



POLICY

In-vitro techniques are to be used when appropriate to produce monoclonal antibodies (mAbs). The use of the ascites method to produce mAbs must be appropriately scientifically justified. Documentation on why in-vitro methods and/or a commercial source of mAbs is not adequate for mAb production must be presented in the IACUC protocol.

To prevent introduction and transmission of diseases into the research animal colonies, hybridoma cultures must be tested for microbiologic contamination before they are injected into animals. Refer to the IACUC Policy for Hazard Management.

After inoculation with an ascites-producing tumor line, mice 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 must be removed by peritoneal tap before abdominal distention is great enough to cause discomfort, respiratory difficulty, or interfere with normal activity. An 18 gauge or smaller needle is to be used for the tap. The tap may be performed without anesthesia by skilled personnel. Anesthesia is to be used when training personnel. Observation of the animal for an hour after the procedure is required to observe for possible signs of shock. The maximum number of survival taps per mouse is two. A third tap can be done as a terminal procedure.

Animals should be euthanized before the final tap if:

- The fluid becomes blood-tinged or infected;
- The abdominal tap does not relieve abdominal distention;
- The animal develops a solid tumor;
- The animal display signs of pain or distress such as but not limited to ruffled coat, hunched posture, anorexia, dehydration, pallor, loss of body condition, difficulty moving, tachypnea, or dyspnea.

BACKGROUND

Monoclonal antibodies (mAb) are important reagents used in biomedical research, in diagnosis of diseases, and in treatment of such diseases as infections and cancer. Production of monoclonal antibody production can be done by two methods:

- In vitro methods, using tissue cultures to grow hybridoma cells that will secrete monoclonal antibodies in the culture media.
- In vivo methods, hybridoma cells are injected into the peritoneal cavity of a mouse where they will multiply. Ascites fluid is produced inside the abdomen of the mouse. The ascites fluid accumulates and distends the abdomen and causes discomfort and pain to the animal.

In 1997, the National Institute of Health issued, through their Office for Protection from



Research Risks (OPRR), guidelines on monoclonal antibody production. The document states that prior to approval of proposal that include the mouse ascites method, IACUCs must determine that (1) the proposed use is scientifically justified, (2) methods that avoid or minimize discomfort, distress, and pain (including in vitro methods) have been considered, and (3) the latter have been found unsuitable. In 1999, the National Research Council's Committee on Methods of Production Monoclonal Antibodies concluded that in vitro methods should be used whenever possible. It recommended that the ascites method should not be banned but when used every effort should be made to minimize pain or distress, including frequent observations, limiting the number of taps, and prompt euthanasia if distress appear.

REFERENCES

National Institutes of Health, OPRR Report 98-01, Production of Monoclonal Antibodies using Mouse Ascites Method. 1997

ILAR Report on Monoclonal Antibody Production. A report of the Committee on Methods of Producing Monoclonal Antibodies. Institute for Laboratory Animal Research, National Research Council. 1999

REGULATION

The Public Health Service (PHS) Policy, 2002, IV.C. 1a. "Procedures with animals will avoid or minimize discomfort, distress and pain to the animals, consistent with sound research design".

U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, IRAC, 1985, Principle 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." Principle IV "Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative."

PROCEDURE

- 1) **To obtain IACUC approval for monoclonal antibody production using the ascites method, provide the following on the IACUC form:**
 - a. Scientific justification for the use of the in-vivo method of mAbs.
 - b. Documentation of what alternatives were considered and why they will not work.
 - c. Proposed procedure including tapping frequency and monitoring plan.