Rodent Tumor Production and Monitoring Guidelines

General

Tumor production in research animals is a critically important experimental activity, which requires consideration of the effect of the tumor or tumors on the animal. The University of Maryland School of Medicine (UM SOM) Institutional Animal Care and Use Committee (IACUC) is committed to supporting all personnel engaged in research involving tumor burden in animals by supplying guidelines that will ensure their projects follow appropriate regulations and conduct their studies as humanely as possible. Effective monitoring systems and endpoints, which are crucial components of any spontaneous or implantable tumor model, should include limits on the tumor burden and severity of tumor-associated disease. The criteria for undertaking research utilizing tumor models are detailed below. Deviations from the guidelines presented here must be addressed and justified when submitting your proposals to the IACUC for review.

Implantable and inducible tumors

Rodent Pathogen Testing[1]

Because transplantable tumors, hybridomas, cell lines, and other biologic materials can be sources of murine viruses that can contaminate rodents, all transplantable murine tumors must be assayed for contamination with adventitious murine viruses to prevent the possible spread of pathogens into our rodent colonies. Additionally, if tumors are derived from human tissues the requirement for Institutional Review Board (IRB) approval should be discussed, all human cell lines and tissues must be registered with Environmental Health and Safety (EHS) and all staff must take the Bloodborne Pathogen Training[2] from EHS. If animal pathogen testing has not been done, this service is provided by Veterinary Resources on a fee for service basis.[3]

Implantation Sites

Tumor implantation sites should be chosen to minimize damage to adjacent normal structures. The IACUC recommends implanting tumors on the dorsum or flank of an animal, as these areas will likely have the least amount of site-related morbidity. If other sites are to be used, they must be described and justified in the IACUC protocol.

The protocol must include detailed information regarding the preparation and implantation procedures including adherence to aseptic technique. The protocol must also include information regarding gauge of needle to be used, and the specific site of injection / implantation. If more than one site is being implanted, please justify and discuss issues such as immobility, etc. When indicated, describe the volume to be injected including concentration (i.e., 1 x 10^6 cells in 100 µl PBS). Please note that biosafety level 2 practices should be used when handling tumor cells.

Evaluation of Tumors

Evaluating tumor burden based only on a percentage of body weight is generally not accurate while the growing tumor(s) may cause an increase in body weight, the general condition of the rodent may be decreased (loss of lean body mass), resulting in a relatively stable body weight but an unhealthy animal.

The evaluation of tumor burden must be clearly described in the protocol. The following criteria should be used for the evaluation.

- Body condition score (BCS). *Refer to the bottom of this document for more information.*
- Objective dimensional criteria (size)
- Anatomical location
- Incidence of multiple tumors
- Tumor ulceration

The guidance below assumes that a normally sized adult rodent will be studied (a ~25 g mouse or a 250+ g rat). The allowable sizes of tumors will be decreased if the tumors are injected into immature or genetically small mice.

Tumor Size and Location

The concern of size for individual tumors is related to central necrosis, ulceration of skin overlying tumors, interference in mobility and abrasions. When tumors occur on the dorsum or flank of adult rodents, tumors may be allowed to grow to a diameter of 2.0 cm (or 4.2 cm^3) in mice and 4.0 cm (33.5 cm^3) in rats at their widest point as long as the rodent remains otherwise healthy.

Multiple Tumors

Multiple tumors, that are individually smaller than the single tumor limit, may not have the same negative sequelae as a single tumor. Multiple tumors may be allowed to grow up 150% (or 6.3 cm^3) of the volume compared with the volume of a single tumor. Please note that the limitation on any single tumor (2.0 cm diameter in mice) will still be valid.

Tumor Ulceration

Ulceration (*overt open lesion or scabbed area*) of a tumor does not necessarily require euthanasia, but it does require more frequent monitoring and potentially treatment, as defined below. The level of follow-up care for ulcerated tumors is based on both the size of the ulceration and the clinical judgment of the veterinarian.

> Pinpoint (≤ 1mm) ulcerations at the site of tumor injection must be monitored at least 3 times per week for worsening of the ulceration site.
>
> Ulcerations (> 1mm) of the surface area of the tumor shall be monitored at least 3 times per week and must be reported to the veterinary staff for evaluation and potential treatment.

Treatment of ulcerated tumors will be determined by the veterinarian or can be described in the approved protocol.

Monitoring and Endpoints

Animals with subcutaneous tumors or tumor implants should be monitored at least 3 times per week and daily as tumor(s) approach 80% of the maximum size limit. Subcutaneous solid tumors can be measured with calipers; as stated above the maximum diameter in any one direction for mice and rats is 2 and 4 cm, respectively. The visible size of the tumor is only one of the criteria used for determination of humane endpoint. The overriding consideration for humane endpoints of oncological experiments as well as spontaneous tumors must be the overall health of the animal.

Evaluating tumors that are not palpable (e.g. leukemia) and tumors located in other areas of the rodent's body (e.g. bone, brain, lungs) can be challenging. Tumor size will likely not be useful due to inability to measure size or because of the sensitivity of areas to compressive lesions. For these models, the BCS and clinical evaluation of the animals take priority regarding decisions on humane endpoints. The expected clinical signs and the humane endpoints of those signs must be clearly described in the protocol. A scoring system may be most helpful in this scenario.

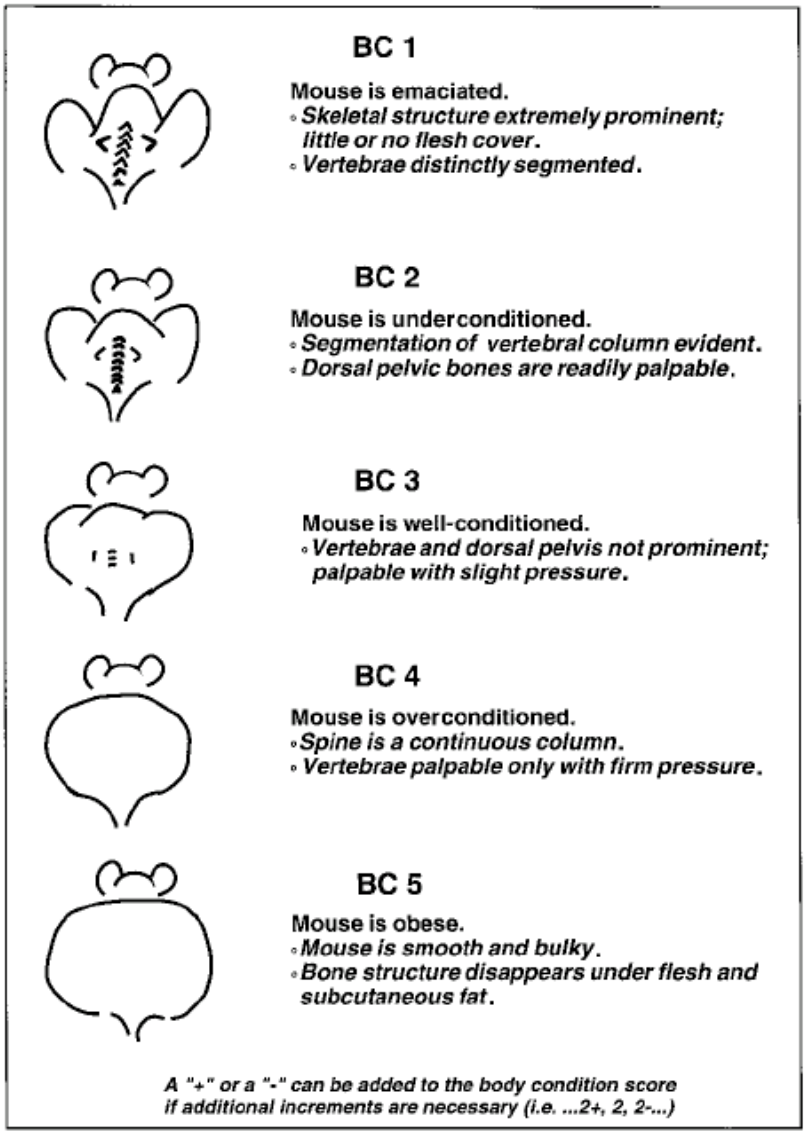
Additionally, the maximum time for the tumor to establish must be described in the protocol. The evaluation of clinical signs in an animal with a tumor burden of this type should include consultation with a Veterinary Resources veterinarian. The overriding concern should be the health and welfare of the animal.

An animal in chronic pain or distress that cannot be relieved by analgesics must be euthanized unless prior scientific justification for these humane endpoints is approved by the IACUC. Endpoint guidelines are available on the Office of Animal Welfare website and should be incorporated into the protocol, e.g., weight loss / gain compared to age-matched animals of the same strain and gender (control animals or published weight curves).

As with all animal monitoring described in an approved protocol, investigators must maintain records of tumor monitoring (including date and size) for review by the IACUC or other regulatory / accrediting body.

The following BCS should be used, especially in rodents with tumors within the body cavity.

Body Condition Score (BCS)



NOTES:

1. Commercial sources do not routinely provide human or animal pathogen testing, so all cells intended for implantation must be tested.
2. Investigators and research staff handling human tissues, cells, or cultures are to assume that they contain bloodborne pathogens. Animal Biosafety Level 2 standards must be applied and adhered to with their use. All staff are required to take the Bloodborne Pathogen Training offered by Environmental Health and Safety (EHS). Please note it is the investigator's responsibility to inform all research staff of the possible risks associated with handling human tissues, cells, or cultures, and to assure this training has been completed. A reference article relative to the risks associated with human xenograft transplantation in animal research is located on the OAWA Website under Rodent Tumor Production and Monitoring Guidelines.
3. Animal pathogen testing is routinely required every 3 years to assure that testing has been performed for all potential pathogens. (A cell line tested 2 – 3 years ago for the presence of animal pathogens may not have included the currently applicable pathogens and/or introduction of pathogen by animal passage.)
4. Studies involving the use of genetically manipulated tumor cells, recombinant DNA or potentially pathogenic microorganisms must be registered with UMB Environmental Health and Safety (EHS) Institutional Biosafety Committee. Please contact EHS and forward a copy of their final approval for the protocol file. IACUC approval will not be granted until EHS approval has been obtained.