Column E Explanation

1. Registration number: 41-R-005

2. Number of animals used in this study for the time period specified.
October 1, 2021 – September 30, 2022: 2 guinea pigs in pain class E during this time period.

3. Species [common name] used in this study: Guinea pig.

4. Explain the procedures producing pain or distress. Include a brief description of the procedures and the animal's experience, such as neurological signs, lethargy, inappetance, respiratory signs, etc.

Anti-CD4 monoclonal antibody injection and guinea pig cytomegalovirus infection: Two pregnant females were treated with 1.5 mg of monoclonal antibody by IP injection and infected by subcutaneous injection into the scruff of the neck with ~$1X10^6$ PFU of virus. mAb injection was repeated at 7 days post-infection. One dam succumbed to the viral infection at 14 days post-infection and the second was euthanized at 14 days post-infection. Both animals failed to gain weight at the expected rate or lost weight relative to the start of the experiment. Viral loads were significantly elevated in maternal and fetal tissues relative to guinea pigs that had been infected after treatment with non-specific rat antibody.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain or distress would interfere with test results:

The objective of this study was to investigate the role of maternal and fetal T cells in controlling primary cytomegalovirus infection during pregnancy. Prior research in $CD4^+$ T cell-depleted macaques led us to anticipate that maternal and fetal viral loads would be elevated relative to infection in immunocompetent animals and that maternal and/or fetal demise could occur by 21 days post-infection.

This experiment is an infectious disease/immunologic study that required continuation until endpoint criteria are met. We compared viral load, maternal weight, placental weight, fetal weight, and maternal/fetal immune cell abundance between T cell depleted and normal guinea pigs. Administration of analgesics to reduce pain or distress could interfere with these parameters and confound our results, thus analgesics were not used to manage pain and we elected to use the earliest feasible experimental endpoints.

Huang, L. and G. Yang (2015). "Repeated exposure to ketamine-xylazine during early development impairs motor learning-dependent dendritic spine plasticity in adulthood." Anesthesiology **122**(4): 821-831.
Peterson, N. C., et al. (2017). "To Treat or Not to Treat: The Effects of Pain on Experimental Parameters." Comp Med 67(6): 469-482.
Rizzi, S., et al. (2008). "Clinical anesthesia causes permanent damage to the fetal guinea pig brain." Brain Pathol **18**(2): 198-210.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number.

N/A

Column E Explanation

1. **Registration number:** 41-R-005

2. Number of animals used in this study for the time period specified.
October 1, 2021 – September 30, 2022: 82 hamsters in pain class E during this time period

3. Species [common name] used in this study: Syrian Hamster (Mesocricetus auratus)

4. Explain the procedures producing pain or distress. Include a brief description of the procedures and the animal's experience, such as neurological signs, lethargy, inappetance, respiratory signs, etc.

Subcutaneous pancreatic tumors were induced in Syrian hamsters with the goal to develop novel Adenovirus-based therapeutics to deliver diagnostic and therapeutic agents to tumor sites with high specificity. Mild ulceration could be observed as a result of intratumoral hypoxic conditions. In addition, tumor necrosis (ulcers) could be also seen as an indicator of the successful therapeutic effect of the oncolytic adenoviruses. Based on our observations, less than 5% of Syrian hamster HP-1 pancreatic cancer xenografts may develop ulceration.

Tumor ulceration was observed in some animals (N=82) receiving subcutaneous tumor cells. These ulcerated tumors were shallow in depth, and we did not observe any bleeding in these animals nor any obvious signs of pain / distress, however there is the potential that the ulcerated tumors could have been causing pain, so the affected hamsters were considered to be in pain class E. Analgesics and antibiotics were not administered.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain or distress would interfere with test results:

We observed the animals with ulcers without antibiotics or analgesia to avoid possible effects of those drugs on the tumor response. In addition, we study the immunological aspects and the ability of Adenovirus-induced immunity to eliminate cancer cells. **The use of antibiotics and / or analgesic drugs would have interfered with the therapeutic / immunomodulatory effect of our adenovirus-based vectors and compromised our data.** Thus, the USDA pain class E was used in order to observe the ulcerated tumors without antibiotics or analgesia.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number.

N/A

Column E Explanation

1. **Registration number:** 41-R-005

2. Number of animals used in this study for the time period specified.
October 1, 2021 – September 30, 2022: Three hamsters in pain class E during this time period

3. **Species [common name] used in this study**: Hamster

4. Explain the procedures producing pain or distress. Include a brief description of the procedures and the animal's experience, such as neurological signs, lethargy, inappetance, respiratory signs, etc.

Using hamsters, we made subcutaneous tumors of pancreatic cancer cells with the goal to develop tumor-targeted therapies using oncolytic adenoviruses. To assess the antitumor effect of oncolytic adenovirus, we performed intratumor injection of virus (or control with tumor but no virus) and continued observation of tumor growth for three weeks. At the end of the experimental period, the subcutaneous tumors in some control hamsters (non-treatment group) developed small ulcerations on the surface of the tumors. The ulcerations caused pain and distress that were observed as reduced activity and appetite of the hamsters. Animals were euthanized within a few days after the incidence of ulceration in accordance with the approved endpoint of the overall experiment. Analgesics were not administered.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain or distress would interfere with test results:

Analgesics were not administered to the animals with ulcerated tumors, because their use would have adversely affected the scientific data obtained from this experiment. NSAID treatment can affect the tumor growth of pancreatic cancer cells. In addition, we avoided usage of buprenorphine or topical analgesics because these drugs can affect liver function and we needed to assess any hepatic dysfunction due to the oncolytic virus treatment compared to the control group.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number.

N/A