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INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
Policy #10
EXPERIMENTAL NEOPLASIA IN RODENTS

In concurrence with
TTUHSC El Paso Assurance #D19-01056
and Federal Regulations and Guidelines

1. Purpose

Experimental induction of neoplasia presents concerns for animal welfare. In particular, the endpoint for the animal bearing the tumor must be clearly described and that endpoint must be approved by the IACUC prior to the initiation of any procedures.

Proposals that involve experimental neoplasia usually involve three types of studies, including those that 1) increase our understanding of biological mechanisms, 2) aid in the design of efficacious treatments, and 3) facilitate production of antibodies via ascites production.

A. The first goal describes studies of how cancer cells grow and behave. This policy is intended to limit the tumor burden an animal experiences to that which does not cause excessive pain or distress, but achieves the research goal.

B. The second goal involves studies of the response of neoplasms to chemical, radiologic or immunologic therapy. In this class of study, not only must the tumor burden be considered, but the effect of the treatment modality must also be evaluated.

C. The third type of study involves the production of experimental reagents (antibodies) by the injection of cell lines that retain properties of cancer cells. In this special case, the goal is to limit the volume of ascites liquid to that which does not interfere excessively with normal function of the animal.

2. Consideration of Alternatives

Outcomes of tumor studies vary depending on the species and strain of animals used, the route of administration used for the growth of transplantable tumors and the subsequent chemotherapy or other modality employed in cancer treatment studies. It is up to the investigator to determine whether alternatives to using live animals are available and are appropriate for their study.

It is very rare that “death as an endpoint” studies (i.e., survival studies) will be allowed by the IACUC. In considering such studies, the PI must examine all possible alternatives and present evidence to the IACUC that none are scientifically acceptable for the proposed outcome.

NOTE: Citing other studies in which ‘death as an endpoint’ has also been used is NOT a scientific justification.

3. Procedural Guidelines

A. <u>General guidelines</u>
All protocols involving experimental neoplasia in rodents must be consistent with the Humane Endpoints Policy (19). In addition to the Default Endpoints listed in Policy 19, the percentage of tumor mass to body weight and the animals' well-being must be considered for those superficial tumors that can be monitored by palpation and measurement. The following general tumor guidelines must be followed and euthanasia is required when:

a) Solid tumors exceed the sizes listed below (unless larger sizes are justified in the protocol based on the scientific needs of the study):

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Calculate the mass of the tumor using the following formula:
Mass = tumor volume (cm^3) = length x width x height of tumor measured using calipers.
Add the volume of multiple tumors together.
For mice, the total tumor volume may not exceed **1.2 cm^3, or 1.5 cm^3** for therapeutic studies
For rats, the tumor volume may not exceed **2.5 cm^3, or 2.8 cm^3** for therapeutic studies.

Clinical Signs Necessitating Immediate Intervention

b) Failure to eat or drink over a 24- to 48-h period resulting in emaciation or dehydration
c) Consistent or rapid body weight loss reaching 20% at any time or 15% maintained for 72 h compared with the pre-treatment weight of adult mice or age-matched, vehicle-treated controls. With some tumors body weight is a very poor indicator and muscle atrophy or emaciation is more useful. Body condition scoring provides a very useful indication of muscle loss (Ullman-Cullere and Foltz, 1999)
d) Persistent hypothermia
e) Bloodstained or mucopurulent discharge from any orifice
f) Labored respiration, particularly if accompanied by nasal discharge and/or cyanosis
g) Enlarged lymph nodes or spleen
h) Hind-limb paralysis or weakness
i) Anemia as indicated by symptoms such as pale feet, or hematological
j) measures
k) Significant abdominal distension or where ascites burden exceeds 10% of the bodyweight of age matched controls. Accurate determination is difficult but body girth is useful and a 20% increase should be the maximum normally allowed; similar to the appearance of a pregnant mouse
l) Incontinence or diarrhea over a 48-h period
m) Tumors that interfere with locomotion or cause abnormal vocalization, animal behavior or function

B. Hematological tumors

Hematological tumors or tumors induced in body cavities (cranium, orbit, abdomen, or thorax) may be more difficult to monitor for progression and may have additional limitations as to the maximum acceptable size or duration. These animals must be monitored very closely for any severe impairment in physiological or neurological function and be euthanized as soon as such signs become apparent. Humane endpoints pertinent to the model must be given in the protocol. For example, with brain tumors, the endpoints must reflect neurological deficits and/or cranial deformity.

C. Myelomas and Ascites Production

After inoculation with an ascites-producing tumor cell line, animals must be observed at least three times per week for the first week and daily thereafter to monitor the degree of abdominal distention and signs of illness and distress. Ascites fluid should be removed by peritoneal tap (peritoneocentesis) before abdominal distention is great enough to cause discomfort or interference with normal activity. Animals should be tapped before they have gained 20% of their baseline body weight. Three abdominal taps, with the last tap being terminal is permitted.
Animals should be euthanized if they become moribund (i.e., anorexia, palpable abdominal mass, huddling, hunched posture, increased respiratory rate and/or effort, lethargy, hypothermia, difficulty with normal ambulation, or ruffled coat).
Priming agent: each mouse can receive one single i.p. injection of 0.2 ml of pristane. Therefore, doses higher than 0.2 mL should only be used when lower doses have been demonstrated to be inadequate for the antibody to be produced. Freund's incomplete adjuvant can be considered as alternative priming agent and each mouse can receive one single i.p. injection of 0.3 ml.

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Exceptions to these guidelines may be taken when scientifically justified and approved by the IACUC.

D. Interventions

After the study has begun, any deviation from the default endpoints (including death as an endpoint), must be reported immediately to the Attending Veterinarian and LARC veterinary staff for clinical evaluation. For example, if the tumor severely impairs normal bodily functions or the animal appears to be in distress, the veterinarian will prescribe treatment/monitoring that may include humane euthanasia.

E. Pain category assignment

Assignment of pain category by the IACUC for studies involving tumor-bearing animals shall be in accordance with Policy 4: Pain Categories for Experimental Protocols. However, because each study and each tumor line is unique, the TTUHSC IACUC will review each protocol individually and consider circumstances that may impact the assignment of the appropriate pain category. In the event that the procedure being proposed will cause pain or distress and analgesics cannot be administered for scientifically justified reasons, the PI must describe additional methods for ensuring that discomfort, distress, and pain will be limited to that which is unavoidable in the conduct of this project.

Related Policies

Investigators must comply with all other institutional policies at TTUHSC El Paso and Federal Guidelines. This list includes, but is not limited to, the following:

1. IACUC Policy #6: Use of Complete Freund's Adjuvant and Other Adjuvants in Laboratory Animals
2. IACUC Policy #19: Humane endpoints Regarding Severe or Chronic Pain or Distress

References

1. Guidelines for the welfare and use of animals in cancer research. Workman, P., et al. (2010). *Br J Cancer*, 102, 1555-1577.
2. Guidelines for the welfare and use of animals in cancer research, P Workman, EO Aboagye, F Balkwill, A Balmain, G Bruder, DJ Chaplin, JA Double, J Everitt, DAH Farningham, MJ Glennie, LR Kelland, V Robinson, IJ Stratford, GM Tozer, S Watson, SR Wedge and SA Eccles. 2010. British Journal of Cancer 102:1555-1577.
3. Jackson, Lynn R., et al. "Monoclonal Antibody Production in Murine Ascites I. Clinical and Pathologic Features." Laboratory Animal Science, vol. 49, no. 1, Feb. 1999.
4. Peterson, Normal C. "Behavioral, Clinical, and Physiologic Analysis of Mice Used for Ascites Monoclonal Antibody Production." *Comparative Medicine*, vol. 50, no. 5, Oct. 2000, pp. 516–526.