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AWARD NUMBER: W81XWH-14-1-0498

TITLE: Gene Therapy for Post-Traumatic Osteoarthritis

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CONTRACTING ORGANIZATION: University of Florida
Gainesville, FL 32611

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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

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a. REPORT

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b. ABSTRACT

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Unclassified

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19a. NAME OF RESPONSIBLE PERSON

19b. TELEPHONE NUMBER

(include area code)
352-273-7059

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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-1Ra 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% |
| <i>Milestone # 1 ACURO** approval obtained</i> | 4 | 100% |
| Subtask 2: Produce scAAV.eqIL-1Ra* Vector | 1-23 | 50% |
| Subtask 3: Perform arthroscopy and induce OCF*** osteoarthritis model in midcarpal joint of 24 horses | 4-19 | 50% |
| Subtask 4: Collect osteoarthritis baseline diagnostics for 24 horses (magnetic resonance imaging, radiography, lameness assessments, kinematics, biological fluids: blood, urine, synovial fluids) | 6-21 | 75% |
| 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 | 30% |
| 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 | 18% |
| 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 | 30% |
| Subtask 8: Perform final diagnostics on 24 horses (magnetic resonance imaging, radiography, lameness assessments, kinematics, biological fluids, arthroscopy) | 18-33 | N/A |
| Subtask 9: Analyze diagnostic readouts (quantify pathologies in magnetic resonance images, radiographs, arthroscopic images; compare lameness assessments, kinematics) | 6-35 | 10% |
| Subtask 10: Collate data from diagnostic and biological sample analyses and perform statistical analyses | 4-36 | 10% |
| <i>Milestone # 2 Define the long-term benefit of local gene delivery of IL-1Ra**** in the treatment of osteoarthritis in a large mammalian joint</i> | 36 | N/A |

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: We have accomplished approximately 50% of this goal. 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 a small amount of virus to inject into animals and are currently producing the remainder of the DNA and virus needed for the study.

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: The horses for this study are staggered and, therefore, this goal is spread out over 19 months. We currently have generated the osteochondral defect in 13/24 horses and are ahead of schedule.

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: We currently have 13 horses who have received the arthroscopic OCF surgery. Of these we have completed baseline diagnostics for 7 animals and are currently collecting information on the remaining 6.

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: We have currently injected 7 animals with either saline or the viral vector and are right on schedule for this goal.

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: As per the protocol, only the current 7 animals that have been injected with either saline or scAAV.eqIL-1Ra are in this stage of monitoring. No animal has completed this portion of monitoring; however, the first animal is scheduled to complete this monitoring in 5 months.

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 have begun analyzing pre-treatment fluids as well as early post treatment fluids for this portion of the study and will continue this activity until the study is complete.

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: Not yet begun. While this activity is part of specific Aim 1, it is scheduled to begin in year 2.

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: Not yet begun. While this activity is part of specific Aim 1, it is scheduled to begin in year 2.

i) Major activity 9: Analyze diagnostic readouts (quantify pathologies in magnetic resonance images, radiographs, arthroscopic images; compare lameness assessments, kinematics).

Objective: Analyze all diagnostic measurements one year after receiving treatment, to determine efficacy.

Results: We are in the process of collecting all readouts for analysis. Final analysis will begin at the end of the one year period for each animal.

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: We are in the process of collecting all diagnostic measurements and readouts for analysis. Final analysis will begin when each animal has completed the treatment period.

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

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

During this reporting period we 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, securing appropriate animal use approvals and recruiting and conditioning healthy Thoroughbred horses for the study. In order to accommodate 24 large animals, the acquisition of animals is spread out over two years. Therefore, during the next reporting period, we plan to finish a number of the goals outlined in the first aim of the study. These goals include the complete production of scAAV.eqIL-1Ra virus needed for the study as well as the recruitment of all of the animals needed for the study. We also plan to have performed the arthroscopy to induce the OCF osteoarthritis model in the midcarpal joint of each of the 24 horses, collected baseline osteoarthritis diagnostics and injected the OCF joints with either scAAV.eqIL-1Ra or saline control. In addition, as horses reach the end of their study period we plan on performing initial efficacy analysis on both fluids and all diagnostic readouts. Part of the next reporting period will also transition the study into the second aim as animals are euthanized and pathology and biodistribution studies are performed.

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?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

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

Nothing to Report

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

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 (Name change from Rachael Watson) |
| Project Role: | Scientific Research Manager (Position change from Post-doctoral Associate) |
| 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