This document has been developed by The Australian National University's (ANU) Research Ethics Office. It has been endorsed by the ANU Animal Experimentation Ethics Committee (AEEC). It is designed to provide guidance regarding current best practice to institutional animal users and carers on the care and use of animals for scientific purposes. It has been prepared in consultation with the Australian code for the care and use of animals for scientific purposes 8th edition 2013.

Document 023: Tumour Models

Background

Tumour studies in mice have been an important tool used in research to learn about tumour biology, the influence of the microenvironment, metastasis and physiological response to perturbations. As such, they are a valuable resource for evaluating cancer progression and trialling treatments for the management of many types of cancers. Tumours in rodents can be experimentally induced, or generated by genetic modifications and/or breeding for a wide range of tumours in an experimental setting. The resulting tumour development may cause primary or secondary symptoms which can have a significant impact on animal welfare. Experimental design should therefore aim to minimise pain, distress and discomfort to the animal. It is important to understand that this area presents unique challenges and special requirements for monitoring and humane endpoints are necessary to ensure high standards of animal welfare.

There are clear guidelines as set out in the Code, which outline specific requirements for experiment involving induction of tumours:

"3.3.23 For animals in studies that involve the induction of tumours, methods used and endpoints chosen must ensure that valid results are obtained with minimal harm, including pain and distress, to the animal. Animal wellbeing must be supported and safeguarded by:

1. *considering potential adverse impacts associated with the development and biology of the tumour (including growth rate, invasiveness, potential for ulceration, development of metastases and cachectic effects), effects of therapeutic agents, side effects of immunotherapy including irradiation, and consequences of surgery involved in transplantation of tumours*
2. *choosing an appropriate implantation site or method of induction of the tumour that causes the least harm, including pain and distress, to the animal. The footpad, tail, brain or eye must not be used unless there is no valid alternative*
3. *monitoring the growth or impact of the tumour and efficacy of therapy, and using early experimental endpoints, to obtain valid results as early as possible. Death from the tumour must not be an endpoint*
4. *establishing and implementing early intervention points and humane endpoints (see Clauses 3.1.26–3.1.28)*
5. *wherever possible, using techniques that facilitate measurement of tumour growth and determination of early endpoints*
6. *monitoring and assessing animals for signs of pain and distress, including changes in body condition and body weight; ulceration; adverse effects of procedures used for induction of the tumour; signs of growth, invasion and metastases of the tumour; and toxic effects of therapeutic agents."*

Definitions

The Code - Australian code for the care and use of animals for scientific purposes 8th Edition 2013

Tumour – abnormal growth of tissue

Cancer – disease caused by uncontrolled division of abnormal cells in a part of the body that can spread to other areas within the body

Benign – non-cancerous, does not invade or spread to other parts of the body

De Novo – In reference to the first occurrence of cancer in the body and mimics the growth patterns within the body.

Malignant – abnormal and uncontrolled cell growth that spreads to other areas of the body

Metastasis – development of secondary growths at a site away from the primary site of cancer

Necrosis – death of cells in a living animal

Neoplasia – process of abnormal and uncontrolled cell growth

Subcutaneous – under the skin

General Information and Considerations

Pilot Studies

Pilot studies using a small number of animals (5-10 depending on experiment) are recommended to ensure that patterns of local and metastatic growth are reproducible and to document any adverse effects associated with the tumour progression, in order to determine appropriate scoring criteria, intervention options and humane endpoints to be identified. For example, in studies involving the injection of subcutaneous tumours, these pilot studies would assist in determining the appropriate scoring parameters, monitoring methods, timeframe of the experiment and endpoints to account for speed of growth, expansion and potential ulceration. For tumours growing as a suspension within the peritoneal cavity, pilot studies should be undertaken to determine clear criteria to ensure that animals are terminated prior to severe compromise. Models involving metastatic tumours should define the extent and expected time course of the dissemination to internal organs. Termination of studies should be aimed at the earliest possible time point to minimise pain and distress to the animal whilst also allowing for meaningful data to be obtained.

Cell Lines and Screening

It is recommended that investigators using cell lines should, based on their own research and information in the literature, develop and maintain records of known features of tumour cell lines, with relevant information which might include origin, sex and strain of original host, treatment

history, name and subclone of the tumour line, source (commercial or private), passage history of all aliquots, in vitro and in vivo growth characteristics, maximum growth rates, histomorphological characteristics, metastatic potential and method, immunogenicity, receptor and oncogene expression, cellular products, microbiological screening history, biohazard potential and unique characteristics (Schuh,2004). This information is important for preventing and investigating issues that may arise, such as microbial or cross contamination, failed implantation, or deviations from the expected behaviour of the tumour line. Screening of cell lines before use in rodents, for pathogens such as rodent viruses, are a mandatory requirement in barrier facilities, as contamination may compromise experimental results and cause outbreaks of disease amongst laboratory staff and animals. Further information regarding screening can be obtained from the animal facilities or from the ANU veterinarians.

Tumour Induction and Location

- Subcutaneous or intradermal – the tumour must be injected into a site(s) that will minimise interference with normal body functions such as movement, eating, drinking and urination/ defecation. Sites on the back or flank are considered most appropriate to minimise interference. Implantation on the face or limbs should be avoided given the limited space for expansion. Implantation on the ventral surfaces of the body are not recommended due to the risk of irritation from the bedding and increased impediment to movement as the tumour progresses. For intramammary injection of tumours, the use of anaesthesia is recommended to ensure accurate injection technique.
- Intramuscular implantation should be avoided as the associated distension of the muscles as the tumour progresses can cause significant pain.
- Intraperitoneal tumour, where ascites is a side effect, should generally only be utilised where the side effects are comparable in the human disease they are emulating. The AEEC will seek strong justification for any proposal involving intraperitoneal tumours.
- In the case of surgical implantation of tumours – best standard practice in terms of anaesthesia, analgesia and aseptic technique (see ANU AEEC Approved Document 006_Animal Surgery Standards) must be followed.
- De novo and metastatic tumour models – the Primary Investigator (PI) must investigate the possible adverse effects and their likely incidence, set appropriate humane endpoints to minimise suffering and set out management for controlling the severity of the adverse effects.

Sites such as the footpad, eye, tail or bone are not recommended unless special justification and earlier endpoints due to the increased amount of pain or distress that the animal would endure.

Humane Endpoints

The overall health and wellbeing of the animal must take precedence over the size of the tumour induced. Death as a final endpoint can never be used under any circumstances. The humane endpoint of the animals in the experiment must consider the characteristics of the tumour, the methods used to induce the tumour as well as the adverse impacts on the animal's clinical

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 (LxW)/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 or 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 (LxW)/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

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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