This document provides information to be used when planning and performing studies involving the maintenance of tumors and hybridomas in rodents for research, teaching, or other purposes at The University of Texas at Austin. It is organized into four sections:

Section A – Requirements and Specific Considerations
Section B – Recordkeeping
Section C – References
Section D – Acknowledgements

### Section A – Requirements and Specific Considerations

1) Cell lines or tumor fragments of rodent origin (or non-rodent origin if they have a history of being directly passaged in rodents) must be tested for presence of murine viruses prior to initial introduction into the animal colony. Other biologicals obtained from animal sources should also be tested if they are used in conjunction with animal implantation, such as basement cell extract matrix formulations or mouse serum. Certification of negative status based on testing results from the vendor may be accepted, subject to veterinary approval. (Contact the veterinary group for information about testing options).

2) If implanting human cell lines or tissues is a component of the approved protocol, issues regarding occupational health will need to be addressed, which may require testing for human pathogens and/or housing and handling the animals under BSL-2 containment. (Contact the EHS group for more information and to determine if IBC registration is required in addition to IACUC approval.)

3) Transgenic and knockout animals that are genetically predisposed to develop cancer may have unpredictable tumor growth. Careful observation of tumor formation and growth is required when new lines are produced or received (and when mutations are moved onto a new background strain) so that appropriate animal care and routine monitoring parameters can be established.

4) Animals inoculated with cells producing solid tumors must be observed a minimum of three times per week until tumor development becomes evident (via palpation or direct observation), and then daily thereafter until they are euthanized, including weekends and holidays. This monitoring must ensure that animals are euthanized before tumors reach a size that interferes significantly with normal bodily functions or activity. Tumor burden should always be limited to the minimum required for a valid scientific outcome. The general maximum allowable tumor size is 1.5 cm in mice and 2.5 cm in rats, as determined by measuring the tumor’s largest diameter. When multiple tumors per animal are grown, the size should be correspondingly less and should not exceed the maximum burden of a single tumor. When tumor burden has reached the maximum allowable size, rodents should be euthanized within 48 hours, even if this requires euthanasia at a time point that is not consistent with the experimental group. It is recommended that plans for tissue collection be in place as the tumor approaches the maximum allowable
size to prevent unnecessary delays in euthanasia and remain in compliance with the guidelines.

5) Tumor ulceration can lead to an increase in potential pain and distress to the animals, and the associated inflammation may affect the subsequent progression of the tumor. For this reason, ulceration is also considered to be an endpoint for humane euthanasia. Rodents exhibiting ulcerated tumors should be euthanized within 24 hours, or sooner if clinical signs of infection, pain or distress are noted.

6) Projects that require the maintenance of tumors greater than 1.5 cm in size must submit a detailed justification for an exception to these guidelines in the protocol to be reviewed by the IACUC. Likewise, if ulceration cannot be used as an endpoint (e.g. early ulceration is a characteristic of the tumor or ulceration is associated with tumor regression) the protocol must provide additional justification. The justification must specify the scientific reasons for this exception, why an earlier endpoint cannot be used, special husbandry needs that might occur (e.g., single cages for animals with ulcerated tumors), provide a detailed plan for the treatment of health issues that may arise as a result of the advanced cancer (e.g., antibiotics, fluid therapy, supplemental nutritional support, topical treatments, etc.) and define surrogate endpoints/criteria for euthanasia.

7) 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.

8) Following tumor induction, animals must be observed for pain, distress, and abnormal behavior and physiology. The overall wellbeing of the animal takes priority over precise tumor measurements in decisions regarding euthanasia or other interventions. Clinical signs that should be monitored closely include (but are not limited to) rapid weight loss, loss of body condition, anorexia, the absence of normal urine and feces, abnormal respiratory rate/patterns, listlessness, piloerection (ruffled hair coat), abdominal distention, ambulatory difficulties, hemorrhage, infection, self-mutilation/cannibalism, vocalization, paralysis, and/or seizures.

9) 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. Ascites fluid should be removed by peritoneal tap before abdominal distention is great enough to cause discomfort or interference with normal activity. Animals should be euthanized if they become moribund (i.e., huddling, hunched posture, increased respiratory rate and/or effort, lethargy, difficulty with normal ambulation, or ruffled coat). Skilled personnel may do removal of peritoneal fluid with an 18-gauge or smaller needle without anesthesia. New personnel or students must be trained and develop proficiency using anesthetized animals prior to performing the procedure on awake animals. Training is available from the ARC staff. Ascites collection is limited to a maximum of three taps, with the animal being euthanized just prior to the third tap.

Section B – Recordkeeping

Written documentation of dates and times of all observations (including recordings of tumor size progression), status of animals and any procedures performed (i.e. euthanasia, peritoneal tap to drain ascites, supportive treatment, etc.) must be kept for review by the IACUC, the ARC veterinary staff, or regulatory agencies. Contact the ARC for training in proper record keeping or to obtain sample monitoring forms.

Section C – References


**Section D – Acknowledgements**

This document contains content that was adapted from materials obtained from Stanford University and the University of Florida.