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14. ABSTRACT

We have shown that scAAV vectors have the capacity to deliver exogenous transgenes to the joints of horses with high efficiency, enabling sustained localized expression of therapeutic transgenes at biologically relevant levels for at least 6 months. When delivered into joints with OA symptoms, dramatically higher levels of transgene expression are achieved, particularly in regions of damaged articular cartilage. Based on these preliminary data, we expect this system could be of tremendous benefit in OA, a chronic erosive joint disease, for which there are currently no useful treatments. To provide a clear assessment of the clinical potential of this technology we are testing the following hypothesis: scAAV-mediated gene delivery of IL-1Ra to large mammalian joints with chronic, symptomatic OA, will provide sustained long-term therapeutic benefit inhibiting the progression of joint degeneration and improving function and mobility. Additionally, we hypothesize that scAAV.IL-Ra can be delivered to large OA joints with a level of biosafety appropriate for human application. Currently we are half-way through Aim 1 of this proposal and have recruited 18/24 horses for the study. Of the recruited horses, 13 have undergone arthroscopic surgery to create an osteochondral defect and 7 of these animals have undergone treatment. Fluids and diagnostics are being collected and analyzed.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
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INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Osteoarthritis (OA) is a chronic, degenerative, often crippling disease that primarily affects large weight bearing joints. There is strong evidence that interleukin-1 (IL-1) is a primary driver of disease progression in OA, mediating cartilage loss, joint pain and inflammation. Its natural inhibitor, the IL-1 receptor antagonist (IL-1Ra), holds promise as a treatment. We have worked to develop methods for delivering the IL-1Ra cDNA to cells and tissues of diseased joints, which then become endogenous sites of sustained, high-level IL-1Ra production and release. For potential clinical use, adeno-associated virus (AAV) offers significant advantages compared to other vector systems due to its increased safety. The development of self-complementary (sc) vectors have further enhanced the efficiency and versatility of this system, such that AAV currently provides the most favorable profile for use in treating human joint disease. Following exploratory work that showed beneficial effects of scAAV-IL-1Ra gene transfer in the joints of rodents, we initiated studies in horses to assess its efficacy on a clinically relevant scale. We targeted the carpal and metacarpophalangeal (MCP) joints of the equine forelimbs, which are similar in size, weight bearing function and tissue composition to the human knee. The findings from these studies demonstrated the capacity of scAAV vectors to provide therapeutic benefit following delivery of IL-1Ra protein in joints of human scale. Building from this work, the current proposal has been formulated in response to pre-IND meetings with the FDA to address the long-term safety and efficacy of scAAV-mediated gene delivery of IL-1Ra for treatment of OA. We will test the hypothesis that scAAV-mediated gene delivery of IL-1Ra to large mammalian joints with symptomatic OA, will provide sustained, long-term therapeutic benefit- inhibiting the progression of joint degeneration and improving function and mobility. Additionally, we hypothesize that scAAV.IL-Ra can be delivered to large OA joints with a level of biosafety appropriate for human clinical trials.

1. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Osteoarthritis (OA)
Gene Therapy
Equine
Adeno-Associated Virus (AAV)
Interleukin-1 Receptor Antagonist (IL-1Ra)
Post-traumatic OA (PTOA)
Self-complimentary AAV (scAAV)
Cartilage
Synovium
Gene Transfer
Large animal model

2. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1 – To determine the capacity of local treatment with scAAV-eqIL-1Ra* to provide long-term protection from symptoms and progression of disease in an equine model of chronic osteoarthritis	**Timeline**	**Percentage of Completion**
Major Task 1	Months	
Subtask 1: Submit documents for ACURO** approval	0-4	100%
*Milestone # 1 ACURO** approval obtained*	4	100%
Subtask 2: Produce scAAV.eqIL-1Ra* Vector	1-23	100%
Subtask 3: Perform arthroscopy and induce OCF*** osteoarthritis model in midcarpal joint of 24 horses	4-19	100%
Subtask 4: Collect osteoarthritis baseline diagnostics for 24 horses (magnetic resonance imaging, radiography, lameness assessments, kinematics, biological fluids: blood, urine, synovial fluids)	6-21	100%
Subtask 5: Inject OCF*** Joints of 24 Horses with either scAAV.eqIL-1Ra* or saline control [12 horses X 2 groups = 24 horses total]	6-21	100%
Subtask 6: Train and monitor 24 animals for 12 months (perform treadmill training, weekly lameness assessments and kinematics; monthly biological fluid collection, 6 month magnetic resonance imaging)	6-32	90%
Subtask 7: Analyze biological samples (quantify by enzyme linked immunosorbent assay IL-1Ra levels in blood and urine, and IL-1Ra, prostaglandin E2 and collagen II fragments in synovial fluids)	6-34	90%
Subtask 8: Perform final diagnostics on 24 horses (magnetic resonance imaging, radiography, lameness assessments, kinematics, biological fluids, arthroscopy)	24-33	25%
Subtask 9: Analyze diagnostic readouts (quantify pathologies in magnetic resonsance images, radiographs, arthroscopic images; compare lameness assessments, kinematics)	8-35	30%
Subtask 10: Collate data from diagnostic and biological sample analyses and perform statistical analyses	8-36	25%
*Milestone # 2 Define the long-term benefit of local gene delivery of IL-1Ra**** in the treatment of osteoarthritis in a large mammalian joint*	36	0%

What was accomplished under these goals?

a) Major activity 1: Submit documents and obtain ACURO approval for equine studies.
Specific Objective: Obtain ACURO approval
Results: Both ACURO and IACUC approval was obtained in 2014 for the equine studies.

b) Major activity 2: Begin production of scAAV.eqIL-1Ra vector.
Specific Objective: Produce the viral vector needed for the completion of the study.
Results: **Completed**. We have accomplished 100% of this goal. The cDNA sequence for the native eqIL-1Ra cDNA was codon-optimized and synthesized (GeneArt, Life Technologies, Grand Island, NY) with a consensus Kozak sequence immediately upstream of the translation initiation codon and flanking recognition sequences for NotI and SacII. The engineered DNA construct was directionally inserted into the corresponding sites of the pHpa-tr-sk scAAV vector plasmid. In this construct, expression of the transgene is driven by the CMV immediate early promoter/enhancer. The scAAV vector construct was packaged in the AAV2.5 capsid at the University of Florida Vector Core or the University of North Carolina Chapel Hill Vector Core by co-transfection of 293 cells using methods previously described. Prior to experimental use in vivo, scAAV.eqIL-1Ra viral vector preparations were functionally characterized in vitro and validated for titer and transgene expression. We were originally going to make our DNA for viral production in house, but ultimately decided to outsource production to Aldeveron, a custom plasmid production company. This decision saved us time, as they are able to make DNA at a faster rate, and production was slightly less expensive. From this, we ended up with a high quality and uniform product and have made virus to inject into animals.

c) Major activity 3: Perform arthroscopy and induce osteochondral fragmentation (OCF) osteoarthritis model in the midcarpal joint of 24 horses.
Specific Objective: To create an osteochondral defect in horses that will model chronic OA.
Results: **Completed**. The horses for this study are staggered and, therefore, this goal was spread out over 19 months. We currently have generated the osteochondral defect in all horses. For this, a total of 20 healthy thoroughbred horses aged 2-5 yrs were selected. To ensure a uniform level of physical condition, prior to treatment, all horses were exercised 5 days/week for 3 weeks on a high-speed treadmill as follows: trotting 2 min at 16-19 km/hr; galloping 2 min at ~32 km/hr, followed by trotting 2 min at 16-19 km/hr.

At the end of conditioning, both carpal joints underwent MR imaging, followed by arthroscopic examination. During arthroscopy, in one randomly selected joint, an ~8 mm osteochondral fragment was generated in the radial carpal bone. After two weeks, to allow recuperation from surgery the animals resumed the 5 day/week athletic exercise regimen from above, for 10 weeks, which induced symptoms of early OA.

d) Major activity 4: Collect osteoarthritis baseline diagnostics for 24 horses (magnetic resonance imaging, radiography, lameness assessments, kinematics, biological fluids: blood, urine, synovial fluids).
Specific Objective: As each of these animals may respond differently to the generation of the OCF defect it is important to establish baselines for each of the parameters to be measured as part of the study prior to treatment so we can determine how the animals improved or changed with treatment.
Results: **Completed.** All horses have received the arthroscopic OCF surgery. We have completed baseline diagnostics for all animals and are currently analyzing the information. For this, after induction of OA following OCF generation, baseline levels of joint pathology were established for each animal. Clinical evaluation, visual lameness, joint pain and kinematics were also assessed, and both carpal joints were imaged by MRI. Blood was drawn, urine collected, and synovial fluid aspirated from both carpal joints. Diagnostic MR Imaging is performed at the UF Veterinary Medical Center using a scanner with a 1.5 tesla, short-bore superconducting magnet. PD, PD/FS, T2, T2/STIR and SPGR/FS scans from dorsal, sagittal, and axial views will be taken of both carpal joints. Kinematic assessment of the horses is performed using the Lameness Locator™, a wireless inertial sensor-based motion analysis system. Normal and impaired gait are assessed by measuring vertical acceleration of the torso to determine asymmetries in head and pelvic position between left and right halves of stride. Following attachment of the sensors to the head, right forelimb and pelvis, the horse is evaluated at a trot for 3 minutes. Repeated sequences of at least 25 strides are captured for analysis. Lameness is recorded as A1/A2 ratio as well as maximum and minimum head height difference with forelimb stride. For equine OA, clinician-assessed pain and lameness scores are the standards of practice. The overall health of the animals is assessed, as well as joint range of motion and capsule thickening. Pain with and without joint flexion is graded on a sliding scale from 0 to 3. For lameness evaluation, a visual

appraisal is made of the horse at rest for conformation, balance, and weight bearing, and evidence of injury or stress. The horse is then observed walking and trotting, from the front, back, and sides. Deviations in gait are noted, such as failure to land squarely on all four feet, the unnatural shifting of weight, stride shortening, irregular foot placement, head bobbing and stiffness. Lameness is then scored from 0 to 5, according to the guidelines of the American Association of Equine Practitioners.

e) Major activity 5: Inject OCF joints of 24 horses with either scAAV.eqIL-1Ra or saline control.
Objective: To measure the therapeutic efficacy of scAAV.eqIL-1Ra on a chronic equine model of OA ten animals are treated and ten are control.
Results: **Completed.** We have currently injected all animals with either saline or the viral vector. For this, the horses were randomly assigned into two equal groups: 1) treatment with scAAV-eqIL-1Ra and 2) placebo control. In a blinded fashion, a dose of 1×10^{13} vg of scAAV.eqIL-1Ra was injected into the OA joint of the animals in the treatment group; an equivalent volume of saline will be injected into the OA joint of the placebo controls. Afterward, the animals were exercised twice per week for the next 12 months to enable gradual progression of the long-term OA model.

f) Major activity 6: Train and monitor 24 animals for 12 months (perform treadmill training, weekly lameness assessments and kinematics; monthly biological fluid collection, 6 month magnetic resonance imaging).
Objective: Perform diagnostic tests to measure any improvements or changes in joint function as determined by pain assessment, lameness score and kinematics.
Results: All of the animals that have been injected with either saline or scAAV.eqIL-1Ra and are in this stage of monitoring. Nine animals have completed this portion of monitoring. For this, over the course of the yearlong study the animals are monitored daily for changes in behavior, and periodically evaluated for the effects of treatment. Peripheral blood was collected for toxicology studies. Synovial fluid was aspirated from the carpal joints and analyzed for eqIL-1Ra levels and pathologic markers. Clinical evaluations, visual lameness score, joint pain and kinematic assessments was performed monthly. After 12 months, in addition to radiograph and MRI, the joints will be examined arthroscopically and digitally recorded. Synovial and articular cartilage biopsies will be taken for histologic examination.

1) Clinical Evaluation: Joint Pain and Lameness: For equine OA, clinician-assessed pain and lameness scores are the standards of practice. The overall health of the animals is assessed, as well as joint range of motion and capsule thickening. Pain with and without joint flexion is graded on a sliding scale from 0 to 3. For lameness evaluation, a visual appraisal is made of the horse at rest for conformation, balance, and weight bearing, and evidence of injury or stress. The horse is then observed walking and trotting, from the front, back, and sides. Deviations in gait are noted, such as failure to land squarely on all four feet, the unnatural shifting of weight, stride shortening, irregular foot placement, head bobbing and stiffness. Lameness is then scored from 0 to 5, according to the guidelines of the American Association of Equine Practitioners.

To date, two animals from Aim 1 remain and are due for endpoint in September. Once these animals have completed the study, the subjective visual lameness assessments that were performed by two qualified evaluators, appropriately blinded, will be logged for each of the animals. This data will be sent to our collaborator Dr. David Nickerson, Professor and Chair of the Department of Statistics & Actuarial Sciences at the University of Central Florida to assist with data management and statistical analysis of the large dataset generated. Estimated completion: March 2018.

2) Kinematic Analysis: For this, kinematic assessment of the horses was performed using the Lameness Locator™, a wireless inertial sensor-based motion analysis system. Normal and impaired gait are assessed by measuring vertical acceleration of the torso to determine asymmetries in head and pelvic position between left and right halves of stride. Following attachment of the sensors to the head, right forelimb and pelvis, the horse is evaluated at a trot for 3 minutes. Repeated sequences of at least 25 strides are captured for analysis. Lameness is recorded as A1/A2 ratio as well as maximum and minimum head height difference with forelimb stride.

To date, two animals from Aim 1 remain and are due for endpoint in September. For each weekly session, at least 3 measurements were taken at a ~4 m/sec trot on a treadmill. Each measurement will be calculated from a minimum of 30 uninterrupted strides. Lameness will be calculated as a vector sum using the mean maximum head difference (HDmax) and mean minimum head difference (HDmin)

between the left and right strides for every stride in each measurement. For each session the means of the HDmax and HDmin from at least 3 measurements will be used to calculate the vector sum (VS) as follows: VS = √HDmax2 + HDmin2. Once the data is recorded for each of the animals in the study, and the appropriate lameness calculations are performed (~3 months), this data will be sent to our collaborator Dr. David Nickerson, Professor and Chair of the Department of Statistics & Actuarial Sciences at the University of Central Florida to assist with data management and statistical analysis of the large dataset generated. Estimated completion: April 2018.

g) Major activity 7: Analyze biological samples (quantify by enzyme linked immunosorbent assay IL-1Ra levels in blood and urine, and IL-1Ra, prostaglandin E2 and collagen II fragments in synovial fluids).
Objective: Measure and analyze fluids for reduced levels of inflammatory and degradative signaling molecules. Periodic quantitation of eqIL-1Ra levels in synovial fluid will be used to correlate the biological and functional responses at each level of analysis with temporal patterns of therapeutic transgene expression.
Results: We are analyzing fluids for this portion of the study and will continue this activity until the study is complete. Equine IL-1Ra levels in recovered fluid samples will be measured using commercially available kits (R&D Systems; # DY2466). 2) Leukocytic infiltration/ WBC count using a haemocytometer will be used as an absolute and relative index of inflammation. Normal=100-150 cells/ml. 3) Fluids will also be analyzed for treatment-associated changes in collagen II (Chondrex, #6009) and PGE_2 using commercially available ELISA.

Synovial fluids were diluted 1:1 with buffered saline containing hyaluronidase at 50 u/ml, and incubated at 37°C for 30 minutes prior to analysis. For serum and synovial fluid, two-fold serial dilutions in reagent diluent (R&D Systems) were generated over a wide range to account for assay variability. Each dilution series was generated in duplicate, and each diluted sample was assayed in triplicate wells. Means were calculated from samples with readouts within the boundaries of the standard curves of the respective assays. To date we have seen continued and stable expression of our transgene within the treated joints for over 1 year (Figure 1).

1) Synovial Fluid Analysis: 90% completed. Waiting for final samples. 1) Equine IL-1Ra levels in recovered fluid samples will be measured using commercially available kits (R&D Systems; # DY2466). 90% completed. Waiting for final samples. 2) Leukocytic infiltration/ WBC count using a haemocytometer will be used as an absolute and relative index of inflammation. Normal=100-150 cells/ml. 90% completed. Waiting for final samples. 3) Fluids will also be analyzed for treatment-associated changes in collagen II (Chondrex, #6009) and PGE2 using commercially available ELISA. Estimated completion: October 2017.

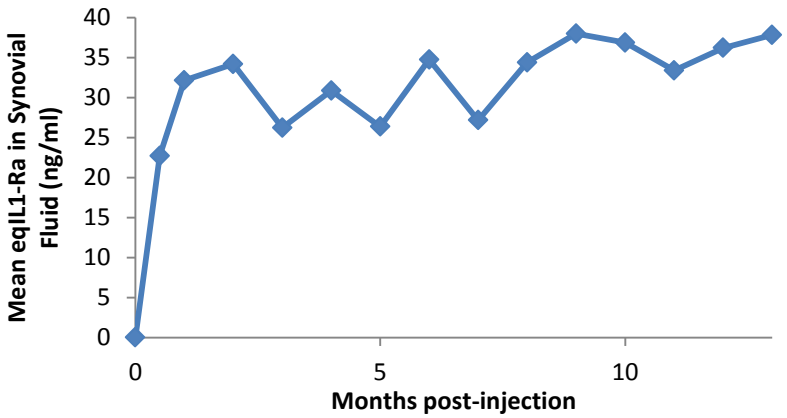

Figure 1. Mean eqIL-1Ra levels in synovial fluid of the OCF joints from the *Treated* group (n=6) shows elevated levels of eqIL1-Ra that are maintained over the course of one year.

h) Major activity 8: Perform final diagnostics on 24 horses (magnetic resonance imaging, radiography, lameness assessments, kinematics, biological fluids, arthroscopy).
Objective: One year after receiving treatment, final diagnostics are performed to determine efficacy.
Results: After 12 months, in addition to radiograph and MRI, the joints will be examined arthroscopically and digitally recorded. Synovial and articular cartilage biopsies will be taken for histologic examination.
We are nearly done with fluid analysis and are organizing MRI images for pathological scoring as well as lameness readouts for statistical analysis and efficacy measurements. * see (i) below for full details.

i) <u>Major activity 9:</u> Analyze diagnostic readouts (quantify pathologies in magnetic resonance images, radiographs, arthroscopic images; compare lameness assessments, kinematics).
<u>Objective:</u> Analyze all diagnostic measurements one year after receiving treatment, to determine efficacy.
<u>Results:</u> We are still in the process of collecting readouts for analysis. Final analysis has begun and will continue over the next 6 months – one year.

During treadmill training the horses were evaluated monthly for lameness by both visual scoring and motion analysis. Kinematic assessment of two of the horses (one control, one treated) is shown below. Data collected using the Lameness Locator™, a wireless inertial sensor-based motion analysis system. Normal and impaired gait are assessed by measuring vertical acceleration of the torso to determine asymmetries in head and pelvic position between left and right halves of stride. Following attachment of the sensors to the head, right forelimb and pelvis, the horse is evaluated at a trot for 3 minutes. Repeated sequences of at least 25 strides are captured for analysis. Lameness is recorded as A1/A2 ratio. To date, it is difficult to draw any conclusions on lameness as all of the animals are not complete. In the figure below we show an example of one animals improved lameness after treatment as opposed to one untreated animal (Figure 2).

Both intercarpal joints of the horses in the Treated and Control groups were examined and imaged arthroscopically following generation of the osteochondral lesion and again at the endpoint of the experimental protocol. Digital images collected during the procedures were scored by three blinded evaluators for the size of the lesion and degree of fragment repair, integration of border zone of the defect with surrounding cartilage, appearance of surface cartilage overall, and appearance of synovium and ligaments. Based on criteria from Dymock et al.[40] a scoring system from 0 to 10 was used where 0 represented normal, and 10 represented severe pathology. As seen in Figure 3, it appears as if the treated Osteochondral fragment has completely healed in the treated animal. Over the next year we will grade and score both treated and untreated to determine statistical significance.

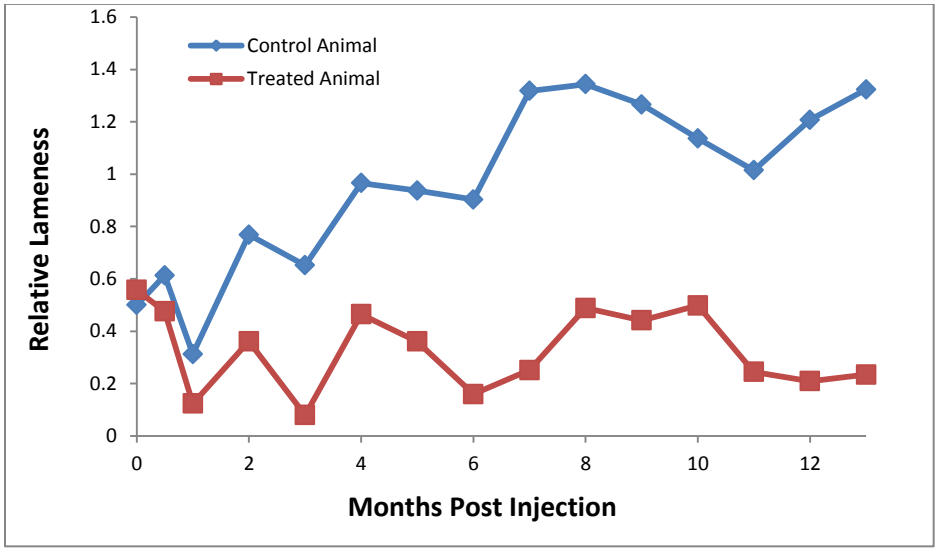

Figure 2. Relative change in lameness (vector sum) assessed by the inertial sensor motion analysis system (Lameness Locator®). Relative lameness of the OCF joints from the *Treated* group & Control group (n=2) shows improved lameness over the course of one year in the treated animal as opposed to worsening lameness in the untreated animal. Over the next year we will perform detailed statistical analysis of all the lameness data to determine if there were any statistically significant improvements in the treated group.

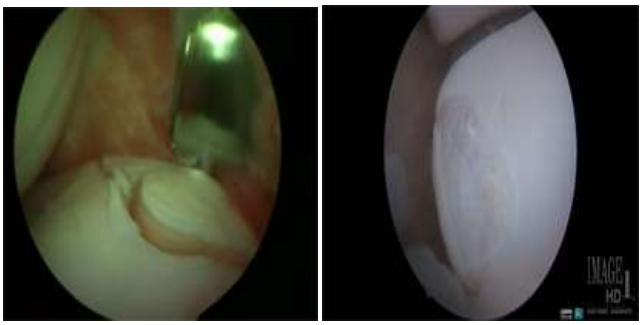

Figure 3. The above arthroscopic images are of the osteochondral lesions in a *Treated* horse following the generation of the osteochondral lesion (Week -2) and at endpoint (Month 12) and show a healed lesion in a treated animal. Images of treated and control animals will be scored and evaluated over the coming year to determine if any statistically significant changes occurred between treatment groups.

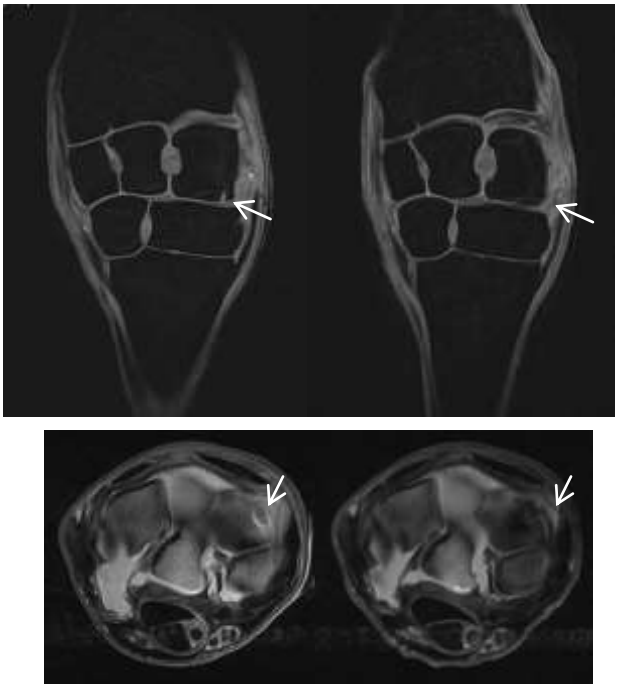

Figure 4: Both intercarpal joints the horses in the Treated and Control groups were scanned by MR imaging immediately prior to treatment and at the end of the experimental protocol (12 months). Images above are from a treated animal. Images to the left are at the start of the experimental protocol and those to the right are at 12 months. White arrows indicate location of osteochondral defect.

1) Magnetic Resonance Imaging: Diagnostic MR Imaging will be performed of both carpi as a fee for service at the UF Veterinary Medical Center using a scanner with a 1.5 tesla, short-bore superconducting magnet. Under general anesthesia, the horses were placed in left lateral recumbency with each carpus in partial flexion (15-25 degrees) in a quadrature transmit/receive knee coil (QD Knee). The MR coil and sequences were selected and optimized to be clinically applicable in live horses, and included sagittal and axial proton density (PD), dorsal T2-weighted, axial T2 short-tau inversion recovery (STIR), sagittal proton density with fat suppression (PD-FS), and sagittal spoiled gradient echo with fat suppression (SPGR-FS). The MR scans for each horse will need to be examined by blindly by certified veterinary radiologists on staff, as well as Drs. Colahan and Smith blinded to treatment group assignment. Following review of the scans from the 6 MR sequences for each intercarpal joint and time point, scores will be assigned for the predominant pathologies associated with the model, including synovial effusion, synovial proliferation, severity of the osteochondral lesion, damage to articular cartilage, marrow edema in the radiocarpal bone, sclerosis of the radial carpal and third carpal bones, joint capsule edema and capsular fibrosis, using a scale from 0 to 10, where 0 represented

normal and 10 represented severe pathology. To date, two animals from Aim 1 remain and are due for endpoint in September. Once all animals have completed the study, MR images will be organized into files and categorized. Due to the large volume of MR images and the number of sequences performed for each time point and each joint, and based on experience with our previous study, we expect an Estimated completion: April 2018.

2) Digital Radiography: Radiographs (5 views) of the carpal joints will be taken using a Kodak digital radiographic system. Drs. Colahan, Smith and Broome will evaluate the images blinded and will score pathology for in the following manner. Osteophyte formation will be scored on a sliding scale from 0-3, grading for new peri-articular bone and alteration of joint margin. Subchondral bone lesions will be classified from 0-4 for loss of trabecular structure and delineation between cortex and medulla. To date, two animals from Aim 1 remain and are due for endpoint in September. Once all animals have completed the study, digital radiographs will be blindly scored for treatment effects. Estimated completion: December 2017.

3) Arthroscopic Evaluation: Diagnostic arthroscopy will be performed by Drs. Colahan and Smith. For this, both intercarpal joints of the horses in the Treated and Control groups were examined and imaged arthroscopically following generation of the osteochondral lesion and again at the endpoint of the experimental protocol. Video recordings and digital images collected post-fracture at Week -2 and prior to repair of the lesion at endpoint will be scored by three evaluators (blinded to the identity of the animals and their treatment) using the SFA grading system for the size of the lesion and degree of fragment repair, integration of border zone of the defect with surrounding cartilage, appearance of surface cartilage overall, and appearance of synovium and ligaments. A scoring system from 0 to 10 will be used where 0 represents normal, and 10 represents severe pathology. To date, two animals from Aim 1 remain and are due for endpoint in September. Once all animals have completed the study, arthroscopic images will be organized into files, we will take individuals pictures from several aspects of each joint for each time point. These images will then be blindly scored for treatment effects. Estimated completion: January 2018.

4) Clinical Evaluation: Joint Pain and Lameness: For equine OA, clinician-assessed pain and lameness scores are the standards of practice. The overall health of the animals is assessed, as well as joint range of motion and capsule thickening. Pain with and without joint flexion is graded on a sliding scale from 0 to 3. For lameness evaluation, a visual appraisal is made of the horse at rest for conformation, balance, and weight bearing, and evidence of injury or stress. The horse is then observed walking and trotting, from the front, back, and sides. Deviations in gait are noted, such as failure to land squarely on all four feet, the unnatural shifting of weight, stride shortening, irregular foot placement, head bobbing and stiffness. Lameness is then scored from 0 to 5, according to the guidelines of the American Association of Equine Practitioners. To date, two animals from Aim 1 remain and are due for endpoint in September. Once these animals have completed the study, the subjective visual lameness assessments that were performed by two qualified evaluators, appropriately blinded, will be logged for each of the animals. This data will be sent to our collaborator Dr. David Nickerson, Professor and Chair of the Department of Statistics & Actuarial Sciences at the University of Central Florida to assist with data management and statistical analysis of the large dataset generated. Estimated completion: March 2018.

4) Kinematic Analysis: For this, kinematic assessment of the horses was performed using the Lameness Locator™, a wireless inertial sensor-based motion analysis system. Normal and impaired gait are assessed by measuring vertical acceleration of the torso to determine asymmetries in head and pelvic position between left and right halves of stride. Following attachment of the sensors to the head, right forelimb and pelvis, the horse is evaluated at a trot for 3 minutes. Repeated sequences of at least 25 strides are captured for analysis. Lameness is recorded as A1/A2 ratio as well as maximum and minimum head height difference with forelimb stride. To date, two animals from Aim 1 remain and are due for endpoint in September. For each weekly session, at least 3 measurements were taken at a ~4 m/sec trot on a treadmill. Each measurement will be calculated from a minimum of 30 uninterrupted strides. Lameness will be calculated as a vector sum using the mean maximum head difference (HDmax) and mean minimum head difference (HDmin) between the left and right strides for every stride in each measurement. For each session the means of the HDmax and HDmin from at least 3 measurements will be used to calculate the vector sum (VS) as follows: $VS = \sqrt{HDmax2 + HDmin2}$. Once the data is recorded for each of the animals in the study, and the appropriate lameness calculations are performed (~3 months), this data will be sent to our collaborator Dr. David Nickerson,

Professor and Chair of the Department of Statistics & Actuarial Sciences at the University of Central Florida to assist with data management and statistical analysis of the large dataset generated. Estimated completion: April 2018.

j) Major activity 10: Collate data from diagnostic and biological sample analyses and perform statistical analyses.
Objective: Compare all diagnostic measurements and readouts for each animal to determine overall efficacy.
Results: The efficacy of treatment will be assessed by 1) improvement in joint function as determined by pain assessment, lameness score and kinematics 2) reduced progression of articular pathology via joint radiographs, MRI, and diagnostic arthroscopy. 3) Reduced pathology at the cellular and extracellular matrix levels by histologic examination of synovial and cartilage biopsies acquired post-treatment, and 4) reduced levels of inflammatory and degradative signaling molecules in synovial fluid.
We are in the process of collecting all diagnostic measurements and readouts for analysis. Final analysis has begun and will continue over the next year.

Statistical analysis will begin over the next 6 months – one year, once every animal has completed the study. The experimental layout is a two-sample repeated measures design with horses randomly assigned to the treatment groups (placebo or scAAV-eqIL-1Ra). The data will be analyzed using a multivariate approach based a one-way MANOVA model. For power calculations, we used data generated from a pilot study in the context of the equine OA model to estimate the repeated measures correlation. With a Type error of 0.05, 80% power, a repeated measures correlation of 0.55 and an effect size f = 0.58 (large), a total of 20 horses in the study were required to show a treatment effect after 2 measurements. Under the same conditions the necessary effect sizes were 0.55, 0.54, 0.53 and 0.52 for 3, 4, 5, and 6 measurements, respectively. Since there is anticipated to be a correlation between baseline measurements and the repeated measurements, under the same conditions listed above, the power will exceed 80% with the inclusion of baseline measurements as covariates.

The data gathered from the temporal and end-point analyses will be collated and assembled to develop a comprehensive description of the effects of sustained IL-1Ra delivery in the context of chronic OA, and the merit of this gene therapeutic approach in the treatment of human disease. Based on our previous experience with intra-articular gene transfer in rodent models, we have found that IL-1Ra, when maintained at sufficient levels intra-articularly, can effectively block the pathogenic effects of IL-1, leading to beneficial effects even in severe arthritis.[48] Our preliminary results show that scAAV can provide high levels of transgene expression in normal equine joints for at least 6 months. We have found scAAV gene transfer and expression in joints with OA to be significantly greater, thus we expect that the treated joints will produce IL-1Ra at biologically relevant levels for the duration of the study.

Relative to placebo controls, we expect that treatment with scAAV-eqIL-1Ra will provide significant protection from the progressive degeneration of the articular tissues and pathologies associated with the long term OA model. This should be reflected across all levels of analysis, in the form of reduced cartilage erosion and improved matrix quality, fewer osteophyte and bone lesions as well as reduced synovitis, inflammation and effusion. We expect that the protective effects on the articular tissues will translate into improved joint function, such that the treated animals will have reduced joint pain and improved mobility, resulting in a significantly reduced lameness relative to placebo controls. If successful, the data from these large animals studies will provide compelling evidence for exploration of this approach in humans, and will form an important component of an IND application to the FDA.

1) Study Design and Power Calculations: These calculations will be performed for the experiments noted above by Dr. Nickerson. The experimental layout is a two-sample repeated measures design with horses randomly assigned to the treatment groups (placebo or scAAV-eqIL-1Ra). The data will be analyzed using a multivariate approach based a one-way MANOVA model. For power calculations, we used data generated from a pilot study in the context of the equine OA model to estimate the repeated measures correlation. With a Type error of 0.05, 80% power, a repeated measures correlation of 0.55 and an effect size f = 0.58 (large), a total of 20 horses in the study were required to show a treatment effect after 2 measurements. Under the same conditions the necessary effect sizes were 0.55, 0.54, 0.53 and 0.52 for 3, 4, 5, and 6 measurements, respectively. Since there is anticipated to be a correlation between baseline measurements and the repeated measurements, under the same conditions listed above, the power will exceed 80% with the inclusion of baseline measurements as covariates.

What opportunities for training and professional development has the project provided?
Nothing to Report

How were the results disseminated to communities of interest?
We are collecting and compiling data received from the first group of horses. We expect to present our interim data and preliminary findings at a national meeting in the coming year.

What do you plan to do during the next reporting period to accomplish the goals?

During this reporting period we have accomplished a great deal and are on schedule as outlined in our statement of work. Much of the work during the current reporting period involved the completion of the acquisition of test subjects, the completion of the production of scAAV.eqIL-1Ra virus, recruiting and the conditioning of healthy Thoroughbred horses for the study, surgically inducing the OCF osteoarthritis model in the midcarpal joint of each of the study animals, performing intra-articular injections of all study animals, and performing final necropsy for toxicology analysis.

In addition to what is listed above, we have several experiments from Aim 2 of this study that remain:

Specific Aim 2: To establish formal toxicology and biodistribution profiles for scAAV.IL-Ra gene transfer to a large mammalian joint.

1) Complete Blood Count: As appropriate for each study group, blood collected at pre-surgery, post-surgery, pre-vector delivery, and days 1, 3, 7, 14, 30, 60, 90 and sacrifice, will be analyzed (including WBC and RBC counts, and percent neutrophils, lymphocytes, monocytes, eosinophils, and basophils, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet count and mean platelet volume). 90% completed. Waiting for final samples. Estimated completion: October 2017.

2) Serum Chemistries: (including creatinine kinase, asparatate aminotransferase, blood urea nitrogen, alanine aminotransferase, alkaline phosphatase and creatinine, as well as gamma-glutamyl transferase, total protein, sodium, chloride and potassium, pending sample availability). The variable of body weight, complete blood counts and serum chemistries will be assessed by a 1-way ANOVA comparison of baseline and necropsy data. 90% completed. Waiting for final samples. Estimated completion: October 2017.

3) Circulating anti-AAV antibodies: (IgG, IgA, IgM) will also be assessed in serum and synovial fluids at baseline, days 14 and 90. Estimated completion: October 2017.

4) Biodistribution: Animals in both cohorts will also be used for evaluation of vector distribution in a tissue-only GLP study. Genomic DNA (gDNA) will be extracted from frozen tissue collected at necropsy or blood collected at various intervals. The entire panel of tissues is to include the Testis (R), Ovary (R), Seminal Vesicle, Oviduct, Epididymis, Uterus, Prostate, Vagina, Urinary Bladder, Kidney (R), Adrenal Gland (R), Lymph Node (Mesenteric Draining Lymph Node), Stomach, Duodenum, Jejunum, Ileum, Spleen, Pancreas, Diaphragm, Lung, Heart, Eye, Cerebellum, Cerebrum, Spinal Cord (lumbar), Bone Marrow (R and L Femur), Carpus (Injected and Contralateral):Extensor carpi radialis, Common digital extensor, Lateral digital extensor, Superficial digital flexor, Deep digital flexor, Biceps brachii, Triceps brachii, Suspensory ligament and Synovium. DNA will be quantified and analyzed by real time PCR for vector sequences, typically using 1 ug of DNA for each sample and running PCR assays in triplicate, at minimum sensitivity of 50 vg/ug. Tissue collection, gDNA isolation, quantitation, and real-time PCR quantification of AAV vector genomes will be conducted according to standard operating procedures established at the Powell Gene Therapy Center resulting in an audited report for inclusion in the IND. Analysis has begun by the University of Florida Toxicology Core. Estimated completion: October 2017.

During the next reporting period, we plan to finish each of goals outlined in the first and second aims of the study. These goals include finish measurements of IL-1Ra & analysis of biological fluids, obtain final measurements and perform final diagnostics for MRI, lamness assessment/kinematics and arthroscopy.

We will also quantify pathologies and perform statistical analysis. Images and video recordings from the procedures will be evaluated using the SFA grading system. Cartilage lesions are graded for swelling and softening, fibrillation and exposure of subchondral bone. The presence of osteophytes, inflammation and hypertrophy of synovium and ligamentous tissue is graded on a similar scale.MRI series will be graded for location and severity of

defects of cartilage, bone marrow edema, osteophytes, subchondral cysts, sclerosis, joint effusion, synovitis, and capsular edema and fibrosis. Each will be graded on a linear scale from 0-10. As part of the imaging service, MRIs will be assessed and graded blindly by certified veterinary radiologists on staff, as well as Drs. Colahan and Smith. At the end of the final reporting period we will have all data completed and begin preparations for manuscript publication.

We have not encountered any problems to date and no specific technical problems are anticipated. All the facilities at the University of Florida are currently housing, caring for and treating the animals. The research team is established and well experienced with animal and each of the technologies and assays described.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The data gathered from the temporal and end-point analyses will be collated and assembled to develop a comprehensive description of the effects of sustained IL-1Ra delivery in the context of chronic OA, and the merit of this gene therapeutic approach in the treatment of human disease. Based on our previous experience with intra-articular gene transfer in rodent models, we have found that IL-1Ra, when maintained at sufficient levels intra-articularly, can effectively block the pathogenic effects of IL-1, leading to beneficial effects even in severe arthritis.[48] Our preliminary results show that scAAV can provide high levels of transgene expression in normal equine joints for at least 6 months. We have found scAAV gene transfer and expression in joints with OA to be significantly greater, thus we expect that the treated joints will produce IL-1Ra at biologically relevant levels for the duration of the study.

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What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report as this study is not yet completed. However, we performed long-term efficacy studies of a local gene-based therapy for post-traumatic osteoarthritis using the horse as a large animal model. We will delivered a self-complementary adeno-associated virus (AAV) containing the cDNA for interleukin-1 receptor antagonist (IL-1Ra) to the forelimb joints of horses with established joint disease, and monitored the effect on disease progression over the course of one year. Formal toxicology and biodistribution studies are also being conducted to assess the safety at both the acute and long-term phases of treatment. These studies are intended to model closely the scale and effects of treatment in humans, and were designed following discussions with the FDA. If successful, the data will comprise critical components of an IND application for phase I clinical trials.

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

We requested (& were approved) a one-time no cost extension for 12 additional months. No changes will be made to the approved objectives or scope of the project. Additional time is needed to complete multiple key aspects of this proposal and ensure completion of the original project scope. This massive project involves over 30 horses. These animals need constant veterinarian observation and require a team of handlers. Because of this we planned to stagger the study animals over 2 years to make this project manageable. While we have not encountered any specific technical problems, the logistics of the treatment procedures and diagnostics did take longer than we anticipated during the initial planning of this proposal. To accommodate such a large number of study animals, these animals were actually staggered over the course of ~3 years. To date, our 2 final animals (from Aim 1) will complete the study at the end of September. Because of this, there are a number of analyses that we have not yet been able to start or complete. Below is a detailed list of the experiments that remain for each Specific Aim and an estimated date of completion. We expect to finish well within 12 months and publish our results.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to Report

  Books or other non-periodical, one-time publications.

  Nothing to Report

  Other publications, conference papers, and presentations.

  Nothing to Report

- **Website(s) or other Internet site(s)**

  Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Nothing to Report

- **Other Products**
  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Steven Ghivizzani
Project Role:	PI
Researcher Identifier:	ORCID ID: 0000-0001-5154-7581
Nearest Person Month Worked:	3
Contribution to Project:	Dr. Ghivizzani is responsible for the overall planning and coordination of all aspects of the equine research study. In addition, he assists with the generation of the osteochondral fragment model and MRIs.

Name:	Patrick Colahan
Project Role:	Co-PI
Researcher Identifier:	ORCID ID: 0000-0001-5278-1564
Nearest Person Month Worked:	5
Contribution to Project:	Dr. Colahan oversees and participates in all aspects of the study as they relate to the care, treatment, surgery and evaluation of the horses. Dr. Colahan generates the surgical defects, and assists with the arthroscopic surgeries, MRIs and radiographies.

Name:	Rachael Watson Levings
Project Role:	Scientific Research Manager
Researcher Identifier:	ORCID ID: 0000-0003-0913-290X
Nearest Person Month Worked:	4
Contribution to Project:	Dr. Watson Levings coordinates the preparation of the DNA required to make the viral vector for the study as well as the generation of the scAAV.eqIL-1Ra vector. She prepares virus/saline for delivery and assists with equine injections and the collection of biological fluids.

Name:	Ted Broome
Project Role:	Graduate Student
Researcher Identifier:	ORCID ID: 0000-0002-9272-8669
Nearest Person Month Worked:	5
Contribution to Project:	Dr. Broome performs clinical evaluation, visual lameness scoring and kinematic assessment of the horses and contributes to all aspects of animal care. He also assists with the induction of the arthritis model and the collection of fluids.

Name:	Andrew Smith
Project Role:	Graduate Student
Researcher Identifier:	ORCID ID: 0000-0003-2772-866X
Nearest Person Month Worked:	5
Contribution to Project:	Dr. Smith performs the arthroscopic procedures to generate the

osteochondral defects for induction of the OA model. He also performs clinical evaluation and visual lameness scoring of the horses and contributes to all aspects of animal care. Dr. Smith also assists with the MR imaging and radiography.

Name:	Brett Rice
Project Role:	Animal Technician
Researcher Identifier:	ORCID ID: 0000-0003-1296-4192
Nearest Person Month Worked:	5
Contribution to Project:	Mr. Rice contributes to the care, treatment and handling of all animals. He assists with the fluid collection and coordinated the procurement of subjects for the study. He also directs and coordinates the exercise and handling of the horses during evaluations, surgeries and injections.

Name:	E. Anthony Dacanay
Project Role:	Technician
Researcher Identifier:	ORCID ID: 0000-0002-0050-7106
Nearest Person Month Worked:	4
Contribution to Project:	Mr. Dacanay is responsible for preparing DNA for viral production. He also helps prepare vectors for injection and assists with the collection and storage of animal fluids.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: No changes.

9. APPENDICES: Nothing to Report