

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 58-R-0003
2. Number 30 of animals used in this study
3. Species (common name) Rabbits of animals used in the study.
4. Explain the procedure producing pain and/or distress.

Myxoma virus causes fatal disease in lab rabbits called myxomatosis. The myxoma virus is used to study the function of particular viral genes affecting virulence and to study how the disease progresses in the rabbit. Rabbits will be infected under a shaved area of skin. If the virus causes significant disease, the rabbit is humanely killed before the disease becomes too severe. If the virus caused little or no disease, rabbits will be infected with wild type virus 21 days later to determine whether or not infection provides protection from infection with the wild type virus. In other studies, infected rabbits will be humanely killed at various times after infection to better determine the effects of the virus on the rabbit.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The reason this study is a Category E is that the analyses require infection of rabbit with myxoma virus by subcutaneous route which produce clinical signs of myxomatosis. Late in infection rabbits may develop acute bacterial infection of the conjunctive and upper respiratory tract and these secondary infections have been postulated as a cause of death. Unfortunately, there is no suitable alternative to this infection process in order to establish pathogenesis. No analgesic or therapeutic treatments can be administered because they would adversely affect the pathogenesis of infection.

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 (e.g., APHIS, 9 CFR 113.102):

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1. Registration Number: 58-R-0003
2. Number 26 of animals used in this study
3. Species (common name) Equine of animals used in the study.
4. Explain the procedure producing pain and/or distress.

To characterize the local and systemic biodistribution of scAAV-eqIL-1Ra following intra-articular delivery, we will deliver an equivalent dose of scAAV-GFP (AAV containing the cDNA for green fluorescent protein) bilaterally to the midcarpal joints of 3 healthy horses and 3 horses with mild to moderate OA in those joints to visualize and map the geographic distribution of virally transduced cells intra-articularly, peri-articularly and systemically. Following injection of scAAV-GFP, the animals will be sacrificed 10 days later, and tissues will be harvested locally and systemically for characterization of transgenic expression, viral trafficking and histopathology.

To evaluate the capacity of scAAV-mediated delivery of eqIL-1Ra to block the pathology of experimental osteoarthritis in the equine joint, we will deliver scAAV-eqIL-1Ra to the midcarpal joints of horses with an osteochondral fragmentation model of OA and evaluate the effects of treatment over a period of 8 weeks. At the end of 3 weeks conditioning, both carpal joints will be arthroscopically examined. During this procedure, in one randomly selected joint, an 8 mm osteochondral fragment (OCF) will be generated in the radial carpal bone. We will monitor and record the degree of lameness before and weekly after surgery in 2 ways. The first is using the American Association of Equine Practitioners lameness scale. The second is using a kinematic evaluation of the gait of the horses. Immediately after surgery, it is expected that all horses will be lame at the grade 2 or 3 level. As the post-operative inflammation subsides, the degree of lameness is expected to drop by at least 1 grade in the scale. Horses that move from a grade 3 or lower score to a grade 4 or 5 lameness level will be treated to reduce their level of pain using cold therapy. If that is not sufficient to reduce the level of lameness, they will no longer be trained and they will be treated including fragment removal and post-operative anti-inflammatory treatment. If that is not sufficient, persistently lame horse will be subjected to euthanasia.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

Horses with moderate naturally occurring osteoarthritis in their forelimb joints are used in tracking the movement of the AAV vector in a disease context. These animals will only be used for short term experiments of less than two weeks and will not be treated for their condition because treatment would adversely affect the progression of disease under study.

In the induced-OA study, reduction in joint pain as evidenced by improved lameness and kinematic assessment scores will be the primary outcome measures of the effectiveness of the gene therapeutic in this study. Therefore, the administration of any drug that reduces pain will adversely affect and artificially influence the results of the study. Furthermore, administration of any analgesic or pain reliever of any type will interfere with the inflammatory process of the disease model and will adversely affect the progression of disease under study.

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1. Registration Number: 58-R-0003
2. Number 38 of animals used in this study
3. Species (common name) Guinea Pigs of animals used in the study.
4. Explain the procedure producing pain and/or distress.

This study has been classified as Category E because this study investigates the mechanisms of gentamicin-induced hearing loss. This aminoglycoside antibiotic is among the critical drugs used in the clinics for gram negative infections and is considered a lifesaving therapy. However, one of the severe side effects of these drugs is damage to the various components of the ear that provide for hearing and balance. Cochlear damage can produce permanent hearing loss, and damage to the vestibular apparatus results in dizziness, ataxia, and/or nystagmus.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

Moderate doses of gentamicin are used for short periods of time to study its effects on the ear and although we do not expect significant hearing loss or vestibular symptoms, the possibility does exist that a few guinea pigs may experience mild hearing loss or dizziness. We are not withholding treatment from any animal that would normally require treatment and our monitoring plan was developed based on our similar protocols, which have had significant veterinary feedback and is consistent with accepted principles. The gentamicin injections may also result in weight loss, however these are short-term insults (10 days). Subjects quickly begin to gain weight once treatments end. All animals will be weighed daily. Any animals that experience weight loss of 10% or greater will receive daily nutritional supplements with ground chow and fluids as appropriate. These animals will be reported to the veterinarians and carefully monitored.

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1. Registration Number: 58-R-0003
2. Number 10 of animals used in this study
3. Species (common name) Dogs of animals used in the study.
4. Explain the procedure producing pain and/or distress.

This study involves a breeding colony of dogs and therapeutic intervention of the disease. The study is Category E because the clinical manifestations of Glycogen Storage Disease Type Ia (GSDI) in neonates is severe hypoglycemia which frequently manifests as seizures, hepatomegaly, lactic acidosis, hyperuricemia, and hyperlipidemia which can result in pancreatitis.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

There is no withholding of anesthesia, analgesia, treatment or timely intervening euthanasia in this study. Hypoglycemia and lactic acidosis develop rapidly even if animals receive very frequent oral supplements of dextrose. Currently there is no cure for the disease. The only treatment is frequent/constant supplementation of glucose or raw cornstarch to significantly reduce the chance of severe hypoglycemia in between feedings that can lead to seizures and even death. While cornstarch therapy does help alleviate the symptoms of disease, it does not correct the underlying defect and complications occur. We have an extensive nursing team for 24 hour support of these dogs however until such time as we achieve the successful gene therapy as proposed by these studies the clinical issues are difficult to control and dogs may experience some distress associated with the clinical symptoms.

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 (e.g., APHIS, 9 CFR 113.102):

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