Background:

Inoculation or implantation of tumor cell lines in rodents is a critical experimental and translational step in characterizing tumor cell biology, and in the discovery, development, and safety and efficacy testing of anti-neoplastic treatments. Unfortunately, these tumor transplants may cause profound physiologic or metabolic disruptions in the rodent host or possibly unintended adverse effects (e.g. infection) that may result in significant pain or distress to the animal. The following guidelines were created to minimize pain and distress for the tumor-bearing animal; to promote sound biosecurity practices for the protection of our rodent colonies; and to reduce experimental variability, which should reduce the number of animals required for these studies.

Minimizing pain and distress in the recipient animal requires the establishment of clearly defined scientific and humane endpoints, and trained personnel willing and capable of performing careful and regular observation and examination of the recipient animal to determine when these endpoints are reached. Endpoints should be based on scientific criteria, because there is no value to terminating an experiment prematurely only to have to use more animals to achieve the desired scientific criteria. However, it is unacceptable to extend the animal’s pain and distress beyond the point required to achieve the scientific criteria. Therefore, the scientific and humane endpoints should be delineated and justified in the animal protocol.¹

Procedures:

1. Prior to inoculation, or implantation of tumor cell lines:
   - Xenotransplantation experiments in mice require an immunodeficient recipient to permit development and/or engraftment of the non-rodent tumor cell line or tissue. These immunodeficient mice, most commonly SCID or nude mice, have a shortened life span, and require special husbandry and biosecurity precautions to prevent diseases caused by opportunistic or adventitious microorganisms. Therefore, all tumor cell lines should be tested and certified free of adventitious rodent pathogens prior to inoculation or
implantation, regardless of origin (rodent or human) or vendor/source. PCR-based diagnostic testing services are readily available (i.e. Research Animal Diagnostic Laboratory, IMPACT profile), and the Animal Resource Center (ARC) can provide more information on testing of cell lines. Additionally, all immunodeficient transplant models should be maintained under strict bioexclusion housing and husbandry conditions in the vivarium, and provided with sterile food, bedding, and water.

NOTE: Implantation of human or non-human primate tissues or cells requires notification to the Biosafety Officer so that occupational health risks may be adequately assessed and mitigated. Additionally, implantation of human embryonic stem cells requires Embryonic Stem Cell Research Oversight (ESCRO) Committee approval to ensure that UCSB meets scientific and ethical standards.

2. After inoculation, implantation, or induction of tumors:

- Animals must be regularly and carefully observed for signs of pain or distress. The next section (#3) contains examples of clinical signs necessitating immediate intervention. These observations must be documented and made available to the IACUC and the Campus Veterinarian. The frequency of these observations will depend on the tumor biology (rate of replication, invasiveness, ability to metastasize, ability to elaborate cachectic factors), and host factors.

- At a minimum, tumor-bearing animals should be observed once a day following tumor implantation or induction, and more frequently as the scientific objectives are being reached, or if clinical signs of painful or distressful conditions are observed in any animals in the same experimental cohort. The frequency of these observations should be maintained during weekend and holidays.

3. An animal (mouse or rat) should be euthanized if any of these clinical signs is observed, and the frequency of observations for the remaining animals in the experimental cohort should be increased.²

- For a rodent carrying a single subcutaneous tumor, the mean diameter of the tumor (determined using calipers to measure two diameters at right angles) should not exceed 1.5 cm in mice, or 2.5 cm in rats. For rodents carrying two or more tumors, the size of each tumor should be correspondingly less.

- Ulceration or necrosis of any solid tumor that persists beyond 48 hours.

- Failure to eat or drink over a 24 – 48 hour period resulting in emaciation or dehydration.

- Consistent or rapid body weight loss reaching 20% at any time or 15% maintained for 72 hours compared to the pre-treatment weight of adult mice or age-matched, vehicle-treated controls.

- Labored respiration, particularly if accompanied by nasal discharge and/or cyanosis.

- Enlarged peripheral lymph nodes.

- Abdominal distension due to an enlarged spleen or liver, or the accumulation of ascitic fluid.
• Incontinence or diarrhea over a 48 hour period.

• Symptoms of severe neurological, physiological or metabolic impairment such as abnormal locomotion (lameness, or circling), posture (head tilt), or behavior (abnormal vocalization, increased aggression, unusually submissive/lethargic).

Reference:

