Guidelines for Monoclonal Antibody Production

Introduction
In vitro (tissue-culture methods) for the production of monoclonal antibodies (MAb) should be used unless there are clear scientific reasons for why they cannot. If in vivo (mouse ascites) methodology is requested, the PI must justify why in vitro methods are not adequate. Scientific reasons may include failure of a cell line to adapt in vitro; purification methods lead to denaturation or decreased antibody activity; contamination of cell lines; and inability of cell line to maintain production of monoclonal antibodies.

Procedure
When the mouse ascites method for producing MAb is used, every reasonable effort should be made to minimize pain or distress, including frequent observation of the animal, limiting the number of taps (i.e. peritoneocentesis), and prompt euthanasia if signs of distress appear. The IACUC considers ascites production to be pain/distress category E, pain with no relief.

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.

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 less than $1 \times 10^5$ cells may elicit fewer ascitic tumors. Cell suspensions must be prepared under sterile conditions in physiological solutions.

Imported hybridomas should be analyzed with a PCR-based or species-specific antibody production test before introduction into the animal host to prevent potential transmission of infectious agents from contaminated cell lines into facility mouse colonies and possibly to humans handling the animals.

If adjuvants are used, Guidelines for the Use of Adjuvants found on the IACUC web site should be followed.

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.

The development of ascites leads to abdominal distention resulting in discomfort and distress. 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 (20-22 gauge) should be used. Ascites fluid must be collected before body weight becomes 20% greater than the weight obtained prior to the
injection, the abdominal distention is greater than a typical pregnant mouse, the body condition score deteriorates or if they are unable to reach food or water.

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 ringers administered subcutaneously.

The number of taps should be limited, based on good body condition of the animal. A maximum of three survival taps (the 4th being terminal) are recommended. Additional taps should have justification.

Animals should be euthanatized appropriately before the final tap or promptly if there is evidence of debilitation, pain or distress. Signs of these include hunched posture, rough hair coat, reduced food consumption, dehydration, loss of body condition, emaciation, inactivity, difficulty in ambulation, respiratory distress, palpable solid tumor growth, and grey-green coloration of the abdomen.