AWARD NUMBER: W81XWH-18-1-0590

TITLE: Novel Strategies to Combat Post-Traumatic Osteoarthritis (PTOA)

PRINCIPAL INVESTIGATOR: Constance R. Chu, MD

CONTRACTING ORGANIZATION: Leland Stanford Junior University

REPORT DATE: OCTOBER 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2023	ANNUAL	1SEPT2022 - 31AUG2023

4. TITLE AND SUBTITLE
Novel Strategies to Combat Post-Traumatic Osteoarthritis (PTOA)

5a. CONTRACT NUMBER
W81XWH-18-1-0590

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Constance R. Chu

E-Mail: chucr@stanford.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Stanford University
450 Broadway
Redwood City, CA 94063

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This program project addresses the overarching clinical need for effective treatments to delay or prevent the development of post-traumatic osteoarthritis (PTOA), a leading cause of disability for military service members and Veterans. The overarching goal is to test the hypothesis that prolonged inflammatory responses to joint injury contribute to progressive cartilage degeneration and subsequent development of PTOA. Consequently, our program project evaluates several innovative strategies to modulate joint inflammation through: [1] cellular and molecular treatments acutely and early after ACL injury in patients and in animal models (Projects 1, 2 and 3), [2] rehabilitation intervention in patients early after ACL reconstruction (ACLR) and prior to OA onset (Project 4), and [3] localized gene therapy for sustained administration of anti-inflammatory therapy in an equine model of PTOA (Project 5). Project 1 will examine the mechanisms by which plasmin and fibrinolysis sustain inflammation and contribute to PTOA. Project 2 will conduct a randomized controlled clinical trial to see whether inhibition of fibrinolysis using tranexamic acid (TXA) acutely after ACL injury reduces inflammation and delays joint degeneration in humans. To address widespread interest in the use of stem cells in the treatment and prevention of OA, Project 3 will evaluate the anti-inflammatory and disease-modifying effects of induced pluripotent stem cell (iPSC)-derived "rejuvenated" human MSC from ACL injured patients. Project 4 will integrate the use of novel quantitative (qMRI) MRI UTE-T2* mapping to evaluate whether an innovative active feedback gait retraining program can reduce both inflammatory and structural markers of elevated OA risk after ACLR. Finally, Project 5 will evaluate the effects of intra-articular anti-inflammatory gene therapy to prevent PTOA. This multidisciplinary program aims to reduce the disease burden of PTOA.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	67	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

Focused Program Award Year End Summary

Substantial progress has been made on all 5 projects with the laboratory and small animal-based projects (Projects 1 and 3) and the equine clinical trial (Project 5) nearing completion. Both clinical trials (Projects 2 and 4) continue to progress with Project 2 achieving 100% of targeted enrollment. Please refer to the individual project summaries that follow for details. The research program has additionally provided employment and training opportunities for several people from under-represented groups, to successfully compete for graduate and medical school positions as noted in the Project 2 summary. Due to COVID-19 related delays to recruitment into the clinical trials, we plan to request a NCE to complete necessary follow-up studies on currently active subjects.

1. INTRODUCTION:

Military service and joint injuries greatly increase the incidence of post-traumatic osteoarthritis (PTOA), a leading cause of chronic pain, morbidity, early separation, and disability. There are currently no disease modifying drugs for OA. Consequently, development of early treatment strategies to prevent or delay the onset of PTOA are needed. The central hypothesis is that prolonged inflammatory responses to joint injury contribute to progressive cartilage degeneration and subsequent development of post-traumatic osteoarthritis (PTOA). Project 1: The Role of Plasmin in Post-Traumatic Osteoarthritis: This project will examine the hypothesis that deregulation of the fibrinolysis system drives the pathogenesis of PTOA by promoting inflammation and cartilage degradation.

2. KEYWORDS:

1. Osteoarthritis (OA)
2. Post-traumatitic OA
3. Anterior Cruciate Ligament (ACL)
4. ACL reconstruction (ACLR)
5. Inflammation
6. Plasmin
7. Fibrinolysis
8. Tranexamic Acid (TXA)
9. Mice

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1. To develop synovial fluid- and/or serum-based fibrinolysis biomarkers for the prediction of recovery outcomes following ACL injuries and the sequent development of post-traumatic OA.

Aim 2: To determine the mechanisms by which fibrinolysis contributes to recovery outcomes following ACL injury, and to development of sequent post-traumatic OA.

Aim 3: To optimize the tranexamic acid dosing regimen for the prevention of PTOA in mice by examination of (i) delayed treatment and (ii) duration of dosing.

What was accomplished under these goals?

Aim 1: To develop synovial fluid- and/or serum-based fibrinolysis biomarkers for the prediction of recovery outcomes following ACL injuries and the sequent development of post-traumatic OA.

ELISA and Luminex analyses were performed on human knee synovial fluids o measure cytokines, immune mediators, and fibrinolysis and coagulation factors. The resulting datasets were bioinformatically integrated with the

associated clinical data and analyzed to identify associations between fibrinolysis and coagulation factors with (i) clinical features, and (ii) inflammatory mediators, with the goal of identifying biomarkers that predict progression to PTOA. We extended these analyses to serum and synovial fluid samples from an additional 165 OA patients, and performed cytokine analysis on these samples along with ELISA analysis of complement, mast cell, coagulation and other mediators. We also performed bulk RNA-Seq analysis of paired synovial lining samples, and identified dysregulation of the fibrinolysis and coagulation pathways as well as evidence of mast cell and type 2 immune activation.

Aim 2: To determine the mechanisms by which fibrinolysis contributes to recovery outcomes following ACL injury, and to development of sequent post-traumatic OA.

Immunohistochemistry and qPCR analysis of joint tissue from TXA and vehicle control treated MMLT or MMLT&ACLT mice. During fibrinolysis, plasmin, the key serine protease of the fibrinolytic cascade, is generated from zymogen plasminogen either by tissue plasminogen activator (tPA) produced by endothelial cells, or by urokinase plasminogen activator (uPA) produced by myeloid cells. Our Aim 1 ELISA analyses demonstrated significant elevations in uPA, tPA and plasminogen in ACLT, DMT and OA synovial fluids. Tranexamic acid (TXA) binds plasminogen thereby reducing conversion of plasminogen to plasmin, preventing fibrin degradation.

TXA-treated MMLT and DMT mice and their joint tissues harvested for immunohistochemical and RNA analyses for uPA, tPA and immune cells, and we demonstrate that TXA-treatment reduced the activation of plasmin and thereby prevented activation of the fibrinolysis pathway.

Aim 3: To optimize the tranexamic acid dosing regimen for the prevention of PTOA in mice by examination of (i) delayed treatment and (ii) duration of dosing.

We performed surgical MMLT and initiated daily treatment with TXA 4 or 6 weeks following surgical MMLT, and continued daily treatment until 3 months post MMLT (e.g. for 8 weeks in the group in which treatment was initiated 4 weeks after MMLT; for 6 weeks in the group in which the treatment was initiated 6 wks after MMLT). Blinded scoring of safranin-O stained joint tissue sections for the severity of cartilage degeneration revealed that TXA reduced the development of OA when initiated either 4 or 6 weeks following MMLT, but exhibited more pronounced protection against cartilage degeneration and osteophyte formation as well as more robustly resulted in reductions in synovitis when initiated earlier, 4 wks following MMLT. These findings validate and replicate our prior findings. We also performed an additional mouse MMLT study that demonstrated that initiation of TXA treatment 2 weeks following MMLT provided even greater efficacy at preventing cartilage degeneration and OA development.

In summary:

- Human OA synovial fluid and joint tissue exhibits dyregulated expression of fibrinolysis pathway proteins
- In mice, genetic deficiency or pharmacological blockade of plasmin attenuates multiple mouse models of OA
- Plasmin contributes to the pathogenesis of OA through multiple mechanisms
- Treatment of mice with transexamic acid started 2 – 4 weeks following surgical-induction reduces the development of OA in both the MMLT and DMM models
- Initiation of transexamic treatment 2 weeks following MMLT joint injury resulted in even greater efficacy at preventing cartilage degeneration and OA development.

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?

In the next reporting period we will:

Aim 1: Continue to validate and extend biomarker results using human synovial fluid and serum samples provided by Projects 2 and 4. Continued integrated analysis of the proteomic findings on the additional 165 OA synovial fluid and serum samples with our bulk and single cell RNA-Seq results on matched synovial lining samples.

Aim 2: MMLT surgery and tranexamic acid treatment on mice. No further studies are planned.

Aim 3: Our results demonstrated that initiation or tranexamic acid 2 wks following surgery results in even greater efficacy at protecting against OA. No further studies are planned.

We have submitted the following manuscript describing our findings to *Journal of Clinical Investigation Insight*, and anticipate that we may need to perform additional experiments to address Reviewer comments.

Wang Q, Shao G, Zhao X, Wong HH, Mao R, Bloom MS, Love ZZ, Chu CR, Cheng Z, Robinson WH. Dysregulated fibrinolysis and plasmin activation promotes the pathogenesis of osteoarthritis. Under review at *JCI Insight.*

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

N/A

Significant changes in use or care of vertebrate animal

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.**

Zhao X, Younis S, Shi H, Hu S, Zia A, Wong HH, Elliott E, Chang T, Bloom MS, Zhang W, Liu X, Lanz TV, Sharpe O, Love Z, Wang Q, Robinson WH. RNA-seq characterization of histamine-releasing mast cells as potential therapeutic target of osteoarthritis. *Clinical Immunology,* Nov 2022

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

Nothing to report

Other publications, conference papers and presentations.

Wang Q, Shao GQ, ZhaoX, Wong H, Hu N, Mao R, Bloom MS, Love ZZ, Chu CR, Cheng Z, Robinson WH. Dysregulated fibrinolysis and plasmin activation promotes the pathogenesis of osteoarthritis. *JCI Insight, under revision.*

The Role of Plasmin and Fibrinolysis Pathways in Osteoarthritis. Poster, ACR Convergence 2023

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: *William Robinson, MD, PhD*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): *0000-0003-4385-704X*
Nearest person month worked: *2.4*
Contribution to Project: *Dr. Robinson oversaw the scientific and fiscal aspects of Project 1.*

Name: *Qian Wang, MD, PhD*
Project Role: *Research Associate*
Researcher Identifier (e.g. ORCID ID): *0000-0002-9665-3982*
Nearest person month worked: *6*
Contribution to Project: *Qian Wang performed the mouse OA TXA treatment, as well as the immunostaining and qPCR analyses of the mouse joints.*

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: *N/A*

QUAD CHARTS: N/A

9. APPENDICES: *N/A*

AWARD NUMBER: W81XWH-18-1-0590

TITLE: (Project 2) Novel Strategies to Combat Post-Traumatic Osteoarthritis: The Effects of Tranexamic Acid (TXA) on Joint Inflammation and Cartilage Health in Anterior Cruciate Ligament Injured Patients

PRINCIPAL INVESTIGATOR: Constance R. Chu, MD

CONTRACTING ORGANIZATION: Stanford University Department of Orthopaedic Surgery

REPORT DATE: September 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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

Anterior cruciate ligament tear (ACLT) is a common knee injury in young active people, and occurs 10 times more frequently in military service members. The injury also leads to PTOA in roughly half of patients about ten years after injury. Intra-articular bleeding accompanies joint trauma, and is a hallmark of ACLT. There is an increasing body of evidence showing that dysregulation of plasmin mediated fibrinolysis by joint bleeding contributes to persistent low-grade inflammation. Fibrinolysis has been associated with the inflammatory processes that have been shown to play a central role in OA pathogenesis. Tranexamic Acid (TXA), an inexpensive fibrinolysis inhibitor routinely used to reduce blood loss in orthopedic surgery may arrest PTOA. We therefore propose an early Phase II double blind randomized controlled trial (RCT) to test the hypothesis that TXA treatment acutely after joint injury will reduce synovial fluid markers of inflammation and cartilage degradation and will improve patient reported outcomes (PROs) and cartilage subsurface matrix structure assessed by quantitative magnetic resonance imaging (qMRI) ultrashort echotime enhanced T2* (UTE-T2*) mapping 6 weeks, 1 year, and 2 years after ACL reconstruction (ACLR).

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

1. Osteoarthritis (OA)
2. Post-traumatitic OA
3. Anterior Cruciate Ligament (ACL)
4. ACL reconstruction (ACLR)
5. Inflammation
6. Plasmin
7. Fibrinolysis
8. Randomized Clinical Trial (RCA)
9. Tranexamic Acid (TXA)
10. Quantitative Magnetic Resonance Imaging (qMRI)
11. Ultrashort echo-time T2* mapping (UTE-T2* mapping)
12. Patient Reported Outcomes (PROs)

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1: Study Start-up
All start-up tasks have been completed.
Standard Operations Protocol is complete with IV dosage chain of custody established by working with the Stanford Healthcare Pharmacy, Mariner Pharmacy, the OSC Chief of Anesthesiologists and OSC Nurse Manager.

Major Task 2: Subject Recruitment

Project recruitment was delayed by COVID-19 related research closures, campus and sports club closures, and a period of lower injury and surgical volumes but is now complete for the originally proposed cohort.

Due to COVID-19 related recruitment challenges, approval was obtained to recruit up to 50 patients seen after the acute injury window (within 7 days of injury) but were within 3 months of ACL injury who would complete an abbreviated version of the full protocol consisting of randomization to receive TXA or placebo at the time of surgery only, as an alternative approach.

Enrollment into the original protocol was completed in July 2023:

Stanford: 26/26. 5 subjects were enrolled at Stanford pre-COVID. 29 additional subjects have been enrolled at Stanford since clinical research resumed May 27, 2020. To date, 410 patients have been considered with 59 found to be eligible and 34 subjects enrolled. Of the 33 enrolled Stanford subjects, 26 remain active because 2 failed final screening, 1 was advised by the pharmacist to withdraw from the study, 2 could not complete surgery, 1 was dropped because they were unable to complete basic study procedures in March 2020 due to the initial COVID shut-down, and 1 tested positive for COVID leading to a surgery cancellation (decided to pursue surgery at different location).

TRIA: 24/24 subjects are active in the full protocol. 2 subjects were enrolled at TRIA pre-COVID: 25 additional subjects have been enrolled at TRIA since clinical research resumed on November 9, 2020. To date, 397 subjects have been considered with 39 found to be eligible and 27 subjects enrolled. 24 subjects are currently active in the study. 1 subject was withdrawn from the study due to prior medical history, 1 could not complete study follow-up visits, and one 1 due to pharmacy error had to be replaced.

Stanford (Additional 'Augmentation' Cohort):26 subjects have been enrolled in the augmentation cohort of which 21 subjects are active in the study. 1 subject was withdrawn since during surgery, the patient was found to have sufficiently healed the ACL to not need a reconstruction. 4 subjects were withdrawn prior to surgery and did not undergo randomization.

Major Task 3: Clinical Monitoring and Quality Control Procedures

Clinical research coordinators at Stanford and TRIA exchanged recruitment and enrollment updates weekly. TRIA withdrew 1 subject from the study due to the patient's medical history. 1 subject in the original cohort was withdrawn from the study as they decided to seek treatment outside of Stanford and would no longer be followed for this study. At planned ACL surgery, 1 subject in the short cohort was found to have sufficiently healed

the ACL to not need a reconstruction. Therefore, the patient was withdrawn from the study as she no longer met the study criteria. The patient will no longer be followed for the study timepoints. Two SAEs were reviewed on 05/03/2022 and 08/02/2022 by the monitoring entity (DSMB) and determined to not be study related. A report for each SAE has been filed in the study regulatory folder and have been reported to the IRB during continuing renewal.

Major Task 4: Subject Follow-up
Subtasks 1&2, collection of PROs and biospecimens are proceeding according to approved protocols. Subtask 3, collection of MRI scans and patient reported outcomes at 6 weeks, 1 year and 2 years after ACL injury are also proceeding according to approved protocols. The 1-year MRI for one subject could not be obtained due to COVID related restrictions preventing the subject from coming onto campus to complete the research scan, and 4 were lost to follow-up. The 2-year MRIs for one subject were obtained outside of the study window due to the subjects being unable to schedule the MRI until these times. Two subjects were unable to come in for 2-year MRIs due to lost to follow-up or have relocated.

Major Task 5: Study Governance
The Yr 2 Annual Investigator Meeting was held on Oct. 29, 2019. The Yr 3 Annual Investigator Meeting was held on Oct. 9, 2020. The PRMRP Mid-point Review Meeting was held with the DOD on Oct. 4, 2021. In addition, PI are meeting on a monthly basis and the research teams are meeting quarterly for a scientific project review. The PI reviews progress weekly with the research team, and there is a monthly review of ongoing data analysis with the Robinson group and the statistician.

Major Task 6: Analyze and Disseminate Results

Completed preliminary analysis of biospecimens. Presented and discussed the data for this project at the Quarterly Conference involving research personnel at all 4 sites (Stanford, VA Palo Alto, Colorado State University, and TRIA/U Minn.)

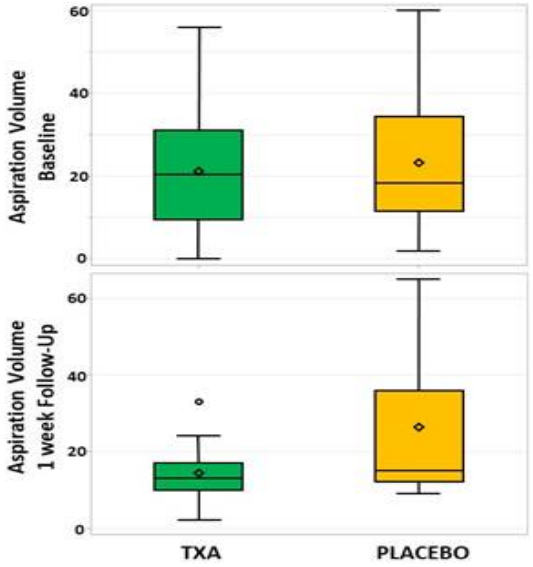
What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

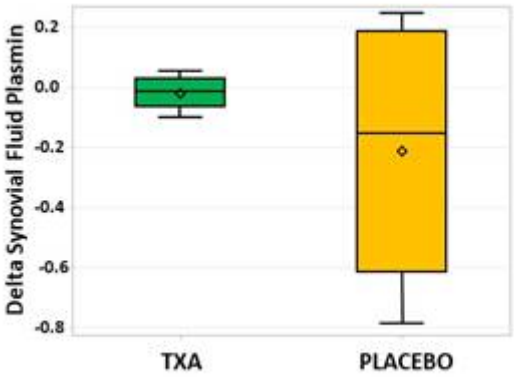
Enrollment for the original protocol is complete. At the Stanford site, one individual has not yet undergone ACLR. Direct research efforts in YR5 focused on continued recruitment in compliance with local, institutional, and national guidance and policies.

At this point all project procedures (recruitment, randomization, medication, biospecimen retrieval and banking) and outcome assessments (PRO, proteomic, qMRI, data analysis) are well established.

While preliminary analysis of biomarkers from less than 20 samples performed by the unblinded Biostatistician showed no differences in changes of RBC, changes in plasmin, changes, in TNF-alpha or COMP, between the TXA-and placebo-treated group, we plan to complete the full analysis now that sample collection is complete.

Preliminary analyses of the data from less than 20 subjects found associations between post-injury changes in serum COMP and cartilage composition assessed with qMRI at 6 weeks post-ACLR. Specifically, increases in serum COMP assessed over 1 week correlated to higher 6-week levels of MRI UTE-T2* in medial tibial cartilage (R=0.67, p=0.006) and also to higher levels of MRI qDESS T2 in patellar and trochlear cartilage (rho=0.56, 0.63; p=0.021, 0.007). Linear regression analyses found no effect of BMI, age or sex on these relationships. The effects of TXA were not assessed as the research team remains blinded to TXA treatment. Nonetheless, these associations suggest that increases in serum COMP early after ACL injury are associated with greater cartilage degeneration as early as 6 weeks post-ACL reconstruction. With the completion of recruitment, this analyses will now be completed with the full cohort.





-

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training"

activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Adam Wadsworth, MS was trained to perform MRI scans. Ashley Williams received training in Python for Healthcare to augment analysis and presentation of study data. Emmanuel Chavez, a Racial Equity to Advance a Community of Health (REACH) scholar who has been accepted to medical school, was trained to recruit ACL injured studies, and to collect and process biospecimens related to this project by Dr. Chu, Christine Hoang, and Adam Wadsworth.

To date, 4 members of under-represented groups in medicine, engineering, and orthopaedic surgery have advanced their professional goals through training and experience from working on this project. Emmanual Chavez (Hispanic male) was accepted to UCLA-Drew Medical School is the first in his family to attend medical school. He was preceded by Sachi Bansal (Southeast Asian female) who is currently a PhD student at the UCLA School of Engineering and Applied Science. She was preceded by Britney Deadwiler (African American female) who is at the USC School of Medicine and interested in Orthopaedic Surgery. She was preceded by Karlos Zepeda (Hispanic male) who is nearing completion of medical school and currently applying for Orthopaedic Surgery residencies. Karlos was the first in his family to graduate from college and attend medical school.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We plan to complete Aim 1 Biomarker studies, continue clinical and MRI follow-up, and prepare additional abstracts and manuscripts reporting on our findings.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We have demonstrated feasibility for multi-site recruitment of acute ACL injured patients into a randomized controlled clinical trial involving medication treatment as well as quantitative MRI follow-up using special research sequences. What we have learned about strengths and weaknesses of this approach will inform future clinical trials involving ACL injured subjects.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

The most significant project challenge has been recruitment which was on-track prior to COVID-19. COVID-19 clinical research restrictions, campus restrictions and the reduction in sports activities were a factor for both recruiting sites extending into 2022.

As recruitment picked up in 2022, we completed recruitment for the originally proposed cohort of 50 by July of 2023. Due to the challenge and delay in recruiting this population within 7 days of injury at both sites, we will be unable to complete 1 and 2-year follow-ups prior to Jan 14, 2024 on recent recruitments. We request NCE of remaining funds and we will need to secure additional funding to complete longitudinal follow-up on subjects recruited into this study.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

COVID related delays in recruitment means we will not be able to complete Aim 2 follow-up on all subjects. We obtained approvals to recruit additional subjects into an "augmentation cohort" consisting of patients who are able to participate in Aim 2 procedures only to enhance achievement of study aims.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No significant changes

Significant changes in use or care of vertebrate animals

N/a

Significant changes in use of biohazards and/or select agents

No changes

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

□ **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Chu CR. Articular Cartilage: Injury, Restoration, and Preservation. Operative Techniques in Orthopaedics. 2022;32(2): 100964.

Williams AA, Koltsov J, Brett A, He J, Chu CR. 3D MRI Bone Shape Indicates Pre-Osteoarthritis of the Knee 2 years after Anterior Cruciate Ligament Reconstruction. Accepted for publication in the American Journal of Sports Medicine.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

n/a

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Chu CR "Bone Shape Changes 2 Years After ACLR Suggest Differing OA Risk Patterns Between Males and Females", Invited Speaker, Magellan Annual Meeting 2022, Denver/Tabernash, CO, July 12, 2022

Chu CR "What I Say to Patients About Reducing Osteoarthritis Risk After ACL Reconstruction", Invited Speaker, 7th Annual Vail Scientific Summit 2022, Vail, CO, August 20-24 2022

Williams AA, Drain NP, Qian Y, Stevens KJ, Chu CR. MRI UTE-T2* of cartilage measured prior to anterior cruciate ligament reconstruction shows associations with patient reported sports activity 11 years later. Ortho Res Soc Trans 48: 0340; Feb 10-14, Dallas TX, (Podium)

Williams AA, Koltsov J, Brett A, He J, Chu CR. MRI detectable bone shape associates with patient symptoms in pre-osteoarthritis. Feb 10-14, Dallas TX, Ortho Res Soc Trans 48: 946; ; Feb 10-14, Dallas TX (Poster)

Williams AA, He J, Bansal S, Wadsworth ALC, Hargreaves B, Chu CR. Higher knee loading correlates to greater cartilage degeneration 2 years after anterior cruciate ligament reconstruction. Osteoarthr Cartil 31: Supplement 1, S128, 2023; March 17-20, Denver CO. (Poster #69)

Williams AA, Chu CR. Posterior medial meniscus composition associates with patient reported outcomes 2 years after anterior cruciate ligament reconstruction. Osteoarthr Cartil 31: Supplement 1, S309-S310, 2023.; March 17-20, Denver CO (Poster #207).

Williams AA, Drain NP, Qian Y, Chu CR. Patellofemoral cartilage MRI UTE-T2* changes over the first 2 years following anterior cruciate ligament reconstruction show associations with patient reported outcomes 9 years later. Osteoarthr Cartil 31: Supplement 1, S273-S274, 2023; March 17-20, Denver CO (Poster #263).

Williams AA, Mahtani G, He J, Wadsworth AL, Chu CR.Repeatability of Cones UTE-T2* Mapping of Cartilage. ISMRM & ISMRT 2023 Annual Meeting & Exhibition in Toronto, ON, Canada, 03-08 (Accepted for Poster Presentation)

Williams AA, Drain NP, Qian Y, Stevens KJ, Chu CR. Meniscus UTE-T2* Measured Prior to Anterior Cruciate Ligament Reconstruction Predicts Radiographic Osteoarthritis 11 Years Later. ISMRM & ISMRT 2023 Annual Meeting & Exhibition in Toronto, ON, Canada, 03-08. (Accepted for Poster Presentation)

Williams AA, Asay JL, Asare D, Desai AD, Mahtani G, He J, Wadsworth AL, Sansal B, Gold GE, Hargreaves B, Chaudhari A, Chu CR. Repeatability and Sensitivity of qDESS T2 Mapping of Cartilage. ISMRM & ISMRT 2023 Annual Meeting & Exhibition in Toronto, ON, Canada, 03-08. (Accepted for Power Pitch Presentation)

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

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

n/a

☐ **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

n/a

☐ **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

n/a

☐ **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- ☐ *data or databases;*
- ☐ *physical collections;*
- ☐ *audio or video products;*
- ☐ *software;*
- ☐ *models;*
- ☐ *educational aids or curricula;*
- ☐ *instruments or equipment;*
- ☐ *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- ☐ *clinical interventions;*
- ☐ *new business creation; and*
- ☐ *other.*

n/a

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Stanford Primary Site:

Name:	Constance R. Chu, MD
Project Role:	Principal Investigator (Stanford)
Nearest person month worked:	1
Contribution to project:	Dr. Chu has overseen all study activities and expended substantial time on COVID-19 related challenges.
Name:	Ashley Williams, MS
Project Role:	MRI Research Associate (Stanford)
Nearest person month worked:	2
Contribution to project:	Ashley coordinated purchase of MRI coil and installation of study MRI sequences for the TRIA site, assisted with establishment of MRI agreement between GE and TRIA site, optimized MRI DESS and Cones sequences for both study sites, assisted with testing and refinement of the MRI protocol, and prepared abstracts and manuscripts.
Name:	Christine Hoang, BS
Project Role:	Clinical Research Coordinator 2
Nearest person month worked:	3
Contribution to project:	Christine assisted with development and distribution of study recruitment materials, development of RedCap alerts, establishment of collaborations with referring physicians and clinics, IRB reporting and modifications, recruitment of study patients, completion of study activities.
Name:	Sun Hyung (Sunny) Kwon, PhD
Project Role:	Chu Lab Research Scientist (PAVIR)
Nearest person month worked:	1
Contribution to project:	Dr. Kwon assisted with specimen processing and storage.

Name:	Adam Wadsworth
Project Role:	Clinical Research Coordinator Associate
Nearest person month worked:	3
Contribution to project:	Adam assists with study recruitment, and study visit follow-up by independently acquiring MRI.
Name:	Emmanuel Chavez*
Project Role:	REACH Research Assistant
Nearest person month worked:	3
Contribution to project:	Emmanuel assists with study recruitment, and specimen processing and storage.
Name:	Henry Truong, Pharm D
Project Role:	Study Pharmacist
Nearest person month worked:	1
Contribution to project:	Dr. Truong assisted with obtaining the FDA exemption and obtained a Minnesota license to permit dispensing trial medications to the Minnesota site.

TRIA Minnesota Site:

Name:	Brad Nelson, MD
Project Role:	Study Co-PI (TRIA, Minnesota)
Nearest person month worked:	1
Contribution to project:	Dr. Nelson oversaw the Minnesota site activities.
Name:	Megan Reams, MS
Project Role:	Research Manager (TRIA/Minnesota Site)
Nearest person month worked:	2
Contribution to project:	Megan assisted with study recruitment and coordination.
Name:	Andrea Lange, MS
Project Role:	Research Coordinator (TRIA/Minnesota Site)
Nearest person month worked:	3
Contribution to project:	Andrea is the primary contact for subjects, IRB reporting. She assists with study recruitment and coordination.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or

domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

AWARD NUMBER: W81XWH-18-1-0590

TITLE: Project 3 of Novel Strategies to Combat Post-Traumatic Osteoarthritis: Cellular Rejuvenation to Combat Post-Traumatic OA

PRINCIPAL INVESTIGATOR: Nidhi Bhutani, PhD/Co-PI Constance R. Chu, MD

CONTRACTING ORGANIZATION: Stanford University Department of Orthopaedic Surgery

REPORT DATE: September 2023

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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

While intra-articular injections of mesenchymal stem cells (MSC) are used abroad to treat OA and there is substantial patient interest in these treatments, the mechanisms of action are not well understood. In addition to their ability to differentiate towards multiple cell types important to musculoskeletal tissue repair, a potential role of MSC in modulating inflammation has now been established. As such, the potential anti-inflammatory benefits of MSC for early treatment of cartilage and joint injuries to facilitate healing and to prevent or delay the onset of PTOA are of substantial interest. Barriers to clinical use of MSC include the paucity of adult stem cells for autologous therapies as well as concern over the reduced potency of adult stem cells. The discovery that somatic cells can be reprogrammed to induce a pluripotent embryonic stem cell like state i.e. iPSC cells by the introduction of four defined factors raises potential solutions to these issues. Our preliminary data shows that cellular reprogramming through iPSC can provide 'rejuvenated' and abundant MSC from adults of any age. We propose to test the central hypothesis that articular injections of iPSC-derived MSC after ACL injury will reduce inflammation and development of PTOA.

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

Knee osteoarthritis, anterior cruciate ligament injuries, mesenchymal stem cells, induced pluripotent embryonic stem cell

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

1. Major task 1: Establishing iPSC lines from donors undergoing ACL reconstruction. Human **iPSC lines established** from **blood cells** collected from **2 consented male donors, expanded** and quality control testing done.
2. Major Task 2: Growth factor-based differentiation of human iPSC to MSC.
3. Major Task 3: Assessment of MSC engraftment and persistence in rats and effects on OA progression.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1. We have procured cells from patients undergoing ACL reconstruction with their consent peripheral blood mononuclear cells (PBMC) were isolated, successfully reprogrammed using a sendai-virus protocol to generate iPSC lines that were expanded and banked. The quality control for the established iPSC lines including karyotyping, pluripotency and tri-lineage differentiation assays have been successfully completed. Growth factor-based differentiation of these iPSC to MSC was performed to generate enough cells for transplantation experiments in rats. Quality control for these cells was performed.

2. We completed in vivo studies testing the ability of MSC and iPSC differentiated into mesenchymal cells to delay OA onset after ACLT in athymic rats. Briefly, 48 6-month old athymic rats were treated with either MSC or iPSC derived MSC either 2 weeks or 1 month after ACL transection. Experimental animals were sacrificed 12 weeks after surgery and the knees were fixed. Histology and microCT analyses and OA disease scoring is ongoing for these animals.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Constance Chu, MD trained Ran Atzman, MD on the performance of the ACLT procedure. She also oversaw Jules Kouki, BS in learning mesenchymal stem cell culture and characterization.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will perform micro-CT and histology-based outcome measures for these rats and prepare abstracts and publications to complete the project.

project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The move originally scheduled for 2020 that occurred in 2022 to the new animal facility at the VA Palo Alto resulted in substantial delay to initiation of animal studies. Stanford was established as a second site and the first set of operations was performed there. The final set of animals was completed in the new VA Palo Alto animal facility.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No changes

Significant changes in use or care of vertebrate animals

No changes

Significant changes in use of biohazards and/or select agents

No changes

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

Publications, conference papers, and presentations
Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

Website(s) or other Internet site(s).

Nothing to report

Technologies or techniques

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

Inventions, patent applications, and/or licenses

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

Other Products

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	Nidhi Bhutani, PhD (Stanford)
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Bhutani supervised the protocols and maintenance/expansion of donor cells.
Name:	Constance Chu, MD (VA Palo Alto)
Project Role:	Co-Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project	Dr. Chu supervised completion of IACUC, specimen collection, culture of human and rat MSC, and performance of animal studies.
Name:	Sunny Kwon, PhD
Project Role:	Chu Lab Research Scientist (PAVIR)
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Kwon assisted with human specimen processing and culture of human and rat MSC, and performance of animal studies.
Name:	Ran Atzmon, MD
Project Role:	Chu Lab Post-doctoral Fellow (Stanford)
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Atzmon assisted with IACUC, and ACLT procedure
Name:	Jules Kouki
Project Role:	Chu Lab Research Assistant (PAVIR)
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Ms. Kouki assisted with human specimen processing and culture of human and rat MSC, and performance of animal studies.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- ☐ *Financial support;*
- ☐ *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- ☐ *Facilities (e.g., project staff use the partner's facilities for project activities);*
- ☐ *Collaboration (e.g., partner's staff work with project staff on the project);*
- ☐ *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- ☐ *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

AWARD NUMBER: W81XWH-18-1-0590

TITLE: (Project 4) Novel Strategies to Combat Post-Traumatic Osteoarthritis: Gait Retraining to Reduce Inflammation, Joint Loading and PTOA Risk

PRINCIPAL INVESTIGATOR: Constance R. Chu, MD

CONTRACTING ORGANIZATION: Stanford University Department of Orthopaedic Surgery

REPORT DATE: August 2023

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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

Following anterior cruciate ligament reconstruction (ACLR), a change in the loading environment at the knee has been suggested as a mechanism for accelerated osteoarthritis development. This study will use a prospective pre-post design to assess the effects of an active-feedback load-modifying gait retraining intervention in patients 2 years after ACLR. The study objective is to determine the magnitude and duration of changes to the knee adduction moment (KAM) following a novel active feedback gait retraining program, and to assess correlations between KAM changes and changes to the serum inflammatory response and cartilage matrix structure in ACLR patients. The gait retraining intervention is based on changing foot position through active feedback to shift pressure from the lateral to medial portion of the foot using pressure sensors in the shoe. Participants will complete 8 weekly laboratory retraining sessions and will be assessed over 6 months post-training.

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

Anterior cruciate ligament reconstruction, gait retraining, osteoarthritis, active feedback, gait analysis, knee adduction moment, magnetic resonance imaging, biomarkers

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1: Prepare for prospective “pre-post” study

Major Task 2: Participant Recruitment, Baseline Assessment, Gait Retraining Program, and Gait Analysis Follow-up Assessments

Major Task 3: Assess all participants at baseline and immediately post-training with the 'Cartilage Stress test' protocol.

Major Task 4: Assess all participants at baseline and 6 months with quantitative MRI.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Efforts in this year of the study continue to focus on subject recruitment and data collection following the remote gait retraining protocol.

Currently 47 subjects have consented to participate in the study, 22 are active (completed or pending to complete).

A total of 25 participants withdrew or discontinued, including 14 failed additional radiograph screening, 2 withdrew due to loss of contact after Covid-19, 2 withdrew due to concerns over Covid-19, 1 loss of contact (unrelated to COVID), 2 withdrew due to becoming pregnant which is an exclusion criterion, and 2 discontinued due to unable to commit further, 1 loss to follow-up, and 1 discontinued due to relocation.

Of the 22 active participants, 18 completed the study and 3 are pending to complete.

- Of the 18 completed the study, all 18 completed MRI and patient reported outcomes (PROs) data at baseline and 6-month. 15 completed gait at baseline and 6-month.
- There are missing data due to COVID19 regulations for the post-training, 3-month, and 6-month visits. Of the same 18 completed the study, 1 missed the post-training cartilage stress test, 1 missed 3- and 6-month cartilage stress test and gait, 2 missed cartilage stress test and 6-month gait, 1 missed post-training cartilage stress test and 3-month gait, and 12 did not undergo the cartilage stress test.
- Of the 3 pending to complete, all 3 are pending for 3-month follow-up visit.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The project has provided postdoc Dr. Jade He training opportunities to mentor three pre-medical students and one high school student on research projects, as well as professional development opportunities to attend conferences including the Orthopaedic Research Society Annual Meeting, World Congress on Osteoarthritis, and the Military Health System Research Symposium. The project has also provided training to support Dr. He in competing for a VA Career Development Award and to receive a VISN 21 Early Career Award to progress towards becoming an independent investigator.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of

these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We plan to continue screening and enrolling study participants to reach the target recruitment number, as well as perform follow-up visits. We will continue to send recruitment letters to potential candidates identified monthly in the clinic database, post study flyers on campuses and in nearby communities, and share our studies on social media and with online interest groups.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

A preliminary analysis on the available data suggested that changes in foot pressure data collected with wireless sensor insoles significantly correlated with changes in knee adduction moment, a mechanical risk factor of osteoarthritis, before and after gait retraining. While the wireless sensor insoles cannot measure knee joint loading directly, the insole pressure data can provide a reasonable proxy for knee joint loading changes in response to interventions like gait retraining. The preliminary finding encourages continued investigation to apply wearables to facilitate rehabilitation to improve joint symptoms and functions in individuals at risk of developing osteoarthritis. This work will be presented at the 2023 Military Health System Research Symposium.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Remote protocol with the help of wearable sensor has the potential to broaden clinical reach to soldiers in the field, VA patients from remote area, and patients with limited mobility.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

As the study recruitment is still recovering from the significantly less anterior cruciate ligament reconstruction cases during 2020 and 2021 and considering the fact the primary outcome measures (i.e., externa knee adduction moment) should not change in a relatively short period of three months, we adjusted the eligibility criteria from 2 years ± 3 months post-surgery to 2 years ± 6 months post-surgery.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Recruitment still a big focus this year. The research team have worked with local universities, and SHC marketing to advertise for the study and are continuously cycled quarterly. PI and team have also worked with the VA facilities and space committee to secure additional space to conduct research activities in case research goes back to stage 2 or 1.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

No changes

there is nothing to report under a particular item, state "Nothing to Report."

Publications, conference papers, and presentations

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

• Fischer AG, Erhart-Hledik JC, **Chu CR**, Asay JL, Andriacchi TP. Changes in stair ascent biomechanics two to eight years after ACL reconstruction are associated with patient-reported outcomes. Gait Posture. 2019 Jan 22;69:91-95.

• Fischer AG, Erhart-Hledik JC, Asay JL, **Chu CR**, Andriacchi TP. Activating the Somatosensory System Enhances Net Quadriceps Moment During Gait. J Biomech. 2019 Jan 3; 82:149-155. doi: 10.1016/j.jbiomech.2018.10.026. PMID: 30381155.

• Andriacchi TP, Griffin TM, Loeser RF, **Chu CR**, Roos EM, Hawker GA, Erhart-Hledik JC, Fischer AG. Bridging Disciplines as a Pathway to Finding New Solutions for Osteoarthritis a Collaborative Program Presented at the 2019 Orthopaedic Research Society and the Osteoarthritis Research Society International. Osteoarthritis and Cartilage Open. 2020 Mar; Volume 2, Issue 1.

• Fischer A, Erhart-Hledik J, Asay J, **Chu CR**, Andriacchi T. Utilizing the Somatosensory System via Vibratory Stimulation to Mitigate Knee Pain during Walking: Randomized Clinical Trial. Gait & Posture. 25 May 2020; 80: 37-43.

• Erhart-Hledik J, **Chu CR**, Asay J, Favre J, Andriacchi TP. Longitudinal changes in the total knee joint moment after anterior cruciate ligament reconstruction correlate with cartilage thickness changes. J Orthop Res. 2018 May; 37(7): 1546-1554. doi: 10.1002/jor.23770. PMID: 28984381

• Erhart-Hledik JC, Mahtani GB, Asay JL, Migliore E, Nguyen MM, Andriacchi TP, **Chu CR**. Changes in Knee Adduction Moment Wearing a Variable-Stiffness Shoe Correlate with Changes in Pain and Mechanically Stimulated Cartilage Oligomeric Matrix Levels. J Orthop Res. 2020 Jun 4. doi: 10.1002/jor.24770. PMID: 32497304

• Chu CR, Williams AA, Erhart-Hledik JC, Titchenal MR, Qian Y, Andriacchi TP. Visualizing pre-osteoarthritis: Integrating MRI UTE-T2* with mechanics and biology to combat osteoarthritis-The 2019 Elizabeth Winston Lanier Kappa Delta Award. J Orthop Res. 2021 Aug;39(8):1585-1595. doi: 10.1002/jor.25045. Epub 2021 Apr 29. PMID: 33788306.

• Erhart-Hledik JC, Titchenal MR, Migliore E, Asay JL, Andriacchi TP, **Chu CR**. Cartilage oligomeric matrix protein responses to a mechanical stimulus associate with ambulatory loading in individuals with anterior cruciate ligament reconstruction. J Orthop Res. 2021 Jun 29. doi: 10.1002/jor.25121. Epub ahead of print. PMID: 34185322.

• **Chu CR**. Can we afford to ignore the biology of joint healing and graft incorporation after ACL reconstruction? J Orthop Res. 2021 Jul 27. doi: 10.1002/jor.25145. Epub ahead of print. PMID: 34314066.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

• Fischer AG, Erhart-Hledik JC, Asay JL, Chu CR, and Andriacchi TP. "Activating the Somatosensory System Enhances the Quadriceps Moment and PROs During Gait", Orthopedic Research Society Annual Meeting, Feb 2-5, 2019, Austin, Texas

• Fischer AG, Erhart-Hledik J, Asay JL, Chu CR, Andriacchi TP, "Activating the Somatosensory System Enhances Knee Flexion and Quadriceps Activity During Gait and Stair Climbing",World Congress on Osteoarthritis (OARSI) 2019, May 2-5, 2019 Toronto, Canada

• Erhart-Hledik J, Williams AA, Mahtani G, Asay JL, Andriacchi TP, Chu CR, "Correlations Between Longitudinal Changes in Knee Kinetics and MRI in Patients with Knee OsteoarthritisSuggest the Benefits of Load Reduction Using Variable-Stiffness Shoes", Military Health System Research Symposium (MHSRS) Annual Meeting, August 18-22, 2019, Orlando, Florida

• Erhart-Hledik JC, Titchenal M, Migliore E, Asay JL, Andriacchi TP, Chu CR, "Serum Cartilage Oligomeric Matrix Protein Responses to a Mechanical Stimulus are Associated with Loading During Gait in Individuals with Anterior Cruciate Ligament Reconstruction", Orthopaedic Research Society Annual Meeting, Feb 8-11, 2020, Phoenix, Arizona

• Erhart-Hledik JC, Mahtani G, Migliore E, Asay JL, Andriacchi TP, Chu CR, "Longitudinal Changes in Knee Adduction Moment with a Variable-Stiffness Shoe Correlate with Changes in COMP Responses to a Mechanical Stimulus", Orthopaedic Research Society Annual Meeting, Feb 8-11, 2020, Phoenix, Arizona

• Fischer AG, Titchenal MR, Williams AA, Migliore E, Asay JL, Erhart-Hledik JC, Andriacchi TP,Chu CR*. Elevated TNF-α, Reduced Knee Loading and Increased UTE-T2* 2 Years Post ACL Reconstruction: A Signal for Knee OA in a Subset of Patients. Poster presentation at the ORSAnnual Meeting, Phoenix, AZ Feb 8-11, 2020.

• Asay JL, Erhart-Hledik JC, Mahtani G, Andriacchi TP, Chu CR, "Medial Shift of Foot Center of Pressure Correlates to Knee Adduction Moment Reduction", Orthopaedic Research Society Annual Meeting, Feb 8-11, 2020, Phoenix, Arizona

• Williams A, Erhart-Hledik JC, Mahtani G, Asay JL, Andriacchi TP, Chu CR, "Correlations Between Longitudinal Changes in Knee Kinetics and Cartilage Composition in Patients with Knee Osteoarhritis Suggest the Benefits of Load Reduction Using Variable-Stiffness Shoes", Orthopaedic Research Society Annual Meeting, Feb 8-11, 2020, Phoenix, Arizona

• Erhart-Hledik JC, Chu CR, Asay JL, Mahtani GB, Andriacchi TP, "Side-to-Side Differences in Vertical Ground Reaction Force Two Years After Anterior Cruciate Ligament Reconstruction Predict Longitudinal Changes in Patient-Reported Outcomes", World Congress on Osteoarthritis (OARSI),Accepted but not presented due to COVID-19.

• Fischer AG, Erhart-Hledik JC, Asay JL, Chu CR, Andriacchi TP, "Trunk Movement Patterns are Associated with Knee Flexion Moment Changes While Utilizing the Somatosensory System During Stair Climbing: Clinical Trial Crossover Study", World Congress on Osteoarthritis (OARSI), Accepted but not presented due to COVID-19.

(continued)

• Williams AA, Erhart-Hledik JC, Mahtani GB, Asay JL, Chu CR. Increasing Vertical Ground Reaction Force Correlates To Concurrent Meniscal And Deep Cartilage Matrix Disruption Assessed With MRI UTE-T2* Following ACL Reconstruction. Orthopaedic Research Society Annual Meeting, Feb 12-16,2021. Virtual Meeting.

• Mahtani GB, Erhart-Hledik JC, Asay JL, Andriacchi TP, Chu CR. Gait Retraining Induced Changes in Center of Pressure Associated with Reductions in Knee Adduction Moment Following ACL Reconstruction. Proceedings of the Orthopaedic Research Society 2021. Virtual Meeting. February 2021.

Mahtani GB, He, J., Chu CR. Novel 'App' and wearable insole based remote gait retraining show potential to alter gait mechanics two years after anterior cruciate ligament reconstruction. In: World Congress on Osteoarthritis; April 7-10, 2022; Berlin, Germany.

He J., Mahtani GB, Chu CR. Is remote active feedback gait retraining comparable to in-person retraining 2 years post anterior cruciate ligament reconstruction? In: World Congress on Osteoarthritis; April 7-10, 2022; Berlin, Germany.

• He J, Mahtani GB, Chu CR. Eight Weeks of Active Feedback Gait Retraining 2-Years Post Anterior Cruciate Ligament Reconstruction Orthopaedic. In: Research Society Annual Meeting; February 4-8, 2022; Tempa, FL.

• Mahtani GB, He, J., Chu CR. Novel 'App' and wearable insole based remote gait retraining show potential to alter gait mechanics two years after anterior cruciate ligament reconstruction. In: World Congress on Osteoarthritis; April 7-10, 2022; Berlin, Germany (hybrid).

• He J, Mahtani GB, Chu CR. Is remote active feedback gait retraining comparable to in-person retraining 2 years post anterior cruciate ligament reconstruction? In: World Congress on Osteoarthritis; April 7-10, 2022; Berlin, Germany (hybrid).

• He J, Chu CR. Different intersegmental coordination patterns after anterior cruciate ligament reconstruction as compared to healthy controls. In: International Society of Motor Control – Motor Control Summer School XIX; June 2-6, 2022; Pittsburgh, PA.

• Williams AA, He J, Bansal S, Wadsworth ALC, Hargreaves B, Chu CR. Higher knee loading correlates to greater cartilage degeneration 2 years after anterior cruciate ligament reconstruction. Osteoarthr Cartil 31: Supplement 1, S128, 2023. (Poster)

• Williams AA, Mahtani G, He J, Wadsworth AL, Chu CR. Repeatability of Cones UTE-T2* Mapping of Cartilage. ISMRM & ISMRT 2023 Annual Meeting & Exhibition in Toronto, ON, Canada, 03-08. (Poster)

• Williams AA, Asay JL, Asare D, Desai AD, Mahtani G, He J, Wadsworth AL, Sansal B, Gold GE, Hargreaves B, Chaudhari A, Chu CR. Repeatability and Sensitivity of qDESS T2 Mapping of Cartilage. ISMRM & ISMRT 2023 Annual Meeting & Exhibition in Toronto, ON, Canada, 03-08. (Power Pitch Presentation)

• He J, Erhart-Hledik JC, Chu CR. Medializing rearfoot pressure distribution using wireless sensor insoles improves knee joint loading in patients after anterior cruciate ligament reconstruction. 2023 Military Health System Research Symposium in Kissimmee, FL, Aug 16. (Poster)

Website(s) or other Internet site(s)

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

Technologies or techniques

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

Inventions, patent applications, and/or licenses

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

Other Products

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*

- ☐ *software;*
- ☐ *models;*
- ☐ *educational aids or curricula;*
- ☐ *instruments or equipment;*
- ☐ *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- ☐ *clinical interventions;*
- ☐ *new business creation; and*
- ☐ *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	Constance R. Chu, MD (VA Palo Alto)
Project Role:	Principal Investigator
Researcher Identifier (ORCHID ID):	_____
Nearest person month worked:	1
Contribution to project:	Dr. Chu has overseen study planning, recruitment, procedures, follow-up, and development COVID-19 alternative approaches to include the remote protocol.
Name:	Jade He, PhD (Stanford)
Project Role:	Postdoctoral Scholar
Researcher Identifier (ORCHID ID):	_____
Nearest person month worked:	4
Contribution to project:	Dr. He has worked on subject recruitment and coordination efforts, data collection, processing, and analysis, and conference abstract preparation and presentation.
Name:	Christine Hoang
Project Role:	Clinical Coordinator
Researcher Identifier (ORCHID ID):	_____
Nearest person month worked:	1
Contribution to project:	Christine Hoang has assisted with activities related to human subject recruitment, screening, and coordination of subject testing.

Name:	Adam Wadsworth
Project Role:	Clinical Coordinator Associate
Researcher Identifier (ORCHID ID):	_____
Nearest person month worked:	1
Contribution to project:	Adam Wadsworth has assisted with activities related to human subject recruitment, screening, coordination of subject testing*, and assisted with data collection*
Name:	Emiley Kim
Project Role:	Clinical Coordinator Associate
Nearest person month worked:	1
Contribution to project:	Emiley Kim has assisted with activities related to human subject recruitment, screening, coordination of subject testing*, and assisted with data collection.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

No change

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- ☐ *Financial support;*
- ☐ *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- ☐ *Facilities (e.g., project staff use the partner's facilities for project activities);*
- ☐ *Collaboration (e.g., partner's staff work with project staff on the project);*
- ☐ *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- ☐ *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

AWARD NUMBER: W81XWH-18-1-0590

TITLE: **Project 5 of Novel Strategies to Combat Post-Traumatic Osteoarthritis: Localized Gene Therapy for Prolonged Anti-Inflammatory Treatment to Prevent or Delay PTOA in an Equine Model**

PRINCIPAL INVESTIGATOR: **Laurie Goodrich, DVM-PhD**

CONTRACTING ORGANIZATION: **Colorado State University**

REPORT DATE: August 2023

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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

Post traumatic osteoarthritis (PTOA) has been shown to be the primary source of disability in warriors. To date there are no effective disease-modifying therapies and PTOA is still primarily diagnosed with radiographs, frequently after irreversible tissue damage has occurred. A potentially promising therapy involving the blockage of the IL-1β receptor with the administration of and IL-1ra gene therapeutic treatment may result in decreased joint catabolism. Short term clinical trials have indicated that the gene delivery of IL1ra to affected joints have resulted in significant improvements in both clinical and histological outcomes. The achievement of the long-term production of these gene therapies may have significant and extended symptom and disease modifying benefits. Moreover, the identification of reliable biomarkers that accurately represent the stage and progression of PTOA, as well as the extent of response to treatment, are of crucial importance.

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

Gene therapy, post-traumatic osteoarthritis (PTOA), IL-1ra, biomarkers

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

1. Develop a safe and effective scAAV-based gene therapeutic approach to treat PTOA in the equine model.
2. Validate biomarkers in a time-sensitive manner as it relates to exercise in the equine model to reflect PTOA disease status and response to therapy.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

We have successfully obtained ACURO (PR171647.e003) approval in May 2019 and CSU IACUC (18-8255A) approval in December 2018 for this Grant. Stanford University was added and approved as an additional site for animal research in Nov 2020.

We have completed our pilot study with 5 horses in which we set out to determine the appropriate dose of virus needed for the primary study. A dose of $5x10^{11}$vg dose of scAAVIL1ra was identified as the optimal dose, and this will be used for the 16-horse, 1year study.

We have officially started the 1-year study with all 16 horses. We have collected Day -30 baseline serum samples and analyzed them for relevant biomarkers. All horses underwent surgery to create an osteochondral defect in their carpal joint, 30 days after baseline blood draws. Sample collections for the day of treatment (when the horses received the viral vectors 14 days after surgery), and monthly thereafter have also been completed. Analysis of these samples is ongoing.

All treatment horses received AAVIL1ra (14 days after surgery) and the control joints received saline. We have observed detectable levels of IL1ra protein in the synovial fluid samples of the treated joints starting from day 28 (14 days after joints were injected with IL1ra), and IL1ra was not detected in any of the untreated joints. IL1ra levels appear to be decreasing with time after treatment consistent with our previous published studies; however, the average protein levels remain high at ~150 ng/ml 98 days after treatment in the treated joints (**Figure 1**).

The levels of relevant biomarkers tested in the serum were variable. Below are representative graphs for the biomarker analysis from the serum samples from baseline to 4 months post-injury. IL1ra levels in serum samples are close to the minimum detection level of 0.3 ng/ml throughout, suggesting no systemic effects of IL1ra injection into the joints. However, levels of MMP3 (a key mediator of cartilage degradation) were minimal at baseline but increased at 2 weeks and 1 month after injury, followed by a decline to pre-injury levels by 4 months consistent with the pattern of injury and recovery (**Figure 2 and 3**).

Figure 1 – IL1ra levels in synovial fluid samples

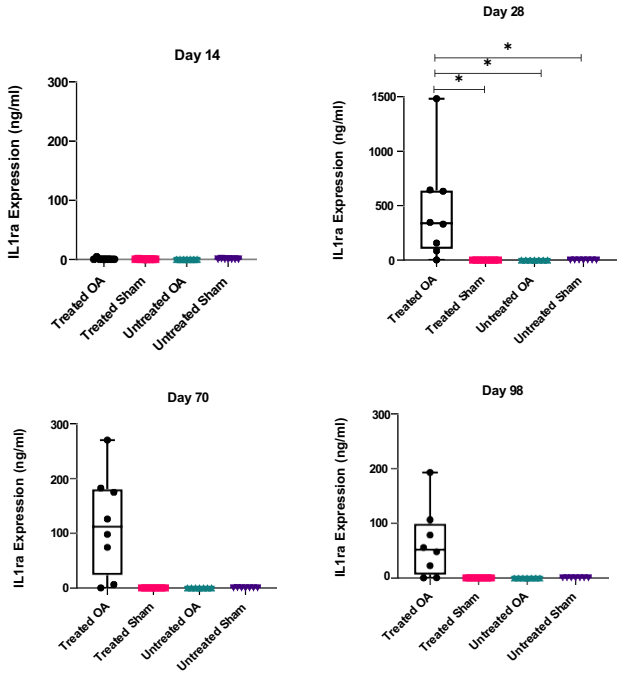


Figure 2 – IL1ra levels in serum samples

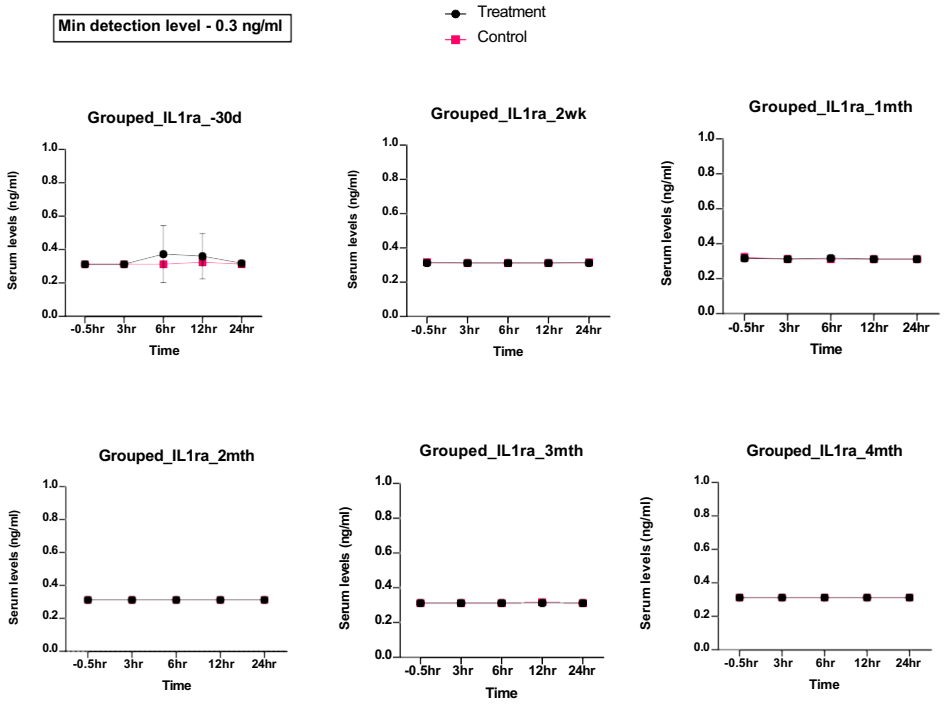
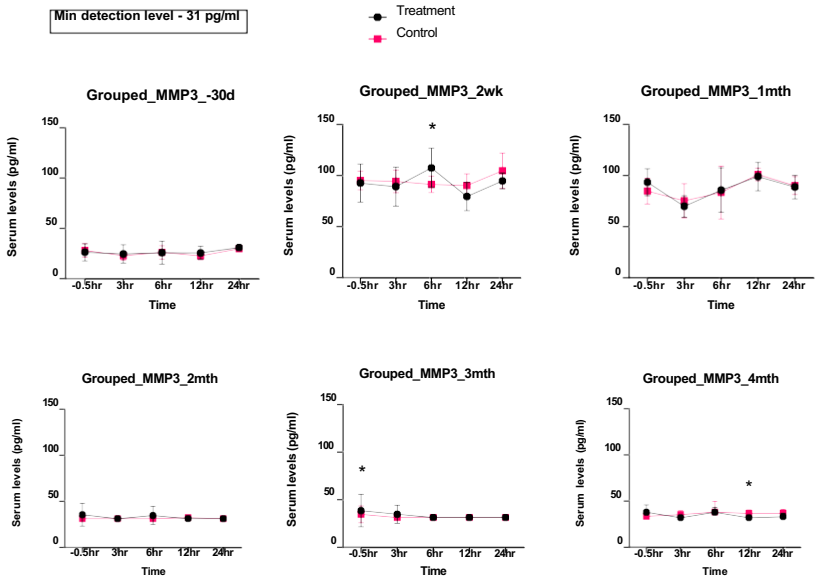
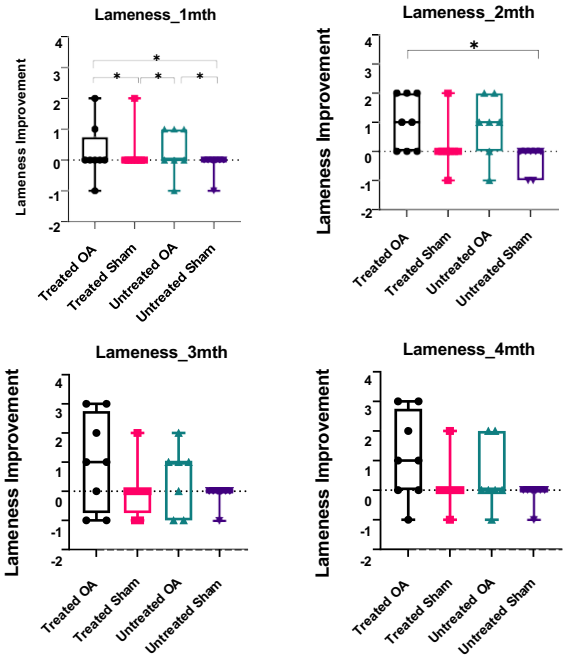


Figure 3 – MMP3 levels in serum samples



Lameness evaluations were performed throughout the study. A numerical improvement in lameness scores was seen in the treated limbs at 3 and 4 months however, these differences did not reach statistical significance (**Figure 4**).

Figure 4 – Improvement in lameness across time



We are currently in the process of analyzing all final serum samples from this study, with an endpoint scheduled at the beginning of the next quarter. All synovial fluid samples, and any remaining serum samples, will be analyzed and compiled for a final report. Final MRI imaging on the carpal joints and histology will be performed on synovial membrane, articular cartilage, and subchondral bone samples.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to report

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No changes

Significant changes in use of biohazards and/or select agents

No Changes

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

Publications, conference papers, and presentations
Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

*Goodrich L, Thampi P,Seabaugh K, Pezzanite L, **Chu CR**, Phillips J, Grieger J, McIlwraith W, Samulski R. "A pilot study to determine the optimal dose of scAAVIL-1ra in a large animal model of post-traumatic osteoarthritis" Accepted manuscript GT-2023-00064RR*

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Parvathy,Thampi; Kathryn A. Seabaugh; Constance R. Chu; Jennifer N. Phillips; Joshua C. Grieger; C. Wayne McIlwraith; R. Jude Samulski; Laurie R. Goodrich. "Optimization Of Scaavil-1ra Dose In A Large Animal Model Of Post-traumatic Osteoarthritis". ORS Conference Feb 2023. Poster

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

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

☐ **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

☐ **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

☐ **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- ☐ *data or databases;*
- ☐ *physical collections;*
- ☐ *audio or video products;*
- ☐ *software;*
- ☐ *models;*
- ☐ *educational aids or curricula;*
- ☐ *instruments or equipment;*
- ☐ *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- ☐ *clinical interventions;*
- ☐ *new business creation; and*
- ☐ *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Dr. Laurie Goodrich
Project role: PI
Research identifier: N/A
Nearest person month worked: 0.25
Contribution to project: Oversaw the submissions of the CSU/DoD/ACURO project approvals; performed surgeries for chip fragment induction in horses.

Name: Jen Daniels
Project role: Submitter of ACUC/Barn manager
Research identifier: N/A
Nearest person month worked: 3.5
Contribution to project: Prepared and submitted project approvals to CSU/DoD/ACURO

Name: Dr. Katie Seabaugh
Project role: Staff veterinarian
Research identifier: N/A
Nearest person month worked: 0.8
Contribution to project: Assist with the chip fragment induction in horses; lameness exams and sample collections.

Name: Jennifer Phillips
Project role: research associate
Research identifier: N/A
Nearest person month worked: 4.25
Contribution to project: Generation, validation and testing of experimental expression vectors planned for use in this project (ie, IL1ra). Scheduled sample analysis & preparation of samples; histology for endpoint samples; contributions to reports and final manuscripts for publication.

Name: Dr. Parvathy Thampi
Projet Role: research scientist
Research identifier: N/A
Nearest person month worked: 3.0
Contribution to project: Generation, validation and testing of experimental expression vectors planned for use in this project (ie, IL1ra). Scheduled sample analysis & preparation of samples; generation of reports and final manuscript for publication.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*