

**AWARD NUMBER:** W81XWH-19-1-0515

**TITLE:** Development of a Novel AAV Vector Capsid Optimized for OA Gene Therapy

**PRINCIPAL INVESTIGATOR:** Steven C. Ghivizzani

**RECIPIENT:** University of Florida  
207 Grinter Hall  
Gainesville, FL 32611-0001

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<b>1. REPORT DATE</b> SEPTEMBER 2020	<b>2. REPORT TYPE</b> Annual Report	<b>3. DATES COVERED</b> 08/15/19 - 08/14/20
<b>4. TITLE AND SUBTITLE</b>  Development of a Novel AAV Vector Capsid Optimized for OA Gene Therapy		<b>5a. CONTRACT NUMBER</b> W81XWH-19-1-0515
		<b>5b. GRANT NUMBER</b> 12712000 / PR180272
		<b>5c. PROGRAM ELEMENT NUMBER</b>
<b>6. AUTHOR(S)</b>  Steven Ghivizzani  E-Mail: ghivisc@ortho.ufl.edu		<b>5d. PROJECT NUMBER</b> 0011331694-0001
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		<b>5f. WORK UNIT NUMBER</b>
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  <b>AND ADDRESS(ES)</b>  Brian Prindle University of Florida 207 Grinter Hall Gainesville, FL 32611-0001		<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>  W81XWH-19-1-0515 Year 1
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<b>13. SUPPLEMENTARY NOTES</b>		

**14. ABSTRACT**

Osteoarthritis (OA) is a chronic, degenerative, often crippling disease that primarily affects large weight-bearing joints. OA is most commonly linked to wear and tear of the joints from old age. In younger people, though, it arises from a significant joint injury, one that damages the bones and cartilage (post-traumatic osteoarthritis; PTOA). Indeed, OA one of the primary causes of disability among active duty soldiers & veterans. Despite the constant claims in the media, there is no drug or dietary supplement capable of slowing joint destruction in OA and although some drugs can ease OA joint pain, they can't stop the erosive biological processes.

We have been working to develop a gene-based therapy for OA. Using a harmless virus, we can deliver the genes for these naturally-occurring anti-arthritic proteins to the cells and tissues in diseased joints. Following a single injection, the diseased joints continually make their own medicine for well over a year.

This experimental treatment has worked exceptionally well, first in small animals and then in the joints of horses similar in size to human knees that are also highly prone to OA. Following detailed toxicology studies this treatment was approved by the FDA for safety testing in humans with knee OA. In the current project we will use a highly advanced, state-of-the-art system developed by a member of our research team, to develop a new, custom-made AAV vector, optimized specifically for use in gene therapy for human OA. This system has been used to generate vectors with dramatically enhanced potency in treatments for blindness, cancer and liver disease. Based the successes in these applications, we expect to generate a delivery system for human OA 10-100x more effective than our current version. This new vector will be invisible to childhood antibodies, easier to manufacture and most importantly will be safe for use in humans with joint disease. The reagents developed in this project will help to advance OA gene therapy from the proof-of-concept stage to a therapy that is commercially viable. One attractive for development by the pharmaceutical industry that will ultimately result in an improved treatments for service members and civilians with OA.

We are currently working on our first aim and have received approvals to begin animal work.

**15. SUBJECT TERMS: NONE LISTED**

**16. SECURITY CLASSIFICATION OF:**

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**INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.**

In the current project we will use a highly advanced, state-of-the-art system developed by a member of our research team, to develop a new, custom-made AAV vector, optimized specifically for use in gene therapy for human OA. This system has been used to generate vectors with dramatically enhanced potency in treatments for blindness, cancer and liver disease. Based the successes in these applications, we expect to generate a delivery system for human OA 10-100x more effective than our current version. This new vector will be invisible to childhood antibodies, easier to manufacture and most importantly will be safe for use in humans with joint disease. The reagents developed in this project will help to advance OA gene therapy from the proof-of-concept stage to a therapy that is commercially viable. One attractive for development by the pharmaceutical industry that will ultimately result in an improved treatments for service members and civilians with OA.

**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  
Pharmacokinetics  
Biodistribution  
Toxicology  
Directed Evolution (DE)  
AAV Capsid Variant  
AAV Combinatorial Capsid Library

## 2. ACCOMPLISHMENTS:

### What were the major goals of the project?

<b>Specific Aim 1:</b> Using directed evolution screening of highly complex AAV capsid libraries, identify novel variants with enhanced tropism for human articular tissues in OA joints.	<b>Timeline</b>	
<b>Major Task 1:</b> Submit documentation and obtain required approvals; generate reagent libraries for use in Aims 1 and 2.	Months	
Subtask 1: Submit documents for ACURO approval	1-3	Dr. Ghivizzani
Subtask 2: Submit documents for HRPO approval	1-3	Dr. Ghivizzani
<i>Milestone #1 Obtain ACURO and HRPO Approvals</i>	2-4	Dr. Ghivizzani
Subtask 3: Expand combinatorial libraries for selection protocols in explants and equine joints	1-4	Dr. Zolotukhin
Subtask 4: Generate vector barcode library with IL-1Ra/GFP reporter	1-6	Dr. Palmer
<b>Major Task 2:</b> Perform Directed Evolution screening in equine OA joints		
Subtask 1: Acquire animals; register with ACS and complete health examinations	4-14	Dr. Smith
Subtask 2: Deliver combinatorial libraries in OA forelimb joints in equine model; euthanize animals and harvest synovium and cartilage	4-14	Dr. Smith
Subtask 3: Isolate synovial fibroblasts and chondrocytes; seed into culture; amplify AAV genomes; isolate episomal DNA.	4-14	Dr. Ghivizzani
Subtask 4: PCR variant capsid coding sequences; clone sub-library into AAV genome	4-14	Dr. Zolotukhin
Subtask 5: Perform Next- Generation Sequencing of variant sub-library; analyze sequence data using bioinformatics	4-14	Dr. Zolotukhin
Subtask 6: Package variant sub-library into virions, purify and characterize. (repeat Subtasks 2-6 as necessary)	4-14	Dr. Zolotukhin
<b>Major Task 3:</b> Perform Directed Evolution screening in equine joints with naturally occurring disease		
Subtask 1: Acquire OA tissues, process, seed into culture; infect with combinatorial library	4-14	Dr. Ghivizzani
Subtask 2: Isolate synovial fibroblasts and chondrocytes; seed into culture; amplify AAV genomes; isolate episomal DNA.	4-14	Dr. Ghivizzani
Subtask 4: PCR variant capsid coding sequences; clone sub-library into	4-14	Dr. Zolotukhin

AAV genome		
Subtask 5: Perform Next- Generation Sequencing of variant sub-library; analyze sequence data using bioinformatics	4-14	Dr. Zolotukhin
Subtask 6: Package variant sub-library into virions, purify and characterize. (repeat Subtasks 2-6 as necessary)	4-14	Dr. Zolotukhin
<b>Major Task 4: Perform Directed Evolution screening in Humanized equine OA joints</b>		
Subtask 1: Modify synovial fibroblasts and chondrocytes in explant tissues to express GFP transgene, isolate cells, prepare for delivery	9-18	Dr. Ghivizzani
Subtask 2: Implant GFP+ articular cells to OA equine forelimb joint; Inject combinatorial libraries; euthanize animal and harvest synovium and cartilage	9-18	Dr. Smith
Subtask 3: Isolate synovial fibroblasts and chondrocytes; seed into culture; amplify AAV genomes; isolate episomal DNA.	9-18	Dr. Ghivizzani
Subtask 4: PCR variant capsid coding sequences; clone sub-library into AAV genome	9-18	Dr. Zolotukhin
Subtask 5: Perform Next- Generation Sequencing of variant sub-library; analyze sequence data using bioinformatics	9-18	Dr. Zolotukhin
Subtask 6: Package variant sub-library into virions, purify and characterize. (repeat Subtasks 2-6 as necessary)	9-18	Dr. Zolotukhin
<i>Milestone #2: Identify up to 40 variants as candidates for characterization as capsid vectors</i>	17-18	Dr. Zolotukhin Dr. Ghivizzani
<b>Specific Aim 2:</b> Characterize candidate AAV capsid variants for efficiency of packaging, transgene delivery & expression and evasion of neutralizing antibody in articular explants and in vivo.		
<b>Major Task 5: Functional Assessment of Candidate Capsids in Tissue Explants</b>		
Subtask 1: Clone variant capsids into AAV helper plasmid, pair with barcoded reporter vector and package into AAV vectors, characterize titer and packaging efficiency	17-20	Dr. Zolotukhin
Subtask 2: Characterize each vector preparation for efficiency of gene transfer in synovial and cartilage explants	18-21	Dr. Ghivizzani
Subtask 3: Characterize candidate vectors for immune escape	18-21	Dr. Ghivizzani
<i>Milestone #3: Identify up to 9 variants as candidates for characterization in vivo in large animal model</i>	24	Dr. Zolotukhin Dr. Ghivizzani

<b>Major Task 6: Functional Assessment of Candidate Capsids In Vivo in Large Animal Model.</b>		
Subtask 1 Generate large-scale preparations of up to 9 candidate vectors + AAV2.5 as vector control; Characterize titer, packaging efficiency	24-28	Dr. Zolotukhin Dr. Ghivizzani
Subtask 2: Acquire animals; register with ACS and complete health examinations	24-28	Dr. Smith
Subtask 3: Inject vector preparations in equine joints; collect blood & urine; aspirate synovial fluids over 10 weeks. Euthanize animals, collect heart liver lung spleen samples, harvest synovium & cartilage,	25-30	Dr. Smith
Subtask 3: Measure IL-1Ra content in biological fluids, Analyze joint tissues for GFP expression, PCR analysis of tissue samples for AAV barcoded genomes.	28-30	Dr. Ghivizzani
Milestone #3: Identify 2 Optimized Capsids for Biodistribution and Toxicology Testing	30	Dr. Ghivizzani Dr. Zolotukhin
Specific Aim 3: Establish formal toxicology and biodistribution profiles for intra-articular IL-Ra gene transfer using optimized AAV capsids.		
<b>Major Task 7: Biodistribution Studies in Large Animal Model</b>		
Subtask 1 Generate large-scale preparations of 2 optimized vectors containing cDNA for human IL-1Ra for use in safety tests	30-32	Dr. Zolotukhin Dr. Ghivizzani
Subtask 2: Acquire animals; register with ACS and complete health examinations	29-30	Dr. Smith
Subtask 3: Deliver each optimized vector containing human IL-1Ra cassette into OA forelimb joint of 3 animals. Collect blood, urine, aspirate synovial fluids. Euthanize, necropsy and procure tissues for analysis.	30-34	Dr. Smith Ms. Erger-Coleman
Subtask 4: Analyze equine tissues for AAV genomes by qPCR, measure IL-1Ra content in blood and urine, measure AAV capsid antibody	32-36	Ms. Erger-Coleman
<b>Major Task 8: Biodistribution and Toxicology Studies in Rodent Model</b>		
Subtask 1: Acquire rats, inject mono-iodoacetate (MIA) to induce OA model and inject test AAV capsid vector in stifle joint, collect blood, monitor weight.	30-31	Ms. Erger-Coleman
Subtask 2: Euthanize animals, collect blood and 26 tissues for analysis.	31-34	Ms. Erger-Coleman
Subtask 3: Perform pathology and biodistribution analyses of collected tissues: Submit blood for clinical blood count and serum chemistries and tissue samples for pathologic analyses. Isolate genomic DNA from tissue samples and perform qPCR for vector genomes; perform enzyme linked immunosorbent assay for adeno-associated virus antibody	32-36	Dr. Smith Ms. Erger-Coleman

Milestone #4 Deliver final report on the biosafety of local gene delivery via 2 synthetic AAV capsids optimized for the treatment of osteoarthritis.	36	Ms. Erger-Coleman
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**What was accomplished under these goals?**

**What was accomplished under these goals?**

- a) Major Activity 1: Submit documentation and obtain required approvals; generate reagent libraries for use in Aims 1 and 2.

Specific Objective: 1) Obtain IACUC and ACURO approvals. 2) Obtain UF IRB and HRPO approvals. 3) Expand Combinatorial libraries for selection protocols in explants and equine joints. 4) Generate vector barcode library with IL-1Ra/GFP reporter.

Results: We have successfully obtained UF IACUC and DoD ACURO approval for all animal work. Despite submission in April of all requested documents including IRB approval letter, full IRB protocol and Informed Consent forms we still have not received approval from HRPO for the use of discarded human articular tissues in explant culture.

We have generated the IL-1Ra/GFP reporter cassette and 12/40 barcoded constructs have been generated. In addition, a novel, fluorescently tagged capsid library was generated to facilitate identification of regions in the equine joint transduced by variant capsids against the large volume background tissue. In this library, the *rep* gene on the AAV genome has been replaced by the coding sequence for mCherry, such that cells *functionally* transduced by a variant capsid will express the reporter gene and fluoresce RED. Prior to use in the joints of horses, we have recently initiated pilot studies in rats to assess the utility of the reporter in the context of the joint tissues. If reporter activity is readily visible in both cartilage and synovium, it would be extremely valuable to the directed evolution screening as we could locate within the huge volume of capsular and cartilaginous tissues, the specific locations of cells infected and transduced by variant capsids. Regions of tissue containing hotspots of fluorescence can be micro-dissected, and following enzymatic digestion of the ECM, the RED cells can be isolated by FACS. This will result in a pure population of cells containing AAV genomes with coding sequences for variant capsids which can be readily determined by NextGen sequencing.

- b) Major activity 2: Perform Directed Evolution screening in equine OA joints with naturally occurring disease.

Specific Objective: 1: Acquire animals; register with ACS and complete health examinations. 2: Deliver combinatorial libraries in OA forelimb joints in equine model; euthanize animals and harvest synovium and cartilage 3: Isolate synovial fibroblasts and chondrocytes; seed into culture; amplify AAV genomes; isolate episomal DNA. 4: PCR variant capsid coding sequences; clone sub-library into AAV genome. 5: Perform Next- Generation Sequencing of variant sub-library; analyze sequence data using bioinformatics. 6: Package variant sub-library into virions, purify and characterize. (repeat Subtasks 2-6 as necessary)

Results: Due to concerns over the COVID-19 pandemic, research work at UF involving animals was halted in early April, and all animals except for essential breeding lines were euthanized. Following approval for re-engagement of research activities in the beginning of August, two suitable horses have been located from local donors and will be available for use in these studies.

- c) Major activity 3: Perform Directed Evolution screening in human explant tissues collected from OA joints during total knee replacement surgeries.

Specific Objective: 1: Acquire OA tissues, process, seed into culture; infect with combinatorial library. 2: Isolate synovial fibroblasts and chondrocytes; seed into culture; amplify AAV genomes; isolate episomal DNA. 3: PCR variant capsid coding sequences; clone sub-library into AAV genome. 4: Perform Next- Generation Sequencing of variant sub-library; analyze sequence data using bioinformatics. 5: Package variant sub-library into virions, purify and characterize. (Repeat Subtasks 2-5 as necessary)

Results: Due to ongoing delays with HRPO review, this work has not yet been initiated.

- d) **Major activity 4:** Perform Directed Evolution screening in Humanized equine OA joints  
Specific Objective: 1: Modify synovial fibroblasts and chondrocytes in explant tissues to express GFP transgene, isolate cells, and prepare for delivery. 2: Implant GFP+ articular cells to OA equine forelimb joint; Inject combinatorial libraries; euthanize animal and harvest synovium and cartilage. 3: Isolate synovial fibroblasts and chondrocytes; seed into culture; amplify AAV genomes; isolate episomal DNA. 4: PCR variant capsid coding sequences; clone sub-library into AAV genome. 5: Perform Next-Generation Sequencing of variant sub-library; analyze sequence data using bioinformatics. 6: Package variant sub-library into virions, purify and characterize. (Repeat Subtasks 2-6 as necessary). 7: Identify up to 40 variants as candidates for characterization as capsid vectors  
Results: Not yet started
- e) **Major activity 5:** Functional Assessment of Candidate Capsids in Tissue Explants  
Specific Objective: 1: Clone variant capsids into AAV helper plasmid, pair with barcoded reporter vector and package into AAV vectors, characterize titer and packaging efficiency. 2: Characterize each vector preparation for efficiency of gene transfer in synovial and cartilage explants. 3: Characterize candidate vectors for immune escape. 4: Identify up to 9 variants as candidates for characterization in vivo in large animal model.  
Results: Not yet started
- f) **Major activity 6:** Functional Assessment of Candidate Capsids In Vivo in Large Animal Model.  
Specific Objective: 1: Generate large-scale preparations of up to 9 candidate vectors + AAV2.5 as vector control; Characterize titer, packaging efficiency. 2: Acquire animals; register with ACS and complete health. 3: Inject vector preparations in equine joints; collect blood & urine; aspirate synovial fluids over 10 weeks. Euthanize animals, collect heart liver lung spleen samples, harvest synovium & cartilage. 4. Identify 2 Optimized Capsids for Biodistribution and Toxicology Testing.  
Results: Not yet started
- g) **Major activity 7:** Biodistribution Studies in Large Animal Model  
Specific Objective: 1. Generate large-scale preparations of 2 optimized vectors containing cDNA for human IL-1Ra for use in safety tests. 2: Acquire animals; register with ACS and complete health examinations. 3: Deliver each optimized vector containing human IL-1Ra cassette into OA forelimb joint of 3 animals. Collect blood, urine, aspirate synovial fluids. Euthanize, necropsy and procure tissues for analysis. 4: Analyze equine tissues for AAV genomes by qPCR, measure IL-1Ra content in blood and urine, measure AAV capsid antibody.  
Results: Not yet started
- h) **Major activity 8:** Biodistribution and Toxicology Studies in Rodent Model  
Specific Objective: 1: Acquire rats, inject mono-iodoacetate (MIA) to induce OA model and inject test AAV capsid vector in stifle joint, collect blood, monitor weight. 2: Euthanize animals, collect blood and 26 tissues for analysis. 3: Perform pathology and biodistribution analyses of collected tissues: Submit blood for clinical blood count and serum chemistries and tissue samples for pathologic analyses. Isolate genomic DNA from tissue samples and perform qPCR for vector genomes; perform enzyme linked immunosorbent assay for adeno-associated virus antibody. 4: Deliver final report on the biosafety of local gene delivery via 2 synthetic AAV capsids optimized for the treatment of osteoarthritis.  
Results: Not yet started

**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 completed most of our major activity 1. Much of the work during the current reporting period involved, securing appropriate animal use approvals from both the University of Florida IACUC, the DoD ACURO, as well as completing all UF animal care services requirements, training and documentation due to COVID reopening procedures. We have also secured approval from UF IRB to begin collecting human samples and are awaiting HRPO approval. We have not encountered any specific technical problems; however, we were impacted by COVID-19. Due to concerns with the COVID-19 pandemic, research involving animals was halted in April at UF. Fortunately, the university has recently approved our team for animal work, as well as human work (once DoD approves).

During the next reporting period, we plan to finish the goals outlined in the first aim of the study (Major Tasks 1-4). These goals include performing Directed Evolution (DE) screening in equine joints with our OA model and perform DE screening in equine joints with naturally occurring disease. We also plan to begin our DE screening in the humanized equine OA joints.

No problems are anticipated. 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**

We had delays with animals due to COVID-19 pandemic. However, all delays have been resolved and we are continuing with the study as planned.

**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:	0.5
Contribution to Project:	Dr. Ghivizzani is responsible for the overall planning and coordination of all aspects of the equine research study. Together with Dr. Zolotukhin, he will be responsible for the overall planning, coordination and execution of all aspects of the project. He will contribute to the AAV library screening in vivo and explant and will be primarily be responsible for the functional testing in Aim 2. He will

coordinate the equine studies with Dr. Smith and the Toxicology studies with Ms. Erger-Coleman. He will assist in the collection and analysis of tissues and fluids, contribute to evaluation of all diagnostic measures and interpretation of the data. He will assume primary responsibility for progress reports and publication.

Name: Rachael Watson Levings (Name change from Rachael Watson)  
Project Role: Senior Scientist  
Researcher Identifier: ORCID ID: 0000-0003-0913-290X  
Nearest Person Month Worked: 5  
Contribution to Project: Dr. Watson is trained in molecular and cellular biological techniques as well as gene transfer and its application to musculoskeletal diseases. She has worked extensively in the equine model system and will be the technical point person involved in candidate testing. She will coordinate day to day work between VetMed, orthopaedic surgery and Dr. Zolotukhin's group. She will assist with acquisition, culture and infections of the explant tissues, and will assist with library screening in the equine model. She will be primarily responsible for the in vitro testing and analysis of the data from the in vivo studies. She will assist with equine injections and the collection of biological fluids and tissues and will perform biological assays for IL-1Ra, and immune escape from AAV neutralizing antibody.

Name: Glyn Palmer  
Project Role: Co-Investigator  
Researcher Identifier: ORCID ID:  
Nearest Person Month Worked: 0.1  
Contribution to Project: Dr. Palmer is an Assistant Professor in the Department of Orthopaedics & Rehabilitation in the College of Medicine at the University of Florida. His areas of expertise lie in molecular & cellular biology, connective tissue biology & repair, gene transfer technologies and transcriptional reporter systems to track the cellular responses in vitro and in vivo. Dr. Palmer also has significant experience with recombinant vector systems. He will assist with the generation of the lentiviral vector, AAV barcoded reporter constructs, candidate vector packaging and testing, data analyses and manuscript preparation.

Name: E. Anthony Dacanay  
Project Role: Technician  
Researcher Identifier: ORCID ID: 0000-0002-0050-7106  
Nearest Person Month Worked: 0.7  
Contribution to Project: Mr. Dacanay is a Biological Scientist in the Orthopaedic Gene Therapy Laboratory. He will work under the direction of Dr. Ghivizzani. He is trained in molecular and cellular biology including DNA isolation and purification from tissue samples. He is also trained in the production and application of AAV vectors for use in gene therapy. Mr. Dacanay will contribute to all phases of the project. He will help prepare the viral vectors for injection into the animals, assist with the collection and analysis of blood urine and synovial fluid samples, and will assist with their distribution among the research team. He will also perform biological assays, including ELISA for IL-1Ra, and PCR for vector genome quantification. 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.

Name: Rachel Leitz  
Project Role: Clinical Research Coordinator  
Researcher Identifier: ORCID ID:  
Nearest Person Month Worked: 0.1  
Contribution to Project: Ms. Leitz is a Clinical Research Coordinator in the Department of Orthopaedics & Rehabilitation of the College of Medicine at the University of Florida. She has worked in the Adult Arthroplasty and Joint Reconstruction clinical service for the past 5 years. Ms. Leitz will serve as the liaison between the OR and members of Dr. Ghivizzani's lab with regard to tissue acquisition. Ms. Leitz will coordinate scheduling and obtain informed consent from each patient donor prior to surgery.

Name: Sergei Zolotukhin  
Project Role: Co-Principal Investigator  
Researcher Identifier: ORCID ID: 0000-0002-4877-3745  
Nearest Person Month Worked: 2.4  
Contribution to Project: Dr. Zolotukhin is a Professor in the Division of Cellular and Molecular Therapy, in the Department of Pediatrics at the University Of Florida College Of Medicine. He is an expert in the biology, design and construction of AAV vectors, as well as their application in gene-based therapies. He will serve as a coPrincipal Investigator (Co-PI) on the project. He will collaborate with Dr. Ghivizzanni on scientific design, coordination of experiments, analyzing data, and manuscript preparation. Additionally, Dr. Zolotukhin will be responsible for all AAV combinatorial library production and screening and supervising Drs. Kondratov and a post-doctoral associate who will be hired once funding is in place. Dr. Zolotukhin will devote 2.4 calendar months effort per year to the project.

Name: Oleksandr Kondratov  
Project Role: Post-doctoral Associate  
Researcher Identifier: ORCID ID: 0000-0002-6390-7158  
Nearest Person Month Worked: 6  
Contribution to Project: Dr. Kondratov is in the Division of Cellular and Molecular Therapy, Department of Pediatrics. He has worked with Dr. Zolotukhin since 2016 and is an accomplished molecular biologist. He has extensive experience working with combinatorial AAV capsid libraries, and production of rAAV vectors. His main asset to this project are his unique skills as a software engineer, bioinformatics analyst, and biostatistician. Not only will he assist in the wet lab with AAV library production and directed evolution at every step of the process, but he'll also conduct the bioinformatics analysis in real time as the capsid screening/selection progresses in cycles.

Name: Anatolii Mamchur  
Project Role: Post-doctoral Associate  
Researcher Identifier: ORCID ID: 0000-0001-5569-7579  
Nearest Person Month Worked: 6 months  
Contribution to Project: Dr. Mamchur, PhD, a recently hired Postdoctorate Associate, has a 5 years postdoctoral experience working in leading laboratories in Sweden, and Israel. His main responsibility is developing approaches of culturing mesenchymal stem cells, tissue explants, directed evolution

(DE) in chondrocytes, amplifying combinatorial capsid libraries, and isolating viral DNA after DE.

Name: Liudmyla Kondratova  
Project Role: Technician  
Researcher Identifier: ORCID ID:  
Nearest Person Month Worked: 8  
Contribution to Project: Ms. Kondratova is in the Division of Cellular and Molecular Therapy, Department of Pediatrics. Ms. Kondratova has worked with Dr. Zolotukhin since 2016 and is skilled in molecular biology has extensive experience with rAAV vectors. She will provide technical support in rAAV vector production and rAAV purification.

Name: Andrew Smith  
Project Role: Co-Investigator  
Researcher Identifier: ORCID ID: 0000-0003-2772-866X  
Nearest Person Month Worked: 5  
Contribution to Project: Dr. Smith is an Assistant Professor in the University of Florida, College of Veterinary Medicine. His research interests are in the area of equine sports medicine, with specific interest in equine OA, lameness and diagnostic imaging. He has worked with Dr. Ghivizzani on the previous will perform the injections and fluid aspirations and contribute to all phases of animal care. He will also assist with euthanization and necropsies and tissue dissection.

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.

**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. See Attached PDF.

## **9. APPENDICES: Nothing to Report**