ASCITES METHOD OF MONOCLONAL ANTIBODY PRODUCTION

I-IC-GU-601

A. PURPOSE

To standardize the acceptable means of producing ascites.

B. GUIDELINES

- In vitro techniques should be the default method for monoclonal antibody production (Mab), unless there is strong scientific justification as to why they cannot be used.
- Before IACUC approval, the proposed use of ascites must be scientifically justified. Specifically, when hybridomas fail to grow or fail to achieve a product consistent with scientific goals, the investigator must show that a good-faith effort was made to adapt the hybridoma to *in vitro* growth conditions before using the mouse ascites method.
- When the ascites method is used, every effort should be made to avoid or minimize discomfort, distress, and pain, including frequent observation, limiting the numbers of taps, and prompt euthanasia if signs of distress appear. The following steps are required if using the ascites method, and must be stated on the Protocol:
    - A baseline weight of the animal must be obtained.
    - The maximum priming dose of Pristane (2,6,10,14-tetramethylpentadecane) is 0.2ml intraperitoneally in mice, as higher doses cause significant distress.
    - Hybridomas must be tested before injection to prevent transmission of infectious agents.
    - After development of visible ascites, animals must be evaluated at least daily, including weekends and holidays.
    - Animals must be weighed daily and <20% weight gain is allowed. Animals must be immediately euthanized if the weight gain is >20%.
    - Ascites must be harvested before fluid accumulation becomes distressful, as determined by assessment of weight gain (<20%), posture, body condition, abdominal distension, activity levels, food and water intake and respiration.
    - Before ascites fluid is harvested, the abdomen must be swabbed with antiseptic and a sterile 18-22 gauge needle must be used. A new needle must be used every time the abdomen is tapped (peritoneocentesis).
    - Ascites can be harvested a maximum of three taps over a four day period. The 4th tap must be terminal. Multiple taps are only allowed if the animal does not exhibit signs of distress (above) and must have prior IACUC approval.
    - If the exudate collected from non-terminal taps is bloody, cloudy, or gross particulates are observed, the animal must be euthanized immediately.
    - Analgesics are not recommended due to central nervous system depression, but narcotic agonists, mixed agonist-antagonists or other species appropriate agents may be used to alleviate pain or distress.
    - All individuals involved in observation, handling, injection, or tapping of the animals must be well trained and experienced and wear appropriate personal protective equipment.

C. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

1. Examples of scientific reasons for the use of the ascites method

a.	The hybridoma cell line will not adapt well to in vitro conditions.
b.	In applications where several different mouse MAb at high concentrations are required for injection into mice, the in vitro method can be inefficient.
c.	MAb from mouse ascitic fluids might be essential for experiments in which MAb are used in vivo in mice.
d.	Rat hybridoma cell lines do not generate ascites efficiently in rats, and usually adapt poorly to in vitro conditions, but usually generate ascites in immunocompromised mice.
e.	Downstream purification can lead to protein denaturation and decreased antibody Activity.
f.	Serum-free or low-serum conditions cannot provide sufficient amounts of MAb for some purposes, such as the evaluation of new vaccines against infectious organisms.
g.	Culture methods sometimes yield populations of IgG MAb that are glycosylated at positions different from those harvested from mouse ascites fluid, thereby influencing antigen-binding capacity and important biologic functions.
h.	When hybridoma cells producing MAb are contaminated with infectious agents, such as yeasts or fungi, the cells often must be passed through mice
i.	Some cell lines that do adapt to tissue-culture conditions become unable to maintain adequate production of Mab.

2. The NIH concurs with the findings and recommendations in the 1999 report of the National Research Council Monoclonal Antibody Production (PDF) which indicates that during the accumulation of ascites there is likely to be pain and distress. The Report concluded that there is and will continue to be scientific necessity for this method, but tissue culture methods, to produce monoclonal antibodies should be adopted as the routine method unless there is a clear reason why they cannot be used.
3. https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/ascites.pdf