progression, effects on the animal and other variables, the scope of this document does not extend to blood cancers. For further advice regarding these models of disease, please consult the ANU veterinarians.

Monitoring, Intervention and Reporting

Monitoring

Regular observation is necessary in all tumour models to monitor for deterioration of the clinical condition. Frequency of monitoring is dependent on the characteristic of the tumour model and the experience in the lab with the tumour model. Mice and rats used in transplantation models should be observed three times weekly or more as per their approved protocols, with the frequency thereafter determined by the rate of progression of the tumour being studied and the data points required for the particular study. If the location of the tumour is not palpable, then the frequency and schedule for monitoring must be determined from the literature or pilot studies. Some criteria for monitoring that should be included on the scoresheet may include:

- tumour site – ulceration and expansion.
- general appearance – demeanour, coat condition etc
- level of activity
- appetite and hydration status
- respiratory rate and effort
- mobility
- changes in urination and defecation
- behavioural changes
- abdominal distension
- enlarged lymph nodes weight (include variations if tumours add weight – or ascites/fluid formation may add weight)

All criteria for monitoring should be clearly outlined in an appropriate scoresheet approved in the ethics protocol. For observable tumour sites, a visual score card with the criteria for each score clearly shown is useful for consistency of scoring. Records must be clear, kept up to date and be available in the animal room with all details such as time and frequency of monitoring, name of the person monitoring, identification of the animal, protocol number, symptoms and any treatments administered.

Supportive Therapies and Clinical Interventions

Supportive therapies may be given as indicated by the animal's condition and consistent with research goals. These include the provision of wet food, food supplements, sucrose gels, and administration of subcutaneous fluids. Housing adjustments such as the provision of food on the



cage floor may also assist where there is weakness or reduction in the range of movement. In some circumstances, shaving the fur may keep the area dry, which reduces the risk of bacterial infection and allows for better visualisation in the case of ulceration.

Analgesics such as non-steroidal anti-inflammatories will often alter inflammatory pathways which precludes them from use in many tumour model studies. They may be suitable if currently incorporated into human treatments/ conditions that are being replicated. Topical antibiotic or anti-inflammatory creams can also sometimes be used. Inclusion of these and the potential impact on the experimental data can be discussed with the ANU veterinarians.

Criteria for Immediate Intervention

While the criteria for scoring may vary depending on the model used, there are clinical signs that are indicators for potential adverse effects and should result in the immediate culling of the animal and monitoring of the remainder of the cohort. In a well-designed study, these effects are unlikely to occur and steps should be taken to avoid them when planning an experiment.

- Ulceration can result in loss of body fluid, infection and pain and discomfort to the animal. Necrosis and/or ulceration resulting in skin breakdown and exudation with no evidence of healing within 24-48 hours is grounds for termination of the animal. Cell lines producing highly vascular tumours and central necrosis run the risk of death via exsanguination and so endpoints must be made to terminate prior to this. Additionally, ulceration may cause the tumour to lose its true architecture and characteristics due to the invasion by other inflammatory cell types and so may not be a useful data point.
- Consistent weight loss reaching 20% at any point in the experiment or 15% maintained for 72 hours in comparison to the pre-treatment weight of the adult mouse.
- Laboured breathing and cyanosis with or without nasal discharge
- Enlargement of the lymph nodes or spleen
- Hind limb weakness or paralysis
- Significant abdominal distension corresponding to an increase in 10% of body weight.
- Tumours causing interference with locomotion or abnormal behaviour
- Blood stained or mucopurulent discharge from any orifice
- Evidence of severe persistent self-mutilation

Unexpected Adverse Event Reporting

Any event that has resulted in a negative impact on the welfare of the animals, has resulted in wastage of animals or samples, which was not anticipated or has occurred at a frequency or severity in excess of what was anticipated in the approved ethics protocol can be classified as an unexpected adverse event (UAE). In the context of tumour models, this can also include wastage of animals or lines due to poor preparation or contamination, metastasis or impact beyond what was expected for the tumour line resulting in culling of animals or any other variables causing animals to reach a humane endpoint before data can be gathered. Anyone identifying an UAE



must act to immediately remove or minimise the risk to animals and contact the ANU veterinarians immediately, as per the ANU Procedure

Minimum Requirements

- For transplantation models, animals will be expected to be scored three times per week from the start of the experiment with increasing frequency dependant on the tumour progression. Tumour models with spontaneous development later in life will require regular monitoring starting before the expected onset of tumour development. An approved scoresheet must be included to be able to assess welfare risk for the model used.
- Humane endpoints must be set using an approved scoresheet to ensure animals are culled prior to significant welfare compromise
- The location of the tumour implantation must be selected to minimise the impact on the animal
- Tumour size must be measured accurately through the use of callipers, imaging or other precise measuring equipment. Tumour size should not exceed a maximum diameter of 15mm for mice or 28mm for rats (measured by $(L \times W)/2$). Within these guidelines the maximum size may vary depending on the type and location of the tumour and therefore the method and threshold of measurement must be clearly outlined in the ethics protocol.
- Screening of cell line must be undertaken to ensure they are free from contamination which may pose a risk to rodents or laboratory staff
- Where possible, supportive therapies should be incorporated into the experimental plan to alleviate the potential suffering of the animal.

Exceptions

In all circumstances research groups should make an effort to meet the above recommendations. It is not acceptable to risk animal welfare or research quality based on time constraints or personal convenience. Any exceptions must be discussed with ANU veterinarians with consideration to welfare and research quality and must be outlined in an approved animal ethics protocol.

References and Resources

Boston University Office of Research (2014) Tumour Policy for Mice and Rats

<https://www.bu.edu/researchsupport/compliance/animal-care/working-with-animals/procedures>

(Accessed 26 June 2020)

NHMRC. Australian code for the care and use of animals for scientific purposes 8th Edition 2013

(Section 4.4.3) <https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes>

ANU Research Ethics Office Animal Experimentation Ethics Committee Approved Document AEEC Approved Document_023_Tumour_Models_V1.4 Final



Office of Ethics and Compliance – Institutional Animal Care and Use Program, University of California (2016) Tumour induction in mice and rats <https://iacuc.ucsf.edu/guidelines> (Accessed 26 June 2020)

Office of Research Ethics and Integrity, University of Melbourne (2018) Animal Care and Use Standard Tumour Models in Mice and Rats <https://staff.unimelb.edu.au/> (Accessed 25 June 2020)

Procedure for Managing & Reporting Unexpected Adverse Events
<https://services.anu.edu.au/research-support/ethics-integrity/animal-ethics-policies-guidelines-and-forms>

Purdue University (2018) Humane Endpoint Criteria for Rodent Tumour Studies
<https://www.purdue.edu/research/regulatory-affairs/animal-research/> (Accessed 26 June 2020)

Schuh, J.L. (2004) Trials, tribulations and trends in tumour modelling in mice, *Toxicologic Pathology*, 32(Suppl. 1): 53-66.

Wallace, J. (2000) Humane endpoints and cancer research, *ILAR Journal*, 41(2). pp 87-93.

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