



Policy and Guidelines on the Maintenance of Hybridomas in Rodents and the Collection of Ascites Fluid

Background: Monoclonal antibodies represent a powerful research tool, and the use of animals is indispensable to the establishment of monoclonal antibody producing cell lines. Once these cell lines are established, *in vivo* and/or *in vitro* techniques can be utilized for production of necessary volumes of monoclonal antibodies. While there is evidence that the rodent ascites method of monoclonal antibody production causes discomfort, distress, and/or pain, and that practical *in vitro* methods exist which can replace the ascites method in many experimental applications, there are scientifically based reasons why *in vivo* methods for producing monoclonal antibodies must still be used in some situations.

The Institutional Animal Care and Use Committee (IACUC) has developed the following policy intended to determine whether *in vitro* or *in vivo* methods are to be used for specific projects and, when *in vivo* methods are necessary, to eliminate or reduce to a minimum animal discomfort associated with these procedures.

Policy: When proposing to use the rodent ascites method of monoclonal antibody production, Investigators must provide the following information in the animal care and use protocol or amendment: (a) confirmation that the procedures to be used may cause discomfort, distress or pain, (b) description of the search for alternative methods, as stipulated in the IACUC-1 form, and (c) scientific justification of the need for using the *in vivo* method, and explanation of why alternative *in vitro* methods cannot be used.

Immortal cell lines that are to be used in hybridoma formation should be tested for presence of rodent viruses prior to introduction into the animal colony to ensure that they are pathogen free. After inoculation with an ascites-producing tumor line, rodents should be observed by the research staff at least three times per week for the first week and daily thereafter specifically to monitor degree of abdominal distention and signs of illness. Ascites fluid should be removed by tapping the peritoneal cavity before abdominal distention is great enough to cause discomfort or interference with normal activity. Mice should be euthanized when they show signs of poor condition such as huddling and ruffled coat.

The number of abdominal taps are to be limited to a maximum of three, with the last collection being a terminal procedure.

Removal of peritoneal fluid with a sterile 18 gauge or smaller needle may be done without anesthesia by skilled personnel. New personnel or students should be trained using anesthetized mice.

Role of the Investigator:

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When the campus Immunological Resource Center (IRC) produces monoclonal antibodies under its Monoclonal Antibody Production protocol for other investigators, the IRC is responsible for justifying the appropriate methods to be used. In these cases, the IRC Director is responsible for providing the above-mentioned information in the form of an amendment to the IRC protocol to the IACUC for review. These amendments are to be submitted on a project-by-project basis and approved prior to initiation of project work.

Role of the IACUC: Federal regulations require the IACUC to critically evaluate proposed use of the mouse ascites method of monoclonal antibody production on a project-by-project basis. Prior to approval of proposals, which include this method, the IACUC must determine that (i) the proposed use is scientifically justified, (ii) methods that avoid or minimize discomfort, distress and pain (including in vitro methods) have been considered, and (iii) the latter have been found unsuitable. The IACUC fulfills this evaluation during the critical review of newly submitted animal care and use protocols and of amendments to previously approved protocols.

References:

- Guidance Concerning the Production of Monoclonal Antibodies in Animals, OPRR, Notice: OD-00-019, February 3, 2000
- Monoclonal Antibody Production Report, National Research Council, 1999
- Production of Monoclonal Antibodies Using Mouse Ascites Method, OPRR Reports, Number 98-01, November 17, 1997
- Guide for the Care and Use of Laboratory Animals, (Guide), NRC, 1996

Approved July 6, 2000