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## Questions for the investigator

Pain, distress, and study endpoints are always issues of concern for an IACUC. All of these came together when the Great Eastern University IACUC reviewed a protocol from Dr. Sandy Lee that proposed to chemically induce hepatocellular carcinoma (liver cancer) in rabbits by administration of diethylnitrosamine. The protocol called for Lee to perform an open (*i.e.*, non-laparoscopic) abdominal surgical procedure about a year after carcinoma-induction, during which a small volume of a gene construct would be injected directly into the portal vein. The intent was to gain adequate distribution of the construct through the liver so that it would localize within the previously induced carcinoma. The goal of the study was to provide a novel form of gene therapy.

Along with other required information, Lee included the following on her IACUC application relative to the induced tumor

and subsequent surgery:

*Animals will be anesthetized with glycopyrrolate (0.1 mg/kg, s.c.) followed by ketamine (35 mg/kg, i.m.), xylazine (5.0 mg/kg, i.m.), and acepromazine (0.75 mg/kg, i.m.). Once anesthetized, the animals will be intubated with a 5 French endotracheal tube and maintained on isoflurane anesthesia (~1.5–2.0%) for the duration of the procedure. After the surgical procedure is completed, aspirin will be administered orally at a dosage of 100 mg/kg every 8 hours for 48 hours.*

*Animals will be observed at least once every hour for the first four hours following surgery, then twice a day for the next two days. They will then be observed by the research staff twice a week for the duration of the experiment. The animal care staff*

*will observe the animals every day.*

*Indications that an animal requires veterinary intervention or removal from the study will include weight loss, not drinking, teeth grinding, jaundice, rapid respirations, increased heart rate, ruffled fur, and sitting in the corner of the cage and not moving.*

*The animals will be euthanized at three three weeks post-operative, and the livers, lungs, spleen, and several other organs will be examined histologically for primary and metastatic tumors.*

Assuming drugs and dosages are appropriate, what are some questions you might ask of Lee relative to animal monitoring, pain, distress, and study endpoints if you were an IACUC member reviewing this proposal? If you believe the information provided is sufficient, explain your reasoning.

### RESPONSE

#### IACUC needs more information

Alison D. Pohl, MS, MT(ASCP), RLATg

The IACUC at Great Eastern should be concerned with the pain and distress of Lee's laboratory animals, as well as with the study's endpoints. US Department of Agriculture (USDA) and Public Health Service (PHS) requirements dictate that all procedures be performed with appropriate sedatives, analgesics, or anesthetics, unless withholding such agents is justified for scientific reasons<sup>1,2</sup>. In addition, the Animal Welfare Act (AWA) requires that investigators search for alternatives to procedures if those procedures are going to be done on a USDA-regulated species and have the potential to cause more than momentary

pain or distress to the animals<sup>3</sup>.

If we assume that the above was the only information presented in the written protocol, there are many questions that need to be answered. The USDA's 'search for alternatives' requirement, for instance, is not addressed for the laparotomy, which would certainly qualify as a painful procedure. Because the rabbit is a USDA-regulated species, this protocol would need to include a Principal Investigator (PI) narrative of the search procedures employed to justify the painful procedure. Has the PI thought of any alternatives (*e.g.*, Could the portal vein injection be performed under the guidance of ultrasound rather than through a laparotomy? If not, why a laparotomy rather than laparoscopy?). Another question is whether or not the PI has consulted a veterinarian during the course of planning the experiment, as required by the AWA. The veterinarian would be the best authority to determine if aspirin therapy

is enough of an analgesic for the planned surgical procedures.

Diethylnitrosamine-induced carcinoma may also qualify as a painful procedure under the AWA and Animal Welfare Regulations (AWRs). The description of the carcinoma induction would need to be expanded to include answers to the following questions: How aggressive is the carcinoma? Will it be a palpable tumor within the time period of the study? Are metastases expected? Will the carcinoma interfere with the rabbits' daily functions? A veterinarian should be consulted regarding the frequency of animal monitoring.

The AWA and the *Guide for the Care and Use of Laboratory Animals (Guide)* discuss the requirements for major survival surgery<sup>4,5</sup>. Information as to where the surgery is performed, how it is performed, and who will perform it must be described (a good surgeon can often minimize tissue destruction at the time of surgery, thereby reducing pain).