

## UNIVERSITY OF TEXAS AT ARLINGTON

### INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

#### MONOCLONAL ANTIBODIES/MOUSE ASCITES SOP

##### A. PRODUCTION OF MONOCLONAL ANTIBODIES IN MOUSE ASCITES FLUID

###### 1. Introduction:

- a. Monoclonal antibodies (MAb) are important reagents in biomedical research and concept testing, in diagnostics and in treatment of infections, cancer and other immune-mediated diseases. Monoclonal antibodies are secreted from a single clone of B-lymphocytes, with antigen specificity. In the 1970's, techniques were developed in which mice were immunized with a specific antigen (Ag), followed by fusion of those harvested splenocytes with mouse plasmacytoma cells using polyethylene glycol (PEG). The immunization step using mice can sometimes be replaced by phage display and *in vitro* immunization, although this is not generally successful. These resulting hybridomas are immortalized like the plasmacytoma and retain the splenocyte-like secretory ability. Desirable clones are expanded *in vitro* or *in vivo* to produce the MAbs. To expand MAb production *in vivo*, mice are inoculated intraperitoneally with a priming agent, such as pristine. The ascites fluid collects in the peritoneal cavity within the next 1-3 weeks and can be removed by paracentesis, also known as "tapping." It is this expansion step which is often acceptably done *in vitro* and can replace *in vivo* use.
- b. The ascites method illustrates the '3R's' from Russell and Burch:
  - (1) Refinement of techniques to minimize pain and distress.
  - (2) Replacement of the use of animals by *in vitro* technologies.
  - (3) Reduction in the number of animals used.

###### 2. Policy:

- a. *In vitro* methods for monoclonal antibody production are the accepted method. Proposed use of the ascites method in mice will be reviewed on a case-by-case basis and may be permitted to produce monoclonal antibodies only if both of the following have been met:
  - (1) Prior to proposed use *in vivo*, good-faith efforts have been made to use various *in vitro* methods and resulted in poor viability, AND
  - (2) If fully justified and documented by the primary investigator and approved by the IACUC.
- b. Time, cost or convenience cannot be the primary reasons for granting exceptions or providing justification. If limited use of the ascites method is approved, those procedures will adhere closely to the refined techniques and timetables stated below, to minimize potential for pain or distress. All hybridomas or cell lines in

these cases must be MAP-tested to identify any potential murine viruses or mycoplasmas that could interfere with experiments or cause morbidity/mortality in rodents.

3. Guidelines for Gauging Viability of *In Vitro* Attempts:
  - a. Specific *in vitro* conditions may be reviewed for viability by an internal or external area expert designated by the IACUC.
  - b. Some hybridomas do not grow or replicate in culture, especially in serum-free media.
  - c. A very small number of antibodies experience changes in full glycosylation when grown *in vitro*. This could result in diminished antibody function: decreased binding affinity, faster clearance, increased immunogenicity. Such functional changes become obvious through analysis of the Mab.
  - d. High levels of yeast, fungi or mycoplasmas which infect hybridoma cells may only be removed by passing cells from culture through mice. Current antimicrobial drugs cannot accomplish this in some cases.
  - e. Low levels of *in vitro* production of a given hybridoma culture may be characterized as:
    - (1) Stationary batch method: when a dense culture is grown for 7-10 days and Mab concentrations are less than 5 ug/ml.
    - (2) Hollow fiber reactors methods: Mab concentrations fall below 400ug/ml.
    - (3) Semipermeable-membrane systems: Mab concentrations fall below 200ug/ml.
  
4. Statutory and Policy Bases for Consideration of Alternatives:

Many countries now prohibit or restrict ascites production. In the United States a legal petition was filed in 1997 by the American Antivivisectionist Society. The lawsuit asked the NIH (National Institutes of Health) to limit or stop the routine production of Mab in ascites, given that *in vitro* technology can now replace much of that animal use. The NIH advised IACUCs of the responsibility to determine if investigators considered *in vitro* technology to minimize pain or distress and as suitable alternatives (“Dear Colleague” letter from OPRR) (Office for Protection of Research Risk, NIH).

  - a. Health Research Extension Act of 1985, Public Law 99-158, Sec. 495(c): “...(B)scientists, animal technicians and other personnel involved with animal care, treatment and use...have available to them instruction or training in the ...concept, availability, and use of research or testing methods that limit the use of animals or limit animal distress..”
  - b. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training, Principles III, IV and V:
    - III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation and *in vitro* biological systems should be considered.
    - IV. Proper use of animals, including the avoidance or minimization of discomfort,

- distress and pain when consistent with sound scientific practices, is imperative.
- V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia or anesthesia..”
- c. PHS (Public Health Services) Policy on Humane Care and Use of Laboratory Animals: Section IV.A.1.g:[The Assurance shall fully describe...] “a synopsis of training or instruction in the humane practice of animal care and use, as well as training or instruction in research or testing methods that minimize the number of animals required to obtain valid results and minimize animal distress, offered to scientists, animal technicians, and other personnel involving in animal care, treatment or use;” Section IV.C.1.a.[In order to approve proposed research...the IACUC shall determine that...] “Procedures with animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design”
- d. Guide for the Care and Use of Laboratory Animals, 1996, National Academy of Sciences:
- (1) “Although scientists have also developed non-animal models for research, teaching, and testing...these models often cannot completely mimic the complex human or animal body, and continued progress in human and animal health and well-being requires the use of living animals. Nevertheless, efforts to develop and use scientifically valid alternatives, adjuncts, and refinements to animal research should continue.” (page 1)
- (2) “The following topics should be considered in the preparation and review of animal care and use protocols...Availability or appropriateness of the use of less-invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation...” (page 10)
- e. CFR (Code of Federal Regulations), Title 9, Chapter 1, Subchapter A – Animal Welfare, Sec,231(d): “Training and instruction of personnel must include guidance in...(2) The concept, availability, and use of research or testing methods that limit the use of animals or minimize animal distress.”
5. *In Vivo* Procedures:
- a. Proposed Use – If use of the ascites method is fully justified by the primary investigator and approved by the IACUC, the following procedures will be followed, to minimize potential pain or distress experienced by the mice. The mice will be used to generate ascites fluid containing monoclonal antibodies in response to the intraperitoneal (IP) injection of hybridoma cells. VAF (Viral Antibody Free) mice will be injected IP with pristine. Following pristine priming, hybridoma cells will be injected IP. The animals will experience a gradual abdominal swelling due to accumulation of ascites fluid, and this may require up to two harvests per animal prior to euthanasia. Mice are usually kept for a period of 14 days after the initial indication that ascites production has begun. Upon recognition of loss of body condition, pain, or distress, the mouse will be euthanized.
- b. Justification for Number of Mice – The number of mice to be used will be determined by the total amount of antibody required and our experience that, on average, ascites typically contain 1-2 mg/ml of antibody. Consequently, up to 10 mice will be used to generate a target of 15mg of monoclonal antibody.

- c. Methods/Techniques –
  - (1) Restraint: Mice (approximately 20 gms) will be restrained by gently grasping the loose skin over the back of the neck.
  - (2) Injections: Inject 0.25 ml(maximum) pristine, IP once per animal with a 23 gauge needle. Within 60 days after pristine, inject 0.5ml (maximum) IP of  $1 \times 10^6$  to  $1 \times 10^7$  hybridoma cells once per animal with a 23 gauge needle.
  - (3) Ascites fluid collection: Peritoneal taps will be done with a sterile 20 gauge needle. An 18 gauge needle may be used if there is excessive clotting or fibrin formation within the needle. To avoid hypovolemia, the volume of fluid collected should not normally exceed 3ml/tap. Fluid may be collected only twice prior to euthanasia. Administration of replacement fluids will be administered (e.g. Lactated Ringer's solution administered subcutaneously) in equal volume to the volume of the ascitic fluid removed. At the time of euthanasia, the third and final tap will remove the remaining ascites fluid.
  - (4) Euthanasia: CO<sub>2</sub> exposure in a chamber.
- d. Personnel – All animal techniques must be done by personnel with training and experience with these mouse techniques and animal care.
- e. Animal Observations –
  - (1) Mice must be observed twice daily if necessary, as the ascites can develop as quickly as within 10-30 days.
  - (2) The abdomen cannot be allowed to become so distended that the animal is weak and in poor condition. The abdomen may be tapped no more than twice before the mouse is euthanized for final harvest of ascites. Clear criteria for euthanasia as part of a post approval monitoring plan must be included in the animal use protocol. Mice that have a rough hair coat or hunched posture or are reluctant to move are considered abnormal and in need of euthanasia.
  - (3) Mice develop a peritoneal inflammation following pristine injection. While mice may develop ascites within a few days following injection of cells, in some cases initiation of ascites production may be delayed up to two weeks. Gradual swelling of the abdomen that accompanies the accumulation of ascites fluid typically occurs over a period 3-7 days. The ascites procedure is considered to cause pain and distress to the mice. The animals must be euthanized before the swelling interferes with normal daily activity.
- f. Records – Notations must be made on each cage card (or a separate card) to include date of injections, identification of cell line, and dates and volume of ascites collected. All mice must have some method of individual identification.
- g. Animal Health and Housing –
  - (1) Mice must be purchased from an approved vendor source.
  - (2) Mice must not be injected with hybridoma cell lines that are of unknown microbial status until they are verified cleared for common murine pathogens under the direction of the attending veterinarian. This will be done with the standard mouse antibody production technique (MAP test). This usually involves mice being injected

IP with  $1 \times 10^6$  cells each. Three to four weeks later, mice are euthanized with CO<sub>2</sub> and serum is collected and evaluated by enzyme linked immunosorbent assays (ELISA) for presence of murine pathogens, to prevent exposure of current rodent research colonies to agents potentially in the cell lines. MAP testing is performed at an external testing facility and may require several weeks of advanced planning, to receive results. In addition, sentinel mice will be placed in the room/cubicle as controls. Serum from these sentinels will be collected periodically and tested for exposure to virus pathogens.