Attachment (Item 6)

Column E Explanation Form

This form is intended as an aid to completing the Column E explanation. 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.

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1. IACUC approved protocol number and date: 15-04, 2/2/15
2. Number of animals used under Column E conditions in this study: 91
3. Species (common name) of animals used in this study: guinea pig
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (Cut/paste from the approved docket and or amendments).

Guinea pigs are the major animal infection model established for the human herpes virus-2 (HSV-2) to evaluate protective efficacy of potential HSV-2 vaccines and therapeutic test compounds. The Hartley guinea pig model was chosen to observe clinical signs, body weight and mortality post HSV-2 infection and treatment with potential therapeutic test substances. Post infection, animals were monitored daily for clinical signs of disease. The viral infection of guinea pigs with HSV-2 can have varying clinical readouts from no clinical signs to morbidity. To evaluate the viral disease, we followed the established published literature for viral clinical scoring listed in the table below. Animals that reached a moribund state or exhibited a score of (4) or a loss of 30% body weight were immediately euthanized humanely.

Observation	Score
No clinical signs of disease	0
Vaginal erythema	1
Single to a few modest herpetic lesions	2
Large or fused vesicles	3
Severe vaginal ulceration and paralysis	4
Found dead	5

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

Analgesics may interfere with immune responses and physiological processes of the infection and the display of disease symptoms and interfere or obscure immune protection results. The measurement of clinical scores is vital to the determination of the efficacy of the vaccine. Therefore, we did not use analgesics that could interfere with test results. For example, morphine is known to suppress the innate immune system and reduce severity of HSV-1 infection; the opioid derivative buprenorphine may have similar effects; the cyclooxegenase-2 (Cox-2) pathway is required for efficient herpes virus replication: NSAIDS down-regulate this pathway and Cox-2 inhibitors limit the replication of herpes viruses; Lidocaine destabilizes the HSV virion. Therefore, animals exhibiting pain or unrelieved distress (reached a moribund state or exhibit a score of 4) or a loss of 30% body weight were immediately euthanized.

Agency None **CFR** _____

Attachment (Item 9)

Column E Explanation Form

This form is intended as an aid to completing the Column E explanation. 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.

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1. IACUC approved protocol number and date: 15-16, approved 11/12/15
2. Number of animals used under Column E conditions in this study 10
3. Species (common name) of animals used in this study *Callithrix jacchus* (common marmoset)
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (Cut/paste from the approved docket and or amendments).

Experimental autoimmune encephalomyelitis (EAE) is induced by immunization of animals with human recombinant myelin oligodendrocyte glycoprotein (rhMOG) emulsified in complete Freund's adjuvant (CFA) once on Day 1 by intradermal injection to the back in 2 spots (50 - 100 μL/spot). After a variable latency period, animals will develop mild symptoms of EAE (altered vision and gait) followed by rapid progression, including partial to total limb paralysis. Onset of disease symptoms may be associated with pain and distress that was not alleviated to allow for end point analysis (evaluation of Test Article effects). Humane end points were used to minimize possible pain and distress. The marmoset has been published as a reliable NHP model for EAE allowing for efficacy testing of novel biologics that would not be possible to test in small animal species due to immune system cross-reactivity to the test article target and immunogenicity.

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

Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disease model in which the immune system is triggered to attack a self-component. Treatment with Non-steroid anti-inflammatory drugs (NSAIDs) would alter this immune process and thus compromise the model and purpose of the study.

Agency None **CFR** _____

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