Preclinical Research Center
Ben-Gurion University of the Negev

STANDARD OPERATING PROCEDURE 803

HUMANE INTERVENTION POINTS FOR RODENT CANCER MODELS

1. PURPOSE

This Standard Operating Procedure (SOP) provides guidelines for performing cancer research involving rodents.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. CONSIDERATIONS

3.1. In vivo studies in a complex living organism can only answer many questions in oncology. Animals with local or disseminated tumors may experience pain and distress, thus justifying special care and attention for their welfare. At all times, the well-being of the research animals should be balanced against the scientific objectives and requirements of the study.

3.2. For this policy, cancer studies have been divided into two (2) broad categories:

3.2.1. Tumor biology is the study of how tumors grow and behave. In these types of studies, the effect of tumor burden on animals should be evaluated to avoid excessive pain or distress and to achieve research goals.

3.2.2. Tumor treatment studies tumors' response to chemical, radiologic, or immunologic therapy. In these types of studies, the effect of the treatment modality on the animal should be evaluated in addition to the tumor burden.

4. GUIDELINES

4.1. For all *in vivo* cancer research, the animal protocol must contain the following:

4.1.1. Justification of animal numbers based on a straightforward experimental design and a detailed statistical analysis.

4.1.2. When known, information on the proposed model's expected tumor kinetic, growth characteristics, and biology is available. The Ethical committee reserves the right to request a pilot study if these factors are unknown.

4.1.3. Clearly defined experimental endpoints.

4.1.4. Clearly defined clinical intervention points to minimize the potential for pain and distress to the animal (refer to section 6). Selecting clinical intervention points requires detailed knowledge of

the impact of tumor biology and tumor treatment on the animal. The FACC may request a pilot study if these factors are unknown. Animals reaching clinical intervention points must be euthanized unless otherwise approved by the FACC or veterinarian.

4.2 Models presenting multiple tumors:

4.2.1. The presence of multiple tumors must be described in the approved animal protocol.

4.2.2. Tumor burden should be limited to the minimum required for a valid scientific outcome.

4.3 Mouse models of metastasis:

4.3.1. Metastatic models must be described in the approved animal protocol.

4.3.2. Consider resecting primary tumors where possible.

4.3.3. Consider imaging techniques to facilitate the development of more defined intervention points.

4.3.4. Specific experimental mouse metastasis models originating from multiple, palpable mammary tumors will not develop metastasis at the conventional intervention points. These multiple mammary tumors tend to be relatively small and grow simultaneously in some or all mammary glands. Exceptional intervention points for these mouse models are described in section 6.2.

5. MONITORING

5.1. Monitoring:

5.1.1. Monitoring is the responsibility of the PI and research staff.

5.1.2. All mice potentially developing tumors must be monitored at least once weekly.

5.1.3. Detailed monitoring logs should be kept for tumor-bearing mice from when a tumor is palpated until euthanasia. When possible, records should be kept in the animal housing room. Records should contain the following information:

5.1.3.1. Animal identification

5.1.3.2. Tumor measurements

5.1.3.3. Monitoring frequency

5.1.3.4. General observations concerning the health of the animals

5.1.4. Cages of mouse models of experimental metastasis originating from multiple, palpable mammary tumors should be identified, preferably using a distinct cage card.

5.1.5. The frequency of monitoring should be increased during critical phases of the study, e.g., from weekly to twice a week to daily, as the tumor volume or tumor burden increases and the humane intervention points are approaching.

5.1.6. The use of a color-coded system may be helpful for the monitoring of tumor-bearing mice. For example, the system can use colored stickers or markers as follows:

5.1.6.1 Green: mice with small palpable tumors or a low tumor burden are monitored weekly, but tumors are not necessarily measured.

5.1.6.2 Yellow: mice have reached approximately 50% of tumor volume or burden endpoint. Monitoring should occur twice weekly, and tumors should be measured weekly.

5.1.6.3 Red: mice are approaching the endpoint. Daily monitoring is necessary, and tumors are measured at least twice weekly.

5. CLINICAL INTERVENTION POINTS

MEASURABLE OBSERVATION	CLINICAL INTERVENTION POINT	ASSESSMENT
General Condition	Hunched Rough hair coat Anorexia Cachexia Hypothermia Abnormal behavior or vocalization Unresponsive to touch	Behavioral and physical examination by qualified personnel.
Tumor Clinical Properties	Ulceration Necrosis Infection	Refer to section 6.1. Scabbing, ulceration, exudates, color (deep red, purple, blue, or black), heat, pain upon palpation. Animals should be individually caged and monitored for carnivorism or excessive chewing.
	Interference with normal functions	Inability to access or ingest food, drink, keep clean, or ambulate.
	Local invasiveness	Inability to access or ingest food, drink, keep clean, or ambulate. Pain upon palpation.
	Distant metastasis	Specific organ failure is assessed by physical examination and, where possible, ancillary tests (hematology, biochemistry, imagery, etc.).
Organ-specific impairment or failure	Respiratory: Dyspnea, tachypnea, apnea Alimentary: Chronic diarrhea, constipation, rectal prolapse, distended abdomen (ex.: ascites, ileus), jaundice Neurological: Circling, blindness, dementia, convulsion, loss of consciousness. Urogenital: Anuria, polyuria, hemorrhage, discharge Myoarthroskeletal: fracture, abnormal gait, or mobility	Behavioral and physical examination by qualified personnel. Specific organ failure is assessed by physical examination and, where possible, ancillary tests (hematology, biochemistry, imagery, etc.).
Body weight (BW)	Adult: weight loss over 20% of initial BW, weight loss over 10% between consequence measures	% = BW – cumulative tumor weight x 100 / Baseline BW
	Young: failure to maintain weight gain within 15% of age-matched control animals	% = BW / Average, age-matched, control BW x 100
Body condition score (BCS)	Body condition score less than 2	Physical examination by qualified personnel.
Tumor Volume (**see File SOP803-A**)	Mice: 1500 mm3 (1.5cc) (2.0 cc exceptionally for some metastatic models) Rats: 4000 mm3 (4.0cc)	4/3 π × [(Length × Width × Height)/2].
Tumor Burden	Mice: 10% baseline BW (6.0 cc exceptionally for some metastatic models) Rat: over 5% baseline BW	% = Cumulative tumor weight* x 100 Baseline BW

6.1. Multiple tumors and tumor burden:

6.1.1. When multiple tumors are present, the total tumor burden is calculated by adding the volume of each tumor.

6.1.2. The total tumor burden should not exceed 10% of the animal's baseline body weight, excluding the importance of the tumor. Tumor weight and tumor volume are equivalent to a presumed tissue density of 1, i.e., 1 cm^3 = 1g. Refer to Annex 2 for sample calculations.

6.1.3. No individual tumor can exceed 1.5cm in long diameter in mice and 3.5 cm^3 in long diameter in rats.

6.2. Experimental mouse models of metastasis originating from palpable, multiple mammary tumors:

6.2.1. Where multiple tumors are present, the total tumor burden is calculated by adding the volume of each tumor.

6.2.2. The total tumor burden must not exceed **1500 mm^3**.

6.2.3. No individual tumor can exceed 1.5cm.

6.3 Tumor ulceration/necrosis:

6.3.1. Some primary tumors injected subcutaneously tend to produce local skin ulceration and necrosis. The presence of ulceration of the tumor is generally a criterion for euthanasia. However, there are circumstances in which maintaining mice passed the time when ulceration of tumors first appears may be necessary. In these cases, the presence of ulcerated tumors must be justified in the AUP as being essential to meet the scientific goals of the study and approved by the FACC.

6.3.2. The proposed scoring method demonstrates vigilance in monitoring the level of ulceration of tumors. It considers the tumor's skin condition to consider the research goals in addition to the pain/distress caused by the experimental procedure.

6.3.3. For scoring of tumor ulcerations, refer to Annex 3:

DESCRIPTION OF LESION	SCORE
No lesion	0
Redness at the site of the tumor; skin looks intact	1
Superficial skin abrasions (scratches) at the site of the mass	2
Small skin ulceration present without necrosis	3
Small skin ulceration (<3mm) with necrosis	4
Extensive skin ulcerations (>3mm) with/without the presence of necrosis	5

6.3.4. Intervention points:

6.3.4.1 A score of 0, 1, or 2: no treatment required.

6.3.4.2 A score of 3 or 4: treatments can be attempted to enhance skin healing and protect the underlying tumor from getting infected. Treatments may include daily skin disinfection, application of topical antibiotics, or spray bandages. If the cancer is located ventrally, soft bedding can minimize the friction caused by standard bedding.

6.3.4.3 A score of 5, or if tumor ulcerations are bleeding, infected (presence of pus), or if the underlying muscle layer is exposed: euthanasia within 24 hours.

6.3.5. Frequency of monitoring:

6.3.5.1. A score of 1 or 2: twice weekly.

6.3.5.2. A score of 3 or 4: daily.

6.4. Pulmonary metastasis:

6.4.1. Longitudinal experiments to characterize metastasis to the lungs can be essential to studying a wide range of cancers. Good intervention points for such studies should allow the development and maturation of advanced metastatic burden while avoiding pain, distress, or mortality from metastasis as an experimental endpoint.

6.4.2. Clinical signs of lung metastasis can be vague, including rough coat, hunched posture, anorexia, dehydration, decreased activity, decreased grooming behavior, and dyspnea.

6.4.3. Whenever possible, consider monitoring animals for lung metastasis using different imaging techniques.

6.4.4. In mice, the Pulmonary Assessment of Advanced Metastasis (PAAM) technique can be used to assess respiratory distress during the progression of metastatic pulmonary disease. The method causes a temporary reduction in breathing capacity that normal mice can quickly compensate for but causes respiratory pain in mice with advanced lung metastasis.

6.4.4.1. Restrain the mouse using the thumb and forefinger of the non-dominant hand.

6.4.4.2. Using the forefinger of the dominant hand, apply gently to moderate digital pressure just caudal to the xiphoid sternum for 3 seconds.

6.4.4.3. In normal mice, no response or a mild increase in respiratory rate is observed.

6.4.4.4. 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.