IACUC Policy for the Utilization of Rodents in Experimental Neoplasia and Ascites Production
GUIDELINES FOR THE UTILIZATION OF RODENTS
IN EXPERIMENTAL NEOPLASIA AND ASCITES PRODUCTION

The use of laboratory animals, especially rodents, as animal models in experimental neoplasia has led to significant increases in the understanding of tumor biology, therapeutics, radio- and immunobiology, carcinogenesis, and other areas of study. In 1975 Kohler and Milstein determined that fusion between myeloma cells (a tumor of bone marrow) and antibody-producing cells produces a hybridoma which secretes large quantities of monoclonal antibody. This discovery has had immeasurable effects on biology and medicine and led to a Nobel Prize. Although the use of animal models in these studies are justifiable, investigators must recognize that animals may experience pain and distress when employed in this manner.

Recommendations provided in this discussion are intended to reduce the pain and distress that can occur when rodents are utilized as animal models for experimental neoplasia or for ascites production. It is extremely difficult to establish precise guidelines that apply to all cases and tumor types used in in vivo systems without being severely restrictive. These guidelines should be used as a foundation for designing and completing an Animal Care and Use Protocol, and for conducting experimental procedures. Appendix I contains highlights of the discussion below and specific recommendations for experimental studies. Deviations from these guidelines should be addressed and justified when submitting your proposals to the Institutional Animal Care and Use Committee (IACUC) for review. Contact a member of the Research Animal Resource Center’s veterinary staff @ (646) 888-2400 or (212) 746-1022 for additional information or for consultation on experimental design.

Understanding the biology of the tumor or system you intend to employ is critical to the study design and to ensure that pain and distress are minimized for the animal subject. For example, tumors which have a propensity to metastasize may have entirely different effects on the animal as compared to tumors which infiltrate locally. Replication times differ with tumor type and will determine the frequency at which the animals must be observed and the duration of the study. Tumors induced by carcinogens or viruses compound the situation in that these inducing agents may pose additional problems for the animal host. Before an Animal Care and Use Protocol (ACUP) is submitted, a literature review must be conducted, and other investigators who have used similar (if not identical) experimental methods and/or tumors should be consulted. If using a new model with little or no historical data, a pilot study, approved on an ACUP, using a small number of animals (5-10) is recommended to establish the pattern of local and metastatic growth. Additionally, a pilot will help define any adverse effects of the tumor and enable humane endpoints to be identified.

The site of tumor implantation is an important consideration regarding potential pain and distress. Sites should be chosen that minimize damage to adjacent normal structures and will not interfere with normal body functions such as ambulation, eating, drinking, defecating, and urinating. Sites involving the special senses, such as the eye, should be avoided. Intramuscular implantation should not be routinely utilized as distention of muscle with the growing tumor is painful. Subcutaneous or intradermal implantation in the flank is least painful and preferred. Always utilize the least number of cells in the smallest volume possible. For example, when inoculating into subcutaneous sites, 1-5 million cells in 100µL is recommended. For orthotopic
sites, smaller volumes are preferred (see administration guidelines). If the method of tumor implantation involves a surgical procedure, the procedure itself may result in pain which should be managed appropriately with analgesics.

**Tumor burden** is one of the most important factors to consider in regards to animal health and well-being. It is extremely difficult to provide precise guidelines for upper limits of tumor burden as these are dependent upon a number of factors, including but not limited to tumor biology, implantation site, and host status. Animals with tumor burdens large enough to interfere with ambulation, eating, drinking, defecating, and urinating should be euthanized. Tumors generally should not exceed 10% of the animal's body weight but it is crucial to recognize that an animal may require euthanasia with smaller tumor burdens.

The *in-vivo* tumor volume calculation is always an approximation. The natural growth of a subcutaneous tumor most closely resembles that of an ellipsoid (or oblate spheroid). Derived from the basic formula for an ellipsoid, the most accurate calculation for the volume of a tumor using calipers is arrived at by measuring its length (L) and width (W) and calculating:

\[
\text{TUMOR VOLUME} = \frac{(W^2 \times L)}{2}
\]

If the dimensions are determined in centimeters, the resultant volume in cubic centimeters is roughly equivalent to the tumor weight in grams.

Ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and bioluminescence imaging are other, more advanced and non-invasive methods for measuring tumor size or volume which are particularly useful for models that do not use subcutaneous tumors (see references for more information).

Tumors may secrete factors which cause severe morbidity independent of tumor size, location, or other aspects of their biology. These animals may become severely debilitated before the tumor has attained appreciable size. Body weight should be monitored when tumors exhibiting these properties are utilized. Study termination will be dependent upon factors other than tumor burden, i.e. percentage of weight loss, etc.

Tumors, like other biologics, may carry viruses or other pathogens which can contaminate animal colonies, infect humans, and introduce additional experimental variables. All tumors, especially those which have been passed in animals, should be screened to determine their microorganism carrying status. Xenografts of human tumors which are typically grown in immunodeficient hosts may be contaminated with human pathogens and require the implementation of appropriate biohazard containment procedures. The use of human xenografts also requires appropriate training of staff, as described on the IACUC Protection and Control Form. Please see the *Guidelines for Conducting Studies in Rodents Transplanted with Human Xenografts, Cell Lines or Cell Strains* for more details. Guidelines are available: MSKCC- ([https://encompass.mskcc.org/Documents/11985214](https://encompass.mskcc.org/Documents/11985214)) and WCMC- ([https://encompass.mskcc.org/Documents/11985278](https://encompass.mskcc.org/Documents/11985278)). For xenografts of rodent origin, the xenograft must be screened as the xenograft may be contaminated with biological agents that can result in colony contamination. A sample of the xenograft must be submitted to the Laboratory of Comparative Pathology (LCP) for analysis. Samples are submitted using the Requisition Form for Screening of Biologicals: MSKCC-
Ascitic tumors such as hybridomas require special consideration. These tumors, when grown in the peritoneal cavity, will produce both a solid mass and ascitic fluid. As the volume of the abdominal cavity is limited, severe distention will develop. Distention interferes with a number of physiological systems including, but not limited to, the respiratory and gastrointestinal systems. Ascitic tumors producing large volumes of fluid can rapidly deplete the animal of essential nutrients such as protein and hasten cachexia from the tumor. Care must also be taken when ascitic fluid is collected from surviving animals as the rapid removal of a large volume of fluid may cause hypovolemia, renal insufficiency, and edema. In general, only tumors that cause ascites in the natural human condition are appropriate for use in animal models.

In vitro alternatives to ascites production are available for production of monoclonal antibodies (Mabs). A variety of tissue culture methods have been employed that frequently yield quantities equivalent to or greater than those obtained in mice. Bioreactors have been successfully employed for production of monoclonal antibodies and have the added benefit of yielding antibodies in concentrations (mg/ml) equivalent to that harvested from mice. Both MSKCC and WCMC’s Animal Care and Use Committees require that production of Mabs must first be attempted in vitro prior to production in mice. MSKCC has a core laboratory for producing monoclonal antibodies. Please call (646) 888-2354 or visit their website at http://www.mskcc.org/research/monoclonal-antibody-core-facility for additional information.

Hybridomas may be grown as ascitic tumors in mice if in vitro production has been attempted and fails. Animals used to produce ascites must be observed at least once daily (including weekends) and often require multiple daily observations. Ascitic fluid must be collected or “tapped” before the abdomen becomes severely distended. In general, ascites fluid volume should not exceed 20% of the animal’s normal body weight. Because of effects of the tumor and ascites withdrawal described above, animals may not be tapped more than three times, with the third tap performed at the time of euthanasia. However, if the animal’s condition necessitates, it may be necessary to euthanize the animal earlier than planned. Abdominal ascites, dyspnea, and piloerection are signs that an animal has reached its endpoint. Ascitic fluid collection is performed by placing the smallest needle diameter possible (e.g. 22 gauge) that allows for adequate flow of fluid from the abdominal cavity of an anesthetized (preferred), or humanely restrained mouse. The abdomen should be swabbed with 70% alcohol before the sterile needle is inserted. The animal should be observed carefully for 15 minutes post collection for signs of distress. The Monoclonal Antibody Core Facility can produce antibodies under their Animal Care and Use Protocol, eliminating delays and the requirement for submission of a PI specific Animal Care and Use Protocol for ascites production.

Pristane (2,6,10,14 tetramethylpentadecane) is commonly utilized to prime the abdominal cavity of rodents prior to implantation of a hybridoma. Pristane is an irritant which prepares the abdominal cavity for seeding with the tumor and interferes with local lymphatic drainage, thus increasing fluid yields. Pristane must be used with caution, as it is toxic at doses only slightly higher than those used to prime mice. Pristane should be administered once seven days prior to tumor implantation with a maximum dose of 0.5 ml intraperitoneally. Studies in mice

https://one.mskcc.org/sites/pub/rarc/Pages/default.aspx or WCMC-http://intranet.med.cornell.edu/research/rarc/. Testing should be scheduled with the LCP in advance by calling (646) 888-2422.
indicate that doses as low as 0.1 ml yield ascitic fluid volumes equivalent to the 0.5 ml dose with less animal distress; therefore, use of a lower dose is strongly encouraged. Because pristane is an irritant and potential carcinogen it should be handled with gloves to minimize skin contact. Once pristane is administered, animals should be observed at least once daily (including weekends) for adverse effects. Moribund animals should be euthanized.

**Incomplete Freunds adjuvant** (IFA) has also been successfully utilized as a priming agent for ascites production. Adverse side effects with IFA are less than those observed with pristane and IFA has the additional advantage that tumors can be implanted into IFA primed mice as soon as 24 hours after its administration. The dosage of IFA is the same as pristane.

There are ethical, legal, and scientific reasons for ensuring that adverse effects are minimized when utilizing rodents in experimental neoplasia. Studies utilizing animals in experimental neoplasia and ascites production must have precise endpoints. **Death as an endpoint**, except in unusual circumstances approved by the IACUC, is not considered acceptable. When designing experiments and preparing Animal Care and Use Protocols, **humane endpoints** should be developed and precise criteria clinical for euthanasia should be provided. Criteria that should be considered include:

- Tissue necrosis or ulceration
- Tumors that interfere with normal activity
- Weight loss greater than 10% of baseline
- Body condition score of two or less (on a scale of one to five)
  - **BC1**
    - Mouse is emaciated
      - Skeletal structure extremely prominent
      - Vertebrae distinctly segmented
  - **BC2**
    - Mouse is under-conditioned
      - Segmentation of the vertebral column evident
      - Dorsal pelvic bones are readily palpable
  - **BC3**
    - Mouse is well-conditioned
      - Vertebrae and dorsal pelvis not prominent; palpable with slight pressure
  - **BC4**
    - Mouse is over-conditioned
      - Spine is a continuous column
      - Vertebrae palpable only with firm pressure
  - **BC5**
    - Mouse is obese
      - Mouse is smooth and bulky
      - Bone structure disappears under flesh and subcutaneous fat
- Clinical signs of illness such as hunched posture, respiratory difficulties, or decreased activity
- Multiple tumors (unless approved in your Animal Care and Use Protocol)
- Signs of infection
Whether animal euthanasia is dependent on tumor size or time post implantation, animals must be observed daily by the investigator, including weekends, to determine that morbidity and distress are minimized. If the biology of the tumor indicates that less frequent monitoring is appropriate, this should be described and justified in the Animal Care and Use Protocol. Attention should be given to the animal's overall appearance, weight, respiratory rate and pattern, pallor fecal and urinary output, size of the tumor, etc. Animals may require euthanasia before established guidelines or scientific endpoints if they appear in distress or moribund. The Research Animal Resource Center’s Veterinary Services staff should be contacted when moribund or distressed animals are noted that cannot be euthanized immediately; appropriate supportive and/or analgesic therapy may allow the experiment to continue humanely.
APPENDIX I

HIGHLIGHTS
of the
Guidelines for the Use of Rodents in Experimental Neoplasia and Ascites Production

A. *In vivo* experimental neoplasia

1. All transplantable tumors should be assayed for contamination with adventitious murine viruses.

2. Tumor implantation sites should be chosen to minimize damage to adjacent normal structures. Sites involving the special senses should be avoided; intramuscular implantation should be avoided as distention of muscle is considered painful. Subcutaneous or intradermal implantation in the flank is considered least painful.

3. Tumors generally should not exceed 10% of the body weight of the animal. However, when utilizing particular sites (i.e., intraocular implantation), growth should be severely restricted.

4. Tumors should not interfere with normal bodily functions (i.e., ambulation, eating, drinking, defecating, and urinating).

5. Animals should be euthanized prior to tumor ulceration.

6. Animals should be examined at least daily, including weekends, after tumor implantation. If the biology of the tumor indicates that less frequent monitoring is appropriate, this should be described and justified in the Animal Care and Use Protocol.

7. Endpoints of experimental neoplasia should be determined and specified in the experimental protocol. Death is generally considered an unacceptable endpoint unless clear justification is provided.

B. Ascites production (rodents)

1. *In vitro* techniques, for example, production of monoclonal antibodies in hollow fiber bioreactors, must be attempted before ascites production.

2. If *in vitro* production fails, rodents should be primed once with a maximum of 0.5 ml pristane. Preferably, a volume of 0.1 - 0.2 ml should be utilized. Incomplete Freunds Adjuvant (IFA) may be substituted for pristane.
3. Rodents should be examined **at least** daily (including weekends) once they have been primed with pristane or IFA and the procedures for myeloma implantation have begun.

4. Ascitic fluid should be tapped prior to **gross** abdominal distention and distress.

5. Rodents should not have ascites fluid harvested more than three times; the smallest diameter needle as possible should be used. The third harvest should be performed after euthanasia. Animals that are in distress (i.e., cachexic, not eating, or with increased respiratory rates) should be euthanized immediately. In general, ascites volume should not exceed 20% of normal body weight.

6. Humane Endpoints should be determined and specified in the experimental protocol. Death is not an acceptable endpoint with ascites production.
REFERENCES


NIH ARAC. Guidelines for Ascites Production in Mice. 2013.


