Category E
10/2018-9/2019 Fiscal Year

Species: Guinea Pig
Studies: MRQ011-PH14 **Number of Animals: 36** **Max Duration: 98 days**

Osteoarthritis (OA)/inflammatory pain

Procedure Summary:

OA pain state that will be treated with the proprietary compound. The use of a negative control group, which will be given only saline, is necessary to determine how well the pain state was induced and how well the test article ameliorates osteoarthritis pain. However, anesthetics (isoflurane) will be used for the surgical procedure for all animals.

Explanation for use of Guinea pigs:

The test article is intended to treat canines and felines and, as such, needs to be tested in animals prior to approval. Guinea pigs with MIA-induced OA are useful studying pain behavior in response to OA. Furthermore, Guinea pigs are relatively easy to handle and have sufficient joint size to evaluate biomarkers. In addition, the Guinea pig has been the most widely used model to evaluate inflammatory biomarkers related with OA. In light of the multifaceted general reasons to use Guinea pig model, it is hoped that this study can establish the model as suitable in particular for assessing the effects of test articles on the pain associated with bearing weight on a weight-bearing joint afflicted with OA. Additionally, the test article has a significantly greater affinity to guinea pig nerve growth factor than to rat nerve growth factor and, as such, testing in rats would not be valid. Finally, due to genetic variations in the targeted protein, the protein homology between Guinea pigs and canine/felines is more closely matched than in rats or mice.

Explanation for why analgesics cannot be used:

Analgesics were not used for two reasons: First, treatment of pain can interfere with the development of the pathology and resultant pain-related behaviors (which would result in those animals not meeting study requirements and being euthanized), and second, these animal models of pain are designed to test novel analgesic agents and as such, require drug-naïve animals.

Category E
10/2018-9/2019 Fiscal Year

Species: Canine
Studies: MRQ008-PH40 **Number of Animals**: 4 **Max Duration**: 14 days/dosing

Inflammatory pain

Procedure Summary:
Beagle dogs underwent unilateral injections of kaolin suspension in order to create a model of inflammatory pain. Lameness and paw edema were assessed ~1 day prior to kaolin injection, and ~3 hours, and 1, 2, 3, 4, 5, 8, 10, 14, 17, 21, 24, and up to 35 days after kaolin injection. Lameness lasted ~14 days following kaolin injection. After minimally a 35 day monitoring period, the dogs were given an additional 14 day washout period, then redosed. Two animals were dosed 5 separate times and two animals dosed twice.

Explanation for use of Dogs:
Dogs are a defined model for the assessment of inflammatory pain and can also serve as a target species for future test article evaluation (that is, dogs may be used as the subjects in this research with the ultimate goal being the development of therapies to treat inflammatory pain in dogs). Further, dogs offer a different dimension to the study of pain and pain therapeutics. While a majority of pain research can be and is done in rodents, rodents notoriously do not show robust affective signs of pain (such as pain-suppressed behaviors), which are key facets of pain in humans. As such, for many later stage investigational pain therapies, assessments in dog models of pain prove to be the most highly translatable preclinical models.

Explanation for why analgesics cannot be used:
The purpose of this study was to determine what pain behaviors are present and over what time course following kaolin injections, as such analgesics could not be administered without compromising the study endpoints. The inflammatory pain state lasts from ~1 to ~2 weeks post-injection. In order to understand the extent and time course of the development of any pain/distress and lameness that develops over the course of this study, animals were assessed frequently by trained staff to determine any changes in lameness and inflammation. This provided a frequent and thorough assessment of the animals' welfare during the course of the study.

The kaolin induced inflammation is expected to resolve over time and so the animals enrolled on this study are expected to return to a baseline state after a period of time. As a result, the animals do not need to be euthanized and can be used again after resolution of the inflammatory pain.

Exception/Exemptions
10/2018-9/2019 Fiscal Year

Species: Ovine

Studies:	**Animal Number:**	**Max Duration:**
HVT025-IS16	6	8 Days

Animals were restrained by a cross ties for the duration of the 8 day study. The study required constant administration of an anticoagulant to meet the endpoints and mitigate risk of clotting, which required the animals to be connected to tubing, along with an externalization of the test article. The animals were restrained from moving freely during the treatment period due to the risk of dislodgement of tubing and subsequent bleeding. A break could not be given to the animals from the cross tie due to the possibility of dislodgement of the test article or medication lines.

The animals were free to stand and lay down but were unable to rotate. Animals were also offered hay *ad libitum* for enrichment and feed.

Species: Ovine

Studies:	**Animal Number:**	**Max Duration:**
SAM001-IS75	4	11 days
SAM002-IS75	4	12 days
SAM003-IS75	5	13 days
SAM004-IS75	5	13 days

Exemption: housing space requirements

Animals were cross-tied for the duration of the experiment, up to 13 days based on study. There are several reasons for this level of restraint: 1) the animals will have a central venous line (CVL) and a lead running from the device in the aorta to the controller coming from their vessels to the outside. If either were to be removed unintentionally (i.e. by chewing or being caught on something), significant to life-threatening hemorrhage could occur. 2) The animals were receiving a heparin infusion to prevent clot formation in their aorta (via CVL) that would increase any bleeding resulting from what would otherwise be minor traumas. As discussed in the previous point, this could lead to life-threatening hemorrhage. On the other hand, should the CRI be disrupted by the animal (i.e. by chewing or being caught on something) and the coagulation time normalize, the animal may develop intra-arterial thrombi that could lead to thromboembolism while on study.

All animals to be used were acclimatized to the cross-ties for 10 sessions prior to the initial procedure. Animals that failed to tolerate the cross-ties for >4 hours were excluded from the study. Animals were given a 10-15 minute break every 4 hours from cross-tying if staff are present to supervise and prevent injury to the animal, lead exteriorization sites and/or CVL exteriorization sites. The animals were free to stand and lay down but were unable to rotate. Animals were also offered hay *ad libitum* for enrichment and feed.

Species: Ovine

Studies:	**Animal Number:**	**Max Duration:**
SDH003-IS21	6	9 days
SDH003-IS21	6	11 days

During the treatment the animals were required to wear a backpack carrying the test article and were connected to multiple devices, including portions of the device, a pump for administering IV fluids and medications and a console for making blood flow measurements. For up to 11 days of the study, the animals were cross tied. In this instance it would be detrimental to animal health and study endpoints for the tubing to become dislodged or removed, in which case would require euthanasia of the animal without meeting study endpoints.

All animals to be used were acclimatized to the cross-ties for 10 sessions prior to the initial procedure. Animals that failed to tolerate the cross-ties for >4 hours were excluded from the study. The animals were free to stand and lay down but were unable to rotate. The animals were monitored during the day and night during this time and allowed to move freely, with observation and guidance, 3-4 times a day for up to an hour. Animals were also offered hay *ad libitum* for enrichment and feed.

Category E
10/2018-9/2019 Fiscal Year

Species: Rabbits
Studies: APS414-IR99 **Number of Animals**: 6 **Max Duration**: 3 days

Skin Irritation Positive Control

Procedure Summary:
Four abrasions (about 2.5 cm X 2.5 cm) in a # shape in the keratinous layer (avoiding injury to the dermis) in the 2 caudal quadrants were made on each rabbits back. Formaldehyde (positive control) or saline (negative control) was applied directly to the skin via a 2.5 cm X 2.5 cm non-occlusive dressing (e.g. sterile gauze) over intact and abraded skin. The application site patches will be secured by wrapping the animals with a semi-occlusive bandage for 24 ± 0.5 hours. After wrapping was removed, the animal was monitored and irritation severity scored for 48 hours.

Explanation for use of Rabbits:
Irritation has been studied extensively in rabbits, and is an approved model for irritation testing among the regulatory bodies. Rabbits are widely used for testing irritation potential of test materials. Rabbits and humans have similarities in their immune system, particularly their skin reactivity that make them a good screening model for compounds that are intended to come in contact with humans. Rabbits are one of the preferred animal species for irritation testing due to their ability to readily display irritation responses.

Explanation for why analgesics cannot be used:
This study is designed to test the irritation potential of medical devices. The use of anesthetics, analgesics, or tranquilizers to alleviate pain is prohibited due to the potential molecular interaction between the drugs and the compounds associated with the medical device. This interaction may cause a response (inhibitory or synergistic) that could affect the outcome of the study. In order to effectively evaluate the characteristics of the device, the use of medications is contraindicated.

Federal Regulatory agency requiring procedure:
Japanese Ministry of Health, Labour, and Welfare (JMHLW) PFSB/ELD/OMDE Notification No. 0301-20 (2012): Part 5 Test for irritation.