

Antibody Production/Neoplasia

Item 3 – Guidelines for the Utilization of Animals in Experimental Neoplasia and Ascites Production

Although, at present, there are often no replacements for utilizing animals to study neoplasia or for producing ascites, investigators must recognize that animals may experience pain and/or distress when employed in this manner. The guidelines set forth here are intended to reduce any pain and distress that may occur. Appendix I contains an overview of these guidelines for easy reference.

It is extremely difficult to establish precise guidelines that apply to all cases without being severely restrictive. These guidelines should be used as a foundation when designing experiments and may require some adaptation to conform to the specific model to be utilized. **Deviations from these guidelines must be addressed and justified when submitting your proposals to the Institutional Animal Care and Use Committee (IACUC) for review.**

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 subjects. For example, tumors with 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. Prior to submitting the IACUC protocol, a literature search and/or consultation with others who have utilized the model should be conducted to gain a complete understanding of how the model will impact the animal.

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, defecation and/or urination. Sites involving the special senses, such as the eye, should be avoided. Intramuscular implantation should be avoided as distention of muscle with the growing tumor may be painful. Subcutaneous or intradermal implantation in the flank is considered least painful and is preferred.

Some tumors have a propensity to ulcerate through the skin when implanted subcutaneously or intradermally, especially those that grow rapidly. Normally, this is secondary to the tumor disrupting and outgrowing its blood supply leading to necrosis of tumor and overlying skin. Factors released by this necrotic tissue may cause systemic illness. The resulting ulceration also compromises the protective barrier of the skin, allowing invasion of microorganisms which may lead to local or systemic infection. In general, tumor ulceration should be a criterion for euthanasia since it predisposes the animal to increased pain and/or distress and may complicate data interpretation since ulcerated tumors may behave differently (especially if infected).

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 an appreciable size. Body weight should be monitored when tumors which exhibit these properties are utilized, and a reasonable endpoint chosen based upon percent weight loss (i.e. 10-20%).

Tumor burden is one of the most important factors in consideration of 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, defecation and/or urination should be humanely euthanized. In general, tumor burden should not exceed 10% of the animal's body weight, although consideration for the above factors should dictate exactly where the endpoint should be set.

Tumors, like other biologics, may carry viruses or other pathogens which can contaminate animal colonies, infect man, and introduce additional experimental variables. All tumors, especially those which have been passed in animals, should be tested 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 institution of appropriate biohazard containment procedures. The Department of Laboratory Animal Resources and the Institutional Biohazard Committee should be contacted for additional information.

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 distension will develop. This distention can interfere with a number of physiologic systems including, but not limited to, the gastrointestinal and the respiratory systems. These tumors can also rapidly deplete the animal of essential nutrients such as protein which may hasten tumor-induced cachexia. 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 shock, renal insufficiency and secondary edema.

Animals with ascitic tumors must be observed at least once daily (including weekends) and often require more frequent observation. In general, ascites should not exceed 20% of the animal's normal body weight. Unless significant justification can be provided (based on tumor biology, collection method, rate of ascites production, etc.), collection of ascites should not exceed one survival tap and a second collection after euthanasia. This guideline is based upon the above complications, the need to use a large-bore needle for collection and the common inability to collect appreciable amounts of ascites once the tumor mass has increased significantly. The preferred method is a single collection at euthanasia. During survival collection of ascites, it is important to swab to abdomen with 70% alcohol prior to needle insertion and to closely monitor the animal for 15 minutes post-collection for signs of distress. Any animal in acute distress should be humanely euthanized.

Pristane (2,6,10,14-tetramethylpentadecane) is commonly used to "prime" the abdomen prior to implantation of hybridomas. It is an irritant which induces granulomatous inflammation and interferes with local lymphatic drainage, thereby increasing ascitic yields. Pristane has a low safety margin and is toxic at levels slightly higher than those used to prime mice. Pristane should be administered *only once* intraperitoneally at a maximum dose of 0.2 ml. A dose of 0.1 ml is usually sufficient and lower doses are associated with less animal distress. Because Pristane is an irritant and potential carcinogen, it should be handled with gloves. After Pristane administration, animals should be monitored at least once daily for adverse effects.

Incomplete Freund's Adjuvant (IFA) has also been used successfully as a priming agent for ascites production at the same dose as Pristane (0.1 ml). Adverse effects appear less than with Pristane and IFA has the added benefit that tumors may be implanted as early as 24 hours after priming (as opposed to 10-30 days with Pristane).

Studies utilizing mice in experimental neoplasia and ascites production must have precise endpoints. **Death as an endpoint is generally unacceptable and requires very strong scientific justification.** The IACUC protocol should include specific endpoints and guidelines for euthanasia. Animals **MUST** be monitored at least daily (including weekends) with particular attention given to the animal's overall appearance, weight, respiratory rate and pattern, color, fecal output, urinary output and tumor size. Animals may require euthanasia prior to the guidelines established in the IACUC protocol if it is determined that they are experiencing significant pain and/or distress. The veterinary staff of the Department of Laboratory Animal Resources should be contacted whenever there is any question concerning an animal's condition. Appropriate supportive and/or analgesic therapy (in consultation with the veterinarian) may allow the experiment to continue in a humane manner.

Adopted: 3/29/1999
Revised: 11/11/2013

Appendix I

Guidelines for the Utilization of Animals in Experimental Neoplasia and Ascites Production

Any deviations from these guidelines MUST be approved by the Institutional Animal Care and Use Committee (IACUC)

A. Experimental Neoplasia

1. All transplantable tumors should be assayed for contamination with adventitious viruses **prior** to use in animals.
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. Subcutaneous or intradermal implantation in the flank is preferred.
3. Tumors should not exceed 10% of the of the body weight of the animal and no one tumor may exceed 2 cm in diameter.
4. Tumors should not be allowed to interfere with normal bodily functions (i.e. ambulation, eating, drinking, urination and/or defecation).
5. Animals should be euthanized if there is tumor ulceration.
6. Animals should be examined **at least** daily after tumor implantation (including weekends).
7. Clearly defined endpoints should be established in the IACUC protocol. Death as an endpoint is generally unacceptable.

B. Ascites Production (Rodents)

1. Rodents should be "primed" **only once** with a maximum of 0.2 ml Pristane. Preferred dose is 0.1 ml. IFA may be substituted at the same volume.
2. Rodents should be examined **at least** once daily after priming.
3. Ascites volume should not exceed 20% of the normal body weight and collection should occur prior to gross abdominal distention or signs of distress.
4. The maximum number of ascites collections should be two, with the second at euthanasia. A single collection at euthanasia is preferred.
5. Clearly defined endpoints should be established in the IACUC protocol. Death as an endpoint is generally unacceptable.