Cancer Models Guidance

The OBSERVE guidelines OBSERVE Guidelines were developed by an expert group of laboratory animal veterinarians, animal welfare officers and researchers involved in preclinical research with an interest in murine cancer studies.

The following document is a summary of the OBSERVE guidelines which is more applicable to the work carried out at the University of Cambridge.

Refinements during Implantation or Induction of Tumours

Consider if there are previously established models, by doing a thorough and critical literature review, including being aware of what's not being reported as well as what is (e.g. success rate, complication rate, adverse effects not reported etc). Communicate with the authors, collaborators, and others in the field to find out any missing information. For UK work, the UBS NVSs and NACWOs can also help make contact.

- The model: tumour growth pattern, metastasis, paraneoplastic syndromes (e.g. cachexia), pilot studies, engraftment success, graft-vs-host-disease (GvHD), predisposition to infectious diseases.
- The mouse: correct background strain, immunocompetent or immunocompromised.
    - **Humanised Mouse Models:** The adverse effects to be expected here is Graft-Versus-Host-disease (GvHD), tumour growth inhibition, efficiency impairment of lymphocytes and anaemia. These can be prevented by considered selection of engraftment strategy and strain of immunodeficient mouse e.g. models with rapid onset of GVHD should not be used when study duration is longer than 4–5 weeks.
- The tumour:
    - PDX – actively dividing cells from non-ulcerated, non-haemorrhagic, non-necrotic tumours and screened for mycoplasma and other agents of human H&S concern.
    - Cell lines – viable, proliferating, <80% confluent, biologically screened for mouse pathogens appropriate to the unit barrier, mycoplasma and other agents of human H&S concern.
- The Surgery: Aseptic (PILh, trained and competent); appropriate anaesthetic and analgesics; appropriately sized surgical instruments, needles and surgical/ incision site; separate surgical tools for handling the tumour tissue to prevent unwanted spread of tumour cells; all in accordance with local guidelines.

Subcutaneous Tissue Implantation – Dorsum or Flank

TO NOTE: Orthotopic implantation gives better growth for some tumours (e.g. breast cancer) and achieves better engraftment (e.g. PDXs).

Single site ideally, but no more than 2 different sites – to enable maximum distance between implants and minimal adverse effects, fusions, effects on vital functions (e.g. locomotion, eating/drinking, defecation/ urination, cleaning, breathing etc).

Mammary Gland Tumour Implantation

Implantation into the 3rd or 4th mammary gland is recommended as these sites are less likely to cause adverse effects or affect vital functions. The most cranial (1st) and most caudal (5th – mice, 6th – rats) should only be used with clear scientific justification.

The mammary fat pad implantation – it is no longer recommended to clear the fat pad for endogenous mammary tissue. The tumour cell suspension is injected by slightly pulling out and exposing the fat pad through an incision size less than 3 mm instead of through a large V-shaped incision.

The maximum volume of Matrigel or Cultrex to be injected into the mammary fat pad in mice is 20ul (2nd gland) and 30ul (4th gland) and tissue fragments should not exceed 3 x 3 mm. For similar work in rats, please discuss with your NVS.

Intraductal (MIND) injection does not necessitate cutting the teat, however dead skin needs to be removed with dissection forceps – a stereoscope and 30-34G needle should be used to inject directly into the teat canal, maximum 10-20ul (mice) and 60ul (rats).

Intracerebral Tumour Implantation/ Injection (Stereotactic Surgery)

Local anaesthetic (LA) of the skin prior to incision should be used, and LA containing adrenaline will reduce skin bleeding during surgery. Non-rupture ear bars should be used, these fit under the ear. The implantation site will be specific to the tumour and scientific requirements and so needs consideration. Be aware to measure the depth of implantation from the dura not the skull (as skull thickness can change with age, e.g. in rats).

Wipe the needle for injection after loading if the cell line is aggressive, to reduce tract seeding. Following needle insertion wait 1-2min for the brain tissue to acclimatise, inject with a maximum speed 1ul/min, maximum volume 5ul. Wait 1-2min before needle extraction after injection. Wash the skull with saline to avoid ectopic tumour growth.

Intrapulmonary Tumour Implantation

- Implantation through the trachea:
    - Surgical Tracheostomy: is an option but comes with adverse effects and surgical risk.
    - Non-surgical installation: through intubation (e.g. with fibreoptic light, surgical microscope, external light source, and/or intubation frame) is the most refined and preferred method and results in a single, not multifocal, tumour which makes volume size and assessment easier. Cannula should

be as delicate and small as possible e.g. intravenous catheter (plastic) altering its length to fit the mouse size so as not to go into the lung. Maximum volumes 30-50ul (mice and neonatal rats) and 100ul (adult rats).

Tumour Implantation into the Abdomen

Orthotopic into organs e.g. intestines, pancreas, liver, urogenital, may require laparotomy. Incision length must be minimised, handle the organs gently using sterile cotton buds if needed, and maintain moisture if organs exteriorised with sterile gauze and warm saline. Avoid accidental spillage of tumour cells into the abdomen – use appropriate needle sizes and volumes to be injected. Close the peritoneum/muscle and skin, separately, with sutures.

Use multimodal analgesia (not just NSAIDS) in consultation with the NVS. This is a painful procedure.

Non-surgical implantations may offer refinement alternatives, e.g.:

- Intrarectal implantation of intestinal tumours, or transurethral implantation of the bladder/ kidneys
- Ultrasound Image Guided injections possible for the liver, spleen, kidney (including subcapsular), pancreas, adrenal glands, bladder

Intravenous Tumour Injection

The lateral tail vein is typically used in mice and the same refinements exist as for IV injection of substances or compounds. Use the smallest possible needle, preheat the animal to circa 37°C, use an appropriate restrainer.

Hydrodynamic Tail Vein Injection

High volumes (≤10% of BW) of cells/ vectors/ plasmids are injected rapidly (≤10s) intravenously to cause expansion of the liver (to promote uptake into the liver of the injected agent), but consequently drops arterial blood pressure, causes cardiac and respiratory disruption (including arrest) and analgesics are required. The UK Home Office also advocates this being performed under recovery anaesthesia. Mice must be under close observation for at least an hour post injection.

Intracardiac Tumour Injection

Used to study brain or bone metastasis. Ultrasound image guidance of needle is required, and there is a high risk of tumour cell clumping thereby causing thrombo-embolisms, cardiac arrest and death.

Intra-bone marrow/intra-osseous Tumour Injection

Intrafemoral is better tolerated by mice than intratibial injections. 30G needle is better than 26G for intratibial injections, however. Intraosseous, rather than para-tibial, does result in a more clinically faithful disease model. This should be limited to only one leg per animal.

Carcinogenic Tumour Models

Carcinogen-induced tumours are unpredictable with timing, location, number of tumours and this variation comes with carcinogen used, dosage, mouse strain, age, sex, individuals. Therefore, literature reviews and pilot studies are essential.

Pilot studies should be used to identify toxicity, lowest effective dose, minimal side effects, optimal route of administration, effects on animal welfare and determining appropriate humane end points.

Tumour Resection Models

Surgical resection of primary tumours can be performed in several settings including models of spontaneous metastasis and models of advanced disease in tumours wherein resection is usually the first treatment in clinic. Pilot studies to ascertain the appropriateness of this approach should look at: recurrence of primary tumour, metastasis development, extended survival time post resection, as well as the optimal time for resection (scientifically and for animal welfare/ experience). Tumour resection timepoints will be unique to the study, and should not be done as standard when the primary tumour reaches maximal allowed sizes.

Tumour-bearing mice are to be considered not-healthy therefore at increased risk of surgical and anaesthetic complications – peri-operative and post-operative care should be adapted accordingly, in consultation with the NVS and Surgical Technicians.

Clinical Signs

Each type of tumour will have its own profile of clinical signs which will be specific to the tumour cells and mouse strain used. Where possible this information should be sought before beginning any studies and reviewed frequently based on what signs are seen in your specific studies.

The table below includes some clinical signs that are frequently seen regardless of tumour type and location, and some signs are more likely based on the body system involved.

Body System	Clinical Signs	What to look for/ monitor
Any	Anorexia/ weight loss	Frequent weighing of animals, body condition scoring, monitoring of food and water intake and faecal output, consider how a growing tumour may impact body weight (see below section).
	Pain	Grimace scales, behaviour changes e.g. poor nest building, reduced movement.
	Fluid in abdomen (ascites)	Pot-bellied appearance, usually soft on palpation.
	Low red blood cells (anaemia) or icterus (jaundice)	Observe colour of skin and mucous membranes e.g. lips, eyelids for paleness or yellowing.
	Dehydration	Change in activity – hyperactivity progressing to reduced movement. Positive dish test (offering water to the animals in a dish, if they immediately drink, they are likely to be dehydrated). Possibly skin tenting, sunken eyes.
Gastrointestinal	Diarrhoea, blood in stool, rectal prolapse, constipation	Check cage for increased or decreased faecal output and any blood. Check mouse for any faeces or blood around anus. A swollen abdomen may suggest constipation.
Urogenital	Blood in urine, increased or decreased urination	Check cage for wet bedding and any blood. Check for increased water consumption (where possible), abdominal palpation to check for enlarged bladder.
Neurological	Circling or rolling, head tilt, wobbly gait (ataxia), paresis, paralysis, seizures	Visual observations either in home cage or on a flat surface, a grip test may allow early detection of paresis.
Musculoskeletal	Lameness	Watch animal moving on a flat surface.
Respiratory	Laboured breathing (dyspnoea), Low oxygen in the blood (hypoxaemia)	Watch breathing pattern for signs such as breathing through mouth, abdominal effort, blue discoloration of skin (cyanosis).
Subcutaneous	Ulceration, impedes movement	Observe animal in home cage.

Monitoring

Tumour Type	What to monitor	How to carry it out
Cutaneous Tumours (SC, Mammary and Melanoma)	Visual/Caliper	Correctly position caliper at widest, longest and deepest tumour region. Gentle manipulation is required, and no direct pressure should be placed on tumour.
Oral Cavity	Visual Inspection and Palpation	Using forceps and light pressure, gently withdraw the tongue. For larger tumours, animals are placed in a supine position. Anaesthesia may be required.
Intra-abdominal	Palpation	Place the mouse or rat on the cage grid and gently lift by the tail. Palpate the abdomen by smoothly going up and down with index finger and thumb of the non-fixing hand. If soft palpation is tolerated, proceed by slowly increasing the pressure. A larger resistance can easily be detected in the upper and/or lower abdomen. Use a scoring system based on tumour size relative to the mouse/rat hemi-pelvis or midline (e.g. zero = nonpalpable, 1 is <25%, 2 is 26–49% and 3 is >50%).
Hematopoietic	Palpation	Restrain the mouse and place in a supine position. The spleen, located in the left quadrant, can be palpated with the index finger and thumb of the non-fixing hand. Use a scoring system based on extension of the spleen (e.g. 0 = normal spleen; 1 = spleen extends halfway between left rib cage and first quadrant line; 2 = spleen extends to the median line; 3 = spleen extends halfway between median line and right rib cage; 4 = spleen extends to the right rib cage).
Firefly-luciferase cell lines/tumours	Light emission	Perform bioluminescence imaging (BLI) to measure luciferase activity. Administer luciferin substrate before imaging. Gently restrain mice/rat to administer via the appropriate route. Anaesthesia is required for imaging.
Fluorescent cell lines/tumours	Fluorescence light emission	Use fluorescence imaging. Calibrate equipment for specific fluorescent markers used. Ensure mice are restrained or anesthetized for consistent imaging conditions.
Orthotopic tumours	Volume	Conduct imaging (CE-CT, CT) and physical examination for localized and metastatic assessment. Anaesthesia is required for imaging. - CT: ionizing radiation from different angles produce cross-sectional images. - CE-CT: administer iodine-based contrast agents via oral gavage, intraperitoneal or intravenous injection to allow tumours to be visualized via negative or positive contrast.

Soft tissue and bone tumours	Magnetic resonance imaging (MRI)	The use of strong magnetic fields to generate images of soft tissues and bone tumours. Anaesthesia is required for imaging.
Oesophagus Colorectal	Volume Luminal occlusion	Endoscopy to evaluate ulceration, inflammation, bleeding and to assess tumour size, number and luminal occlusion. Anaesthesia is required for imaging.
Hematopoietic	Percentage human leukemic cells in blood Total white blood cells	Use flow cytometry to define % of leukemic cells. Haematology analysis to measure total white blood cells.
Transducable cell lines	Secreted Gluc reporter in blood, serum or urine	Gluc reporter assay can be used to monitor tumour growth, therapeutic response and linearity between Gluc reporter signalling and cell number. Only requires a few microliters of blood and assaying for activity. Can be used in combination with bioluminescence to localise tumours.
Cutaneous tumours	Volume	Three-dimensional (3D) through biovolume can be used to calculate tumour volume. Modality currently is only validated for mouse tumours.
Intra-abdominal and lung	Volume	Using ultrasound, sound waves are reflected by the tissues with different properties. Ultrasound waves are therefore used to acquire images. Measure the tumour dimensions (length, width and depth) to calculate the volume. Anaesthesia may be required for imaging.
Radiolabelled cells and tumours	Tumour-specific biomarkers and emission of gamma rays	Positron emission tomography (PET)/single-photon emission computed tomography (SPECT). The visualization of molecular aspects and metabolic alterations in highly metabolically active tumour cells upon injection of radioactive tracers. Anaesthesia is required for imaging.

Welfare monitoring sheets

Creating a welfare sheet to monitor clinical signs, (i.e. adverse effects), supports action to mitigate them and determine the humane endpoints in a robust and consistent manner. Welfare monitoring sheets should be created in consultation with the NACWO, NVS, PIL holders and animal technicians and periodically reviewed to determine if any enhancements can be made.

The signs monitored should be related to tumour type, its location and the expected effects on body system as detailed above. Monitoring of animals should be based on the expected adverse effects, but it is important general signs are also monitored e.g. body weight loss, body condition scoring*, hydration, appearance/behaviour, signs of pain** (some of which are listed below, grimace scales should also be considered***).

- Behaviour: decreased activity, reluctance to move, or aggression when handled,
- Posture: abnormal gait or posture, or back-arching,
- Grooming: excessive licking or chewing of a body part or area, or repetitive grooming,
- Vocalisations: making sounds,
- Withdrawal: withdrawing a body part from a painful touch,
- Appearance: rough, greasy-looking coat, or un-groomed appearance,
- Other: decreased appetite, eating of bedding material, or flight responses.

Body weight and tumour weight****

How to determine baseline body weight (BW)

BW loss (actual weight compared with baseline weight) is often used to determine humane end points. In studies where tumours are implanted in adult animals, baseline BW = BW at the start of the study. When animals are implanted at a younger age, BW at study start might not be the correct value to assess weight loss, especially in slow-growing tumours. Other options could be e.g. weight loss measured over past 7 days or use of growth curves.

Illustrative example: an 18 g mouse is implanted with a slow growing tumour. During tumour development the animal grows to 25 g. Assume the mouse starts to show adverse effects from then on, including weight loss. Humane killing upon 20% weight loss based on 25 g baseline weight should be carried out when the animal reaches 20 g. Considering 18 g as baseline BW would imply humane killing at 14.4 g or 42% BW loss. Therefore, baseline BW in growing animals (especially in slow growing tumours) should be based on growth curves and/or discussed with the NACWO or NVS.

BW increase in tumour studies

Although BW loss often occurs during mouse tumour studies, some models may also cause an increase in BW (e.g., due to ascites or splenomegaly) and can even aid in determining humane endpoints.

Should we consider tumour weight when measuring BW?

Although tumour volume is often measured in studies and we know that 1 cm3 of tumour weighs 1g (see table below), tumour weight will only remarkably influence weight (>10%, indicated in boldface) in very small mice (<20 g) or in very large tumours (exceeding the criteria for humane killing) in mice. Consider using larger mice in long-term studies or when studying fast-growing tumours. This only applies to mice and not rats since the tumour/BW ratio is very different in rats compared with mice. The table below shows the percentages of tumour weight as a function of mouse weight.

Tumor		Mouse weight (g)												
Volume	**Weight**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
		Percentage of tumor weight in function of BW												
0.5 cm^3	0.5 g	2.8	2.6	2.5	2.4	2.3	2.2	2.1	2.0	1.9	1.9	1.8	1.7	1.7
1 cm^3	1 g	5.6	5.3	5.0	4.8	4.5	4.3	4.2	4.0	3.8	3.7	3.6	3.4	3.3
1.5 cm^3	1.5 g	8.3	7.9	7.5	7.1	6.8	6.5	6.3	6.0	5.8	5.6	5.4	5.2	5.0
2 cm^3	2 g	**11.1**	**10.5**	**10.0**	9.5	9.1	8.7	8.3	8.0	7.7	7.4	7.1	6.9	6.7
2.5 cm^3	2.5 g	**13.9**	**13.2**	**12.5**	**11.9**	**11.4**	**10.9**	**10.4**	**10.0**	9.6	9.3	8.9	8.6	8.3
3 cm^3	3 g	**16.7**	**15.8**	**15.0**	**14.3**	**13.6**	**13.0**	**12.5**	**12.0**	**11.5**	**11.1**	**10.7**	**10.3**	**10.0**

Examples of 'traffic light' welfare assessment sheets:

Health monitoring for Brain Tumour M(

Traffic Light System for monitoring papi

Traffic Light System for NSG abnormal b

Traffic Light System for Abnormal Behavi

Further reading

Guidelines for the welfare and use of animals in cancer research: Workman, P., Aboagye, E. O., Balkwill, F., et al. (2010) Guidelines for the welfare and use of animals in cancer research, British Journal of Cancer, 102 (11), pp. 1555-1577

Workman guidelines for the w

References

* Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice

Body Condition Scoring A Rapid anc

** A Review of Pain Assessment Methods in Laboratory Rodents

*** Grimace scale: Mouse | NC3Rs

**** De Vleeschauwer, S. I. et al. *Nat. Protoc.* https://doi.org/10.1038/s41596-024-00998-w (2024).