

AUBURN UNIVERSITY

Institutional Animal Care and Use Committee (IACUC)

Policy on Monoclonal Antibody Production Using the Ascites Method

An *in vitro* method, such as culturing hybridomas in hollow fiber bioreactors or semipermeable plastic bags, is an alternative to *in vivo* ascites production. Assurances must be provided to the IACUC that use of such alternatives has been tried and not been successful before approval for ascites production in animals will be granted.

Typically, monoclonal antibody secreting tumor cells (hybridomas) are injected into the peritoneal cavity of mice 10-14 days after a “priming” dose of pristane has been given. The severity of the reaction and the amount of ascitic fluid produced are related to the doses of the adjuvant (pristane) and hybridoma cells administered. The volume of pristane should not exceed 0.2 ml in mice unless specifically justified. Cell suspensions must be in sterile physiologic solutions and the inoculations must be aseptically performed. Hybridoma cells are usually given at doses averaging 1,000,000 cells (concentrations of 100,000 cells or less generally elicit fewer ascitic tumors, produce less ascitic fluid, and are far less likely to be effective).

The increased volume and pressure resulting from the accumulations of neoplastic cells and ascitic fluid can impair breathing and cause asphyxia if monitoring and removal of fluid are inadequate. Excessive quantities of fluid in the peritoneal cavity may be painful and shock may occur from the sudden decrease in fluid volume following harvest. The number of times such withdrawals should be performed should accordingly be minimized. It is customary to limit withdrawals to a total of two taps, unless the investigator provides evidence that the hybridoma is slow growing and additional taps can be accomplished in a humane fashion.

Monitoring of inoculated animals must be done by investigative staff at least three times a week for the first week after inoculation of the hybridoma and daily thereafter. Monitoring personnel must be trained and experienced in recognizing clinical signs associated with problems.

Ascitic fluid must be aseptically withdrawn before abdominal distension becomes great enough to interfere with normal activity. In no case should such distension be allowed to increase more than 20% beyond the normal diameter of the abdomen. While use of an anesthetic is preferred, manual restraint may be used, particularly in cases where anesthetic risk is increased. The smallest needle that allows for good flow should be used; needles larger than 18 gauge should not be used. The number of taps may not exceed a single survival tap and a second terminal tap, unless justified in the protocol and specifically approved by the IACUC. Following survival taps, animals must be monitored and treated for shock if indicated. The animal must be immediately treated or

ethanized if found to be in respiratory distress or shock due to what is determined to be an excessive accumulation of ascitic fluid.

Animals should be euthanized appropriately before the final tap or at any point if there are signs of uncorrectable debilitation, pain or suffering. Signs can include hunched posture, rough hair coat, reduced food consumption, emaciation, inactivity, difficulty in ambulation, or respiratory problems.

REVISED: December 18, 2012