STANDARD OPERATING PROCEDURE #415

HUMANE INTERVENTION POINTS FOR RODENT CANCER MODELS

1. PURPOSE

The intent of this Standard Operating Procedure (SOP) is to provide guidelines for the McGill University scientific community performing cancer research involving rodents.

2. RESPONSIBILITY

Facility Animal Care Committees (FACC's), principal investigator (PI) and their research staff, veterinary care staff.

3. INTRODUCTION

3.1. Many questions in oncology can only be answered by in vivo studies in complex living organism. Animals with local or disseminated tumors may experience pain and/or distress, thus justifying special care and attention for their welfare. At all times, the wellbeing of the research animals should be balanced against the scientific objectives and requirements of the study. This necessitates the selection of the most appropriate clinical intervention points.

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

3.2.1. Tumor biology defined as 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 is defined as the study of the response of tumors 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 tumor burden.

4. GENERAL GUIDELINES

4.1. For all *in vivo* cancer research, the Animal Use Protocol (AUP) must contain:

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

4.1.2. Information on the expected tumor kinetic, growth characteristics and biology in the proposed model, when known. The FACC 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/or distress to the animal (refer to section 6). The selection of clinical intervention points requires detailed knowledge of the impact of the tumor biology and/or 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 FACC-approved AUP.

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

4.3. Mouse models of metastasis:

4.3.1. Metastatic models must be described in the FACC-approved AUP.

4.3.2. Consider resecting primary tumors where possible. Refer to SOP 205.

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

4.3.4. Certain experimental mouse models of metastasis originating from multiple, palpable mammary tumors will not develop metastasis at the conventional intervention points. These multiple mammary tumors tend to be relatively small in size and grow simultaneously in some or all of the mammary glands. Exceptional intervention points for these mouse models are described in section 6.2. These models and associated intervention points must be clearly outlined in the FACC-approved AUP.

5. MONITORING

5.1. Monitoring:

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

5.1.2. All mice that can potentially develop tumors must be monitored at least once weekly.

5.1.3. Detailed monitoring logs should be kept for tumor-bearing mice from the point a tumor is palpated until euthanasia. When possible, logs should be kept in the animal housing room. Logs 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 clearly 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:

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

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

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

6. 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, to drink, to keep clean or to ambulate.
	Local invasiveness	Inability to access or ingest food, to drink, to keep clean or to ambulate. Pain upon palpation.
	Distant metastasis	Specific organ failure 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 assessed by physical examination and, where possible, ancillary tests (hematology, biochemistry, imagery, etc).
Body weight (BW)	Adult: weight loss over 20% of initial BW	$\% = \frac{\text{BW – cumulative tumor weight}}{\text{Baseline BW}} \times 100$
	Young: failure to maintain weight gain within 15% of age-matched control animals	$\% = \frac{\text{BW}}{\text{Average, age matched, control BW}} \times 100$
Body condition score (BCS)	Body condition score less than 2	Physical examination by qualified personnel.
Tumor Volume	Mice: 2000 mm3 (2.0cc) (2.5 cc exceptionally for some metastatic models) Rats: 5000 mm3 (5.0cc)	$4/3\ \pi \times [(\text{Length} \times \text{Width} \times \text{Height})/2]$.
Tumor Burden	Mice: 10% baseline BW (6.0 cc exceptionally for some metastatic models) Rat: over 5% baseline BW	$\% = \frac{\text{Cumulative tumor weight*}}{\text{Baseline BW}} \times 100$

6.1. Multiple tumors and tumor burden:

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

6.1.2. Total tumour burden should not exceed 10% of the animal's baseline body weight, excluding the weight of the tumor. Tumour weight and tumour volume are considered equivalent based on 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 2.0cm^3 in mice and 5.0cm^3 in rats.

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

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

6.2.2. Total tumour burden must not exceed 6.0cm^3.

6.2.3. No individual tumor can exceed 2.5cm^3.

6.3. Tumor ulceration/necrosis:

6.3.1. Some primary tumors injected subcutaneously have a tendency to produce local skin ulceration and/or necrosis. The presence of ulceration of the tumor is generally a criterion for euthanasia. However, there are special circumstances in which maintaining mice past 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 absolutely necessary 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 takes into account the skin condition of the tumor in order to take into consideration the research goals in addition to the pain/distress caused by the experimental procedure.

6.3.3. 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
Large skin ulcerations (>3mm) with/without presence of necrosis	5

6.3.4. Intervention points:

6.3.4.8. Score of 0, 1 or 2: no treatment required.

6.3.4.9. Score of 3 or 4: treatments can be attempted to enhance skin healing and protect the underlying tumor from getting infected. Treatments may consist of daily skin disinfection, application of topical antibiotics, or spray bandages. If the tumor is located ventrally, soft bedding (e.g., Envigo Teklad Diamond Soft cellulose bedding) can minimize the friction caused by standard bedding.

6.3.4.10. Score of 5, or if tumor ulcerations are found to be 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. Score of 1 or 2: twice weekly.

6.3.5.2. 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 the study of a wide range of cancers. Adequate 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 and may include 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 pulmonary metastatic disease. The technique causes a temporary reduction in breathing capacity that normal mice can easily compensate for but causes respiratory distress in mice with advanced lung metastasis.

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

6.4.4.2. Using forefinger of dominant hand, apply gentle 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.

7. REFERENCES

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7.2. Workman P, Aboagye EO, Balkwill F, et al. Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer*. 2010;102(11):1555-1577. doi:10.1038/sj.bjc.6605642.

7.3. Wallace J, Humane endpoints in cancer research. ILAR Journal, Volume 41, Issue 2, 1 January 2000, Pages 87–93, https://doi.org/10.1093/ilar.41.2.87.

7.4. Morton DB, Griffiths PH. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Vet Rec*. 1985 Apr 20;116(16):431-6.

7.5. Montgomery CA. (1990) Oncological and toxicological research: Alleviation and control of pain and distress in laboratory animals. *Cancer Bulletin*. 42:230-237.

7.6. Workman P, et al. UKCCR Guidelines for the welfare of animals in experimental neoplasia. *Cancer and Metastasis Reviews* 8: 82-88, 1989.

SOP REVISION HISTORY

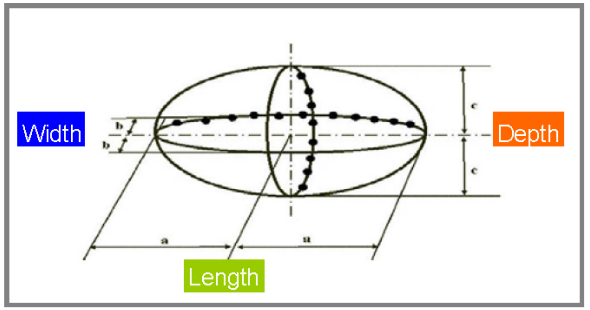
DATE	PREVIOUS VERSION	NEW VERSION
17.11.02	(NO TEXT)	**Addition of section 6.1 Tumor ulceration/necrosis**
17.12.08	(NO TEXT)	**Addition of section 6.2 Pulmonary Metastasis**
18.03.19	(NO TEXT)	**7.2. Workman P, Aboagye EO, Balkwill F, et al. Guidelines for the welfare and use of animals in cancer research. British Journal of Cancer. 2010;102(11):1555-1577. doi:10.1038/sj.bjc.6605642.**
18.03.19	(NO TEXT)	**7.3. Wallace J, Humane endpoints in cancer research. ILAR Journal, Volume 41, Issue 2, 1 January 2000, Pages 87–93, https://doi.org/10.1093/ilar.41.2.87.**
18.05.24	(NO TEXT)	**4.2. Models presenting multiple tumors:** **4.2.1. The presence of multiple tumors must be described in the FACC-approved AUP.** **4.2.2. Tumor burden should be limited to the minimum required for valid scientific outcome.** **4.3. Mouse models of metastasis:** **4.3.1. Metastatic models must be described in the FACC-approved AUP.** **4.3.2. Consider resecting primary tumors where possible. Refer to SOP 205.** **4.3.3. Consider the use of imaging techniques to facilitate the development of more defined intervention points.** **4.3.4. 4.3.4. Certain experimental mouse models of metastasis originating from multiple, palpable mammary tumors will not develop metastasis at the conventional intervention points. These multiple mammary tumors tend to be relatively small in size and grow simultaneously in some or all of the mammary glands. Exceptional intervention points for these mouse models are described in section 6.2. These models and associated intervention points must be clearly outlined in the FACC-approved AUP.**
18.05.24	(NO TEXT)	**6.1. Multiple tumors and tumor burden:** **6.1.1. When multiple tumors are present, total tumor burden is calculated by adding the volume of each individual tumor.** **6.1.2. Total tumour burden should not exceed 10% of the animal's baseline body weight, excluding the weight of the tumor. Tumour weight and tumour volume are considered equivalent based on a presumed tissue density of 1, i.e., 1 cm3 = 1g. Refer to Annex 2 for sample calculations.** **6.1.3. No individual tumor can exceed 2.0cm3 in mice and 5.0cm3 in rats.** **6.2. Experimental mouse models of metastasis originating from palpable, multiple mammary tumors:** **6.2.1. Where multiple tumors are present, total tumor burden is calculated by adding the volume of each individual tumor.** **6.2.2. Total tumour burden must not exceed 6.0cm3.** **6.2.3. No individual tumor can exceed 2.5cm3.**
18.05.24	(NO TEXT)	**7.4. Morton DB, Griffiths PH. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Vet Rec. 1985 Apr 20;116(16):431-6.** **7.5. Montgomery CA. (1990) Oncological and toxicological research: Alleviation and control of pain and distress in laboratory animals. Cancer Bulletin. 42:230-237.** **7.6. Workman P, et al. UKCCR Guidelines for the welfare of animals in experimental neoplasia. Cancer and Metastasis Reviews 8: 82-88, 1989.**
18.05.24	(NO TEXT)	**Addition of Annex 1, Annex 2 and Annex 3.**
18.07.24	5.2 Mouse models of experimental metastasis originating from multiple, palpable mammary tumors: 5.2.1. The cage should be identified with a distinct cage card. 5.2.2. Monitoring logs should be kept in all rooms housing tumor-bearing mice. 5.2.3. Once a tumor is palpated, a file is open for that mouse in the monitoring log book and a colored dot is place on the cage card next to the mouse identification number. 5.2.4. As the tumor burden increases, the color of the dot on the cage card is changed as follows: 5.2.4.1. Green dots: mice with a palpable tumor, with a low tumor burden, small masses < 1 cc: mice are monitored weekly but tumors are not necessarily measured. 5.2.4.2. Yellow dots: mice have reached approximately 50% of tumor burden endpoint. Monitoring should occur twice weekly and tumors are measured weekly. 5.2.4.3. Red dots: mice are approaching endpoint. Daily monitoring is necessary and tumors are measured at least twice weekly.	**5.1.3. Detailed monitoring logs should be kept for tumor-bearing mice from the point a tumor is palpated until euthanasia. When possible, logs should be kept in the animal housing room. Logs 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 clearly 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 with a low tumor burden: mice are monitored weekly but tumors are not necessarily measured.** **5.1.6.2. Yellow: mice have reached approximately 50% of tumor volume or tumor burden endpoint. Monitoring should occur twice weekly and tumors are measured weekly.** **5.1.6.3. Red: mice are approaching endpoint. Daily monitoring is necessary and tumors are measured at least twice weekly.** ~~5.2 Mouse models of experimental metastasis originating from multiple, palpable mammary tumors:~~ ~~5.2.1. The cage should be identified with a distinct cage card.~~ ~~5.2.2. Monitoring logs should be kept in all rooms housing tumor-bearing mice.~~ ~~5.2.3. Once a tumor is palpated, a file is open for that mouse in the monitoring log book and a colored dot is place on the cage card next to the mouse identification number.~~ ~~5.2.4. As the tumor burden increases, the color of the dot on the cage card is changed as follows:~~ ~~5.2.4.1. Green dots: mice with a palpable tumor, with a low tumor burden, small masses < 1 cc: mice are monitored weekly but tumors are not necessarily measured.~~ ~~5.2.4.2. Yellow dots: mice have reached approximately 50% of tumor burden endpoint. Monitoring should occur twice weekly and tumors are measured weekly.~~ ~~5.2.4.3. Red dots: mice are approaching endpoint. Daily monitoring is necessary and tumors are measured at least twice weekly.~~
18.07.24	5.1.2. All mice that can potentially develop tumors need to be monitored at least once weekly.	5.1.2. All mice that can potentially develop tumors ~~need to~~ **must** be monitored at least once weekly.

Annex 1 – Tumor Volume Estimation Chart

Length (largest measurement in cm) \ Width (smallest measurement in cm)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0
0.1	0.00																													
0.2	0.00	0.00																												
0.3	0.00	0.01	0.01																											
0.4	0.00	0.01	0.02	0.03																										
0.5	0.00	0.01	0.02	0.04	0.07																									
0.6	0.00	0.01	0.03	0.05	0.08	0.11																								
0.7	0.00	0.01	0.03	0.06	0.09	0.13	0.18																							
0.8	0.00	0.02	0.04	0.07	0.10	0.15	0.21	0.27																						
0.9	0.00	0.02	0.04	0.08	0.12	0.17	0.23	0.30	0.38																					
1.0	0.01	0.02	0.05	0.08	0.13	0.19	0.26	0.34	0.42	0.52																				
1.1	0.01	0.02	0.05	0.09	0.14	0.21	0.28	0.37	0.47	0.58	0.70																			
1.2	0.01	0.03	0.06	0.10	0.16	0.23	0.31	0.40	0.51	0.63	0.76	0.90																		
1.3	0.01	0.03	0.06	0.11	0.17	0.25	0.33	0.44	0.55	0.68	0.82	0.98	1.15																	
1.4	0.01	0.03	0.07	0.12	0.18	0.26	0.36	0.47	0.59	0.73	0.89	1.06	1.24	1.44																
1.5	0.01	0.03	0.07	0.13	0.20	0.28	0.38	0.50	0.64	0.79	0.95	1.13	1.33	1.54	1.77															
1.6	0.01	0.03	0.08	0.13	0.21	0.30	0.41	0.54	0.68	0.84	1.01	1.21	1.42	1.64	1.88	2.14														
1.7	0.01	0.04	0.08	0.14	0.22	0.32	0.44	0.57	0.72	0.89	1.08	1.28	1.50	1.74	2.00	2.28	2.57													
1.8	0.01	0.04	0.08	0.15	0.24	0.34	0.46	0.60	0.76	0.94	1.14	1.36	1.59	1.85	2.12	2.41	2.72	3.05												
1.9	0.01	0.04	0.09	0.16	0.25	0.36	0.49	0.64	0.81	0.99	1.20	1.43	1.68	1.95	2.24	2.55	2.88	3.22	3.59											
2.0	0.01	0.04	0.09	0.17	0.26	0.38	0.51	0.67	0.85	1.05	1.27	1.51	1.77	2.05	2.36	2.68	3.03	3.39	3.78	4.19										
2.1	0.01	0.04	0.10	0.18	0.27	0.40	0.54	0.70	0.89	1.10	1.33	1.58	1.86	2.16	2.47	2.81	3.18	3.56	3.97	4.40	4.85									
2.2	0.01	0.05	0.10	0.18	0.29	0.41	0.56	0.74	0.93	1.15	1.39	1.66	1.95	2.26	2.59	2.95	3.33	3.73	4.16	4.61	5.08	5.58								
2.3	0.01	0.05	0.11	0.19	0.30	0.43	0.59	0.77	0.98	1.20	1.46	1.73	2.04	2.36	2.71	3.08	3.48	3.90	4.35	4.82	5.31	5.83	6.37							
2.4	0.01	0.05	0.11	0.20	0.31	0.45	0.62	0.80	1.02	1.26	1.52	1.81	2.12	2.46	2.83	3.22	3.63	4.07	4.54	5.03	5.54	6.08	6.65	7.24						
2.5	0.01	0.05	0.12	0.21	0.33	0.47	0.64	0.84	1.06	1.31	1.58	1.88	2.21	2.57	2.95	3.35	3.78	4.24	4.73	5.24	5.77	6.34	6.92	7.54	8.18					
2.6	0.01	0.05	0.12	0.22	0.34	0.49	0.67	0.87	1.10	1.36	1.65	1.96	2.30	2.67	3.06	3.49	3.93	4.41	4.91	5.45	6.00	6.59	7.20	7.84	8.51	9.20				
2.7	0.01	0.06	0.13	0.23	0.35	0.51	0.69	0.90	1.15	1.41	1.71	2.04	2.39	2.77	3.18	3.62	4.09	4.58	5.10	5.65	6.23	6.84	7.48	8.14	8.84	9.56	10.31			
2.8	0.01	0.06	0.13	0.23	0.37	0.53	0.72	0.94	1.19	1.47	1.77	2.11	2.48	2.87	3.30	3.75	4.24	4.75	5.29	5.86	6.47	7.10	7.76	8.44	9.16	9.91	10.69	11.49		
2.9	0.02	0.06	0.14	0.24	0.38	0.55	0.74	0.97	1.23	1.52	1.84	2.19	2.57	2.98	3.42	3.89	4.39	4.92	5.48	6.07	6.70	7.35	8.03	8.75	9.49	10.26	11.07	11.90	12.77	
3.0	0.02	0.06	0.14	0.25	0.39	0.57	0.77	1.01	1.27	1.57	1.90	2.26	2.65	3.08	3.53	4.02	4.54	5.09	5.67	6.28	6.93	7.60	8.31	9.05	9.82	10.62	11.45	12.32	13.21	14.14

Formula Used for Tumour Volume Calculation: $(4/3\pi^*(\text{Length}/2)*(\text{Width}/2)^2)$

Width	Measure the shortest axis
Length	Measure the longest axis
Depth	Not used in calculation, instead use the smallest measurement (width) twice - hence: $(\text{width}/2)^2$
	Tumor volume is approaching $1.5cm^3$
	Tumour volume is approaching or exceeding $2.0cm^3$

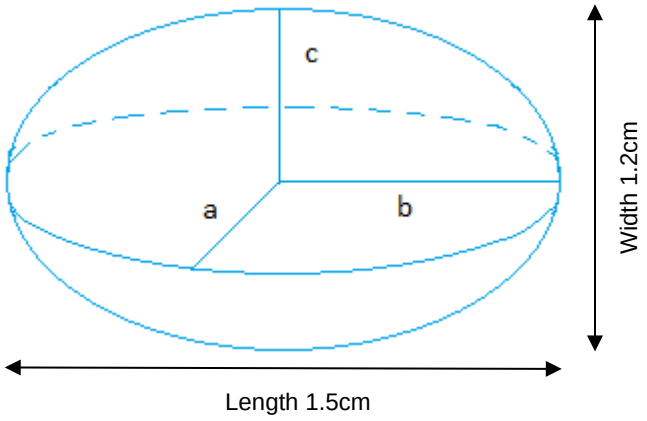


Annex 2 –Tumor Volume Calculations

- External caliper measures are currently the standard technique for tumor volume estimation.
- It should be noted that measurements may vary depending on observer subjectivity, differences in tumor compressibility, skin thickness, subcutaneous fat, and variations in tumor shape. Ideally, the same individual should measure tumors over time.
- Most tumors will take on a spherical or ellipsoid shape. An ellipsoid is a three-dimensional figure whose plane sections are ellipses or circles. The formula used to estimate the tumor volume is that for calculating the volume of an ellipsoid: **$V = 4/3 \times \pi \times a \times b \times c$**, where **a**, **b**, and **c** are the semi-axes. Therefore, when calculating tumor volume using the Length (a), Width (b), and Height (c) of a tumor, the formula is **$V = 4/3\pi \times [(a \times b \times c)/2]$** or **$V = 4/3\pi \times [(\text{Length} \times \text{Width} \times \text{Height})/2]$**.
- Tumor Width and Length can be readily measured on the surface of the skin. However, measuring tumor Height precisely can be challenging. Therefore, the measurement for Height is estimated to be equal to the Width. The formula then becomes **$V = 4/3\pi \times [(\text{Length}/2) \times (\text{Width}/2)^2]$.**
- Tumour weight and tumour volume are considered equivalent based on a presumed tissue density of 1, i.e., 1 cm^3 = 1g.
- If there are multiple tumors present, when calculating the maximum allowable tumor burden of 10%, subtract the weight of the tumor from the weight of the animal or use the baseline body weight, i.e., the weight of the animal prior to tumor implantation or growth.

Example 1: single tumor in mouse

- Single tumor, measurements: Width: 1.2cm, Length: 1.5cm



Volume = $4/3\pi \times [(\text{Length}/2) \times (\text{Width}/2)^2]$

$V = 4/3\pi \times [(1.5/2) \times (1.2/2)^2]$

$V = 4/3\pi \times [0.75 \times 0.6^2]$

$V = 4/3 \times 3.14 \times 0.27$

Volume = 1.13cm^3

(tumor is within guidelines)

Example 2: multiple tumors in mouse

- Two tumors, measurements: Width: 1.2cm, Length: 1.5cm and Width: 1.4cm, Length: 1.7cm.
- Mouse weighs 22.5g.
- Non-metastatic study
- Tumor 1 volume calculated from chart in Annex 1: 1.13cm^3
- Tumor 2 volume calculated from chart in Annex 1: 1.74cm^3
- Total tumor burden: 1.13 + 1.74 = 2.87cm^3
- Tumor burden weight presuming density of 1 cm^3 = 1g: 2.87g
- "True" animal weight = Animal weight including tumor – tumor weight
- "True" animal weight: 22.5 – 2.87 = 19.63g

Maximum allowable tumor burden is 10% of "true" animal weight

19.6 × 10% = 1.96g = 1.96cm^3

2.87cm^3 > 1.96cm^3

(tumor burden exceeds guidelines)

Example 3: model of metastasis originating from palpable, multiple mammary tumors in mouse

- Four mammary tumors
- Tumor 1 volume calculated from chart in Annex 1: 2.12cm^3
- Tumor 2 volume calculated from chart in Annex 1: 0.47cm^3
- Tumor 3 volume calculated from chart in Annex 1: 1.14cm^3
- Tumor 4 volume calculated from chart in Annex 1: 0.38cm^3
- Total tumor burden: 2.12 + 0.47 + 1.14 + 0.38 = 4.11cm^3

Maximum allowable tumor burden is 6.0cm^3

4.11cm^3 < 6.0cm^3

(tumor burden is within guidelines)