A. Purpose

To clarify when the mouse ascites method may be used for the production of monoclonal antibodies and to provide the requirements when it is used.

B. Principle

The Public Health Service Policy on Humane Care and Use of Laboratory Animals (NIH 1996, page 7) requires IACUCs to ensure that approved protocols conform with the PHS requirement that "procedures with animals . . . avoid or minimize discomfort, distress and pain to animals (in a way that is) consistent with sound research design."

The mouse ascites method for the production of monoclonal antibodies has the potential to produce pain, distress, or discomfort in the animals utilized.

Therefore, prior to approval of the use of this method, it is the IACUC's responsibility to 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 implemented whenever possible or determined to be unsuitable.
C. Policy Statement

In vitro techniques are the default method for the production of monoclonal antibodies. The mouse ascites method may only be utilized after appropriate in vitro methods have been tried for that antibody and have failed. The failure of in vitro methods and the subsequent use of the ascites method must be documented in the investigator’s ASAF and approved by the IACUC.

D. Requirements

When the mouse ascites method for producing monoclonal antibodies is used, every reasonable effort should be made to minimize pain or distress, including frequent observation, limiting the number of taps [i.e. peritoneocentesis], and prompt euthanasia if signs of distress appear.

Specific guidelines for consideration by Principal Investigators when developing animal study proposals involving the mouse ascites method are:

1. The volume of the priming agent should be reduced to as small a volume as necessary to elicit the growth of ascitic tumors and at the same time reduce the potential for distress caused by the irritant properties of the priming agent. Although 0.5 ml Pristane has been considered standard for adult mice, the lower dose of 0.1-0.2 ml has been shown to be as effective for many hybridomas. Therefore, doses higher than 0.2 ml should only be used when lower doses have been demonstrated to be inadequate for the antibody to be produced.

2. Although the time interval between priming and inoculation of hybridoma cells as well as the number of cells in the inoculum are determined empirically, inocula generally range from $10^5$ - $10^7$ cells in volumes of 0.1 - 0.5 ml and are usually administered 10 - 14 days after priming. Generally, very high cell numbers
are associated with greater mortality and \(< 1 \times 10^5\) cells may elicit fewer ascitic tumors. Cell suspensions must be prepared under sterile conditions in physiological solutions.

3. In order to prevent potential disease transmission, imported hybridomas should be tested for the presence of infectious agents prior to being injected into mice. (Please contact OCV at 335-6246 if you have any questions)

4. Animals should be monitored at least once daily, seven days a week by personnel familiar with clinical signs associated with ascites production and circulatory shock.

5. Animals should be weighed before being inoculated with the hybridomas, and then daily thereafter. No more than 15% weight gain should be allowed.

6. Ascites pressure should be relieved before abdominal distension is great enough to cause discomfort or interfere with normal activity. Manual restraint or anesthesia may be used for tapping. The tap should be performed by trained personnel using proper aseptic technique. The smallest needle possible that allows for good flow should be used (18 -22 gauge).

7. It is recommended that no survival taps be performed, however, a maximum of two survival taps (the 3rd being terminal) are permitted. Additional taps would have to be specifically justified in the ASAF and approved by the IACUC.

8. If survival taps are performed the animals should be monitored frequently over several hours following the tap to observe possible signs of shock due to fluid withdrawal. Pale eyes, ears and muzzle and breathing difficulties are indicative of circulatory shock. Shock may be prevented or treated with 2 -3 ml warm saline or lactated Ringer’s administered subcutaneously.

9. Animals should be euthanized appropriately before the final tap or promptly if there is greater than a 15% weight gain, or there is evidence of debilitation, pain or distress. Signs of distress include hunched posture, rough hair coat, reduced food consumption, emaciation, inactivity, difficulty in ambulation, respiratory problems, and solid tumor growth.
E. References

2. ILAR Journal Volume 37, Number 3, 141-152, 1995.