MASS GENERAL RESEARCH INSTITUTE
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON MONOCLONAL ANTIBODY PRODUCTION

GENERAL POLICY

The mouse ascites method of monoclonal antibody (MAb) production causes significant discomfort, distress, or pain to an animal. Practical *in vitro* methods exist that can replace the ascites method for many experimental applications without compromising the aims of a study. The IACUC critically evaluates the proposed use of the mouse ascites method as described in the **Adjuvants Use, Ascites/Antibody Production** form in each protocol. Prior to approval of protocols that include the ascites 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 for scientific reasons (see Appendix 1 for examples). Justification based on financial costs or on the length of time required are not acceptable. The Principal Investigator (PI) is responsible for documenting in the protocol that alternative *in vitro* methods for production of the monoclonal antibodies of interest have been attempted and found to be unsuitable.

PROCEDURES

When the mouse ascites method is used for monoclonal antibody production, efforts to minimize pain or distress (i.e. frequent observation, limiting the number of abdominal taps, clinical criteria for euthanasia if signs of distress appear) must be described in the protocol.

The following guidelines must be followed:

- The first step in producing monoclonal antibodies *in vivo* is to prime the mice with an agent that facilitates implantation of the hybridoma cells to be injected and the subsequent development of ascites. The volume of primer used should be the minimum required to stimulate the necessary reaction. Recommended agents and volumes are: Pristane, 0.1-0.2 ml injected intraperitoneally, two weeks prior to inoculation of hybridoma cells, or Incomplete Freunds Adjuvant (IFA), 0.1 ml injected intraperitoneally, 24-48 hours prior to inoculation.

- Prior to use, hybridomas must be tested and demonstrated free of viruses and *Mycoplasma spp*. that could contaminate the animal colony and introduce unwanted variables. Hybridoma cell suspensions should be prepared aseptically.

- Following injection of hybridoma cells, animals must be monitored twice daily (including weekends and holidays) by the PI or his/her designee for signs of abdominal distension (e.g., abdomen becomes tight, body weight has increased 20% from baseline, activity impairment) at which time the abdominal pressure must be relieved by aseptic paracentesis (i.e., abdominal tap) to withdraw ascitic fluid and relieve intra-abdominal pressure.

- Once ascites develops, animals must be monitored daily, at minimum, for signs of distress. Signs of distress include, but are not limited to, rough hair coat, hunched posture, decreased activity, rapid shallow breathing, pallor, and decreased appetite or water consumption. Animals should be monitored for changes in body weight. Rapid weight loss may be a sign of distress; the weight measured after tap should be compared to animal's pre-inoculation weight as the pre-tap weight will be artificially elevated due to fluid retention.

- Animals may be tapped a maximum of three times, the third tap following euthanasia. Post-tap monitoring is critical to avoiding hypovolemic shock. Warm saline or lactated Ringer's solution may be administered subcutaneously at the time of the tap to avoid shock. If multiple taps are used, the animal should be tapped at intervals not to exceed 48 hours in duration in order to avoid excessive abdominal distension. The number of taps and interval between taps must be specified in the protocol.

Deviations from this policy must be scientifically justified and approved by the IACUC.

REFERENCES

Leenaars, M., Hendriksen, C.F.M. Critical Steps in the Production of Polyclonal and Monoclonal Antibodies: Evaluation and Recommendations. *ILAR Journal* **2005**, 269–279.

Stills, H.F., Jr. Antigens, Antibodies, and Blood Collection. In *The IACUC Handbook*, 3rd edition; Silverman, J., Suckow, M.A., Murthy, S., Eds. CRC Press: Boca Raton, FL, 2014; pp 447-459.

U.S. Department of Health and Human Services. Ascites Production in Mice . National Institutes of Health, Animal Research Advisory Committee: Bethesda, MD, January 23, 2019.

v1.1, 16 February 2005
v1.2, 17 March 2014
v1.3, 17 February 2021

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

- The hybridoma cell line will not adapt well to *in vitro* conditions.
- In applications where several different mouse MAb at high concentrations are required for injection into mice, the *in vitro* method can be inefficient.
- MAb from mouse ascitic fluids might be essential for experiments in which MAb are used *in vivo* in mice.
- 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.
- Downstream purification can lead to protein denaturation and decreased antibody activity.
- 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.
- 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.
- When hybridoma cells producing MAb are contaminated with infectious agents, such as yeasts or fungi, the cells often must be passed through mice.
- Some cell lines that do adapt to tissue-culture conditions become unable to maintain adequate production of MAb.