

Institutional Animal Care and Use Committee

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IACUC GUIDELINE: HUMAN EXPERIMENTAL ENDPOINTS

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Humane Endpoints

Animal studies may involve procedures that cause pain or distress to the animals, which cannot be alleviated with analgesia, anesthesia, sedation, or palliative care. In these cases, the application of a humane experimental endpoint is required. The phrase humane endpoint¹ refers to the termination of an experiment, and humane euthanasia of the animal, in order to minimize the pain, distress, or suffering experienced by the animal, while still achieving the scientific goal.

Any animal experiment with the potential for pain or distress should have a humane experimental endpoint. The endpoint criteria will vary depending on the experimental procedure. Specific humane endpoint monitoring and alleviation plans for two such experiments are described below: tumor cell implantation and experimental infections with the potential to cause mortality. For other potentially painful or distressful experiments with humane endpoint considerations, the following general monitoring and alleviation recommendations should be followed:

1. Review as much information as possible about the substance to be tested, or the gene of interest in the case of genetic mutations, or the disease process being modeled, in order to prospectively identify any expected and possible adverse effects the research animals may experience.
2. Determine the most accurate signs of a painful or distressful state, which should involve consultation with the Campus Veterinarian.

Accurate clinical signs should be based on measurable parameters (e.g. body temperature, and body weight), and subjective criteria (e.g. physical appearance, unprovoked behavior, and response to external stimuli).²⁻⁴

3. Ensure that appropriately trained personnel are responsible for the animal evaluation/monitoring, record keeping, and notification of the investigator and/or the Campus Veterinarian.

Checklists or score sheets may be helpful in ensuring appropriate observations are made, consistently interpreted, and properly documented. Written records should be kept of all monitoring and treatment activities.

The frequency of the animal monitoring, including weekends and holidays, should be described in the protocol. The frequency of observation of the experimental cohort (i.e. group of similarly treated animals) should be increased when any animal in the cohort begins to exhibit abnormal clinical signs.

4. Consider moving animals to individual cages when their condition deteriorates to the point that injury from other animals is likely.

Implantation of Tumor Cells/Tissues in Rodents

1. 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 (<https://www.idexxbioanalytics.com/impact-pcr-o>), and the Animal Resource Center (ARC) can provide more information on testing.

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, the animals must be regularly and carefully observed for signs of pain or distress. 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. The experimental (i.e., bearing a tumor implant) animal should be euthanized if any of the following clinical signs are observed, and the frequency of observations for the remaining animals in the experimental cohort should be increased.⁵
 - 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 proportionately less (for example, 2 X 0.75 cm in mice or 2 X 1.25 cm in rats).
 - Ulceration or necrosis of any solid tumor that persists beyond 48 hours.
 - Failure to eat or drink for \geq 48-hours.
 - Consistent or rapid body weight loss reaching 20% at any time or 15% maintained for 48 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).

Experimental Infections with the Potential to Cause Animal Mortality

There may on occasion be an animal study in which the endpoint that is compatible with the scientific requirements of the study requires moribundity or mortality.^{6,7,9} The moribund condition is defined as a clinically irreversible condition leading inevitably to death.⁸ In these types of studies (e.g., sepsis studies), animals are permitted to die or become moribund out of scientific necessity (e.g., the need to study late-stage disease processes or determine survival rates). These studies must demonstrate that the benefits of the research are greater than the harm done to the animal, and that the earliest scientifically valid humane endpoint will be used. Specifically, these protocols must include a scientifically justified experimental design that addresses the following animal welfare concerns:

1. Are any scientifically valid alternatives available? If available, why are they not being utilized?
2. Are measures or treatments (e.g., sedatives, analgesics) available to alleviate the pain and/or distress? "It is no longer acceptable to deny analgesic support to an injured or septic animal without direct experimental evidence that providing analgesic support would invalidate the experimental results."⁹
3. How many animals will be used for experiments in which pain or distress will be unrelieved, and how was it determined that this is the minimum number of animals required?
4. Will animals be euthanized when moribund, and if not, what scientific information (i.e. data) is gained or lost in the interval between moribundity and death?

References:

1. *Guide for the Care and Use of Laboratory Animals, 8th Edition (2011), pg 27-28.*
2. Hendriksen and Morton, ed. (1998), *Humane Endpoints in Animal Experiments for Biomedical Research. Proceedings of the International Conference, 22-25 November 1998, Zeist, The Netherlands.* Laboratory Animals Ltd, by Royal Society of Medicine Press Limited, London, England.
3. Morton DB. (2000), *Humane Endpoints for Animals Used in Biomedical Research and Testing.* Institute for Laboratory Animal Research Journal 41:80-86.
4. Montgomery (1990), *Cancer Bulletin* 42:230-237.
5. Workman, P., et. al. *Guidelines for the welfare and use of animals in cancer research.* British Journal of Cancer. 102:1555-1577. 2010
6. Nemzek JA et. al. (2004), *Humane Endpoints in Shock Research.* Shock 21(1):17-25.
7. Nemzek JA et. al. (2008), *Modeling Sepsis in the Laboratory: Merging Sound Science with Animal Well-Being.* Comparative Medicine 58(2):120-128.
8. Toth LA (2000), *Defining the moribund condition as an experimental endpoint for animal research.* Institute for Laboratory Animal Research Journal 41:72-79.
9. Stortz JA et. al. (2017), *Murine Models of Sepsis and Trauma: Can We Bridge the Gap?* ILAR Journal 58(1):90-105.

Additional Reference Material:

1. Institute for Laboratory Animal Research: *Recognition and Alleviation of Distress in Laboratory Animals* (2008).
2. Institute for Laboratory Animal Research: *Recognition and Alleviation of Pain in Laboratory Animals* (2009).