AWARD NUMBER: W81XWH-18-1-0590

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

PRINCIPAL INVESTIGATOR: Constance R. Chu, MD

CONTRACTING ORGANIZATION: Stanford University

REPORT DATE: October 2021

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
AUGUST 2021	Annual	09/01/2020-08/31/2021

4. TITLE AND SUBTITLE

Novel Strategies to Combat Post-Traumatic Osteoarthritis (PTOA)

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

5b. GRANT NUMBER
PR171647

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 USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	65	

TABLE OF CONTENTS

AWARD NUMBER: W81XWH-18-1-0590

TITLE: Project 1 of Novel Strategies to Combat Post-Traumatic Osteoarthritis: The Role of Plasmin in Post-Traumatic Osteoarthritis

PRINCIPAL INVESTIGATOR: William Robinson, MD, PhD

CONTRACTING ORGANIZATION: VA Palo Alto Health Care System/PAVIR; 3801 Miranda Ave. , Palo Alto, CA 94304

REPORT DATE: October 2021

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.*

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

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

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.

Aim 1: Major Task 1: Subtask 1: ELISA and Luminex assays on the ACLT and DMT cohorts (months 1-24)
Aim 2: Major Task 1: Subtask 1: MMLT or MMLT& ACLT surgery and tranexamic acid treatment on mice (months 1-24)
Aim 3: Major Task 1: Subtask 1: Design and perform Regimen 1 tranexamic acid treatment on mice subjected to MMLT or MMLT&ACLT (months 1-24)

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.

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.

Major Task 1: Subtask 1: ELISA and Luminex assays on the ACLT and DMT cohorts (months 1-24). ELISA and Luminex analyses were previously performed on knee synovial fluids from ACLT, DMT, as well as OA comparators to measure cytokines, immune mediators, and fibrinolysis and coagulation factors. The resulting datasets are being 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.

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

Major Task 1: Subtask 1: Immunohistochemistry and qPCR analysis of joint tissue from TXA and vehicle control treated MMLT or MMLT&ACLT mice (months 1-24). 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 (data previously shown). Tranexamic acid (TXA) binds plasminogen thereby reducing conversion of plasminogen to plasmin, preventing fibrin degradation.

Following MMLT, mice were treated with tranexamic acid (TXA) and their joint tissues harvested for immunohistochemical and RNA analyses – we continue to perform immunostaining to characterize uPA, tPA and inflammatory infiltrates, as well as qPCR to characterize expression of inflammatory and degradative mediators in samples from TXA and vehicle treated mice.

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.

Major Task 1: Subtask 1: Design and perform Regimen 1 tranexamic acid (TXA) treatment on mice subjected to MMLT (months 1-18). 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 are currently performing a additional mouse MMLT study to determine if initiation of TXA treatment 2 weeks following MMLT provides even great efficacy at preventing cartilage degeneration and OA development.

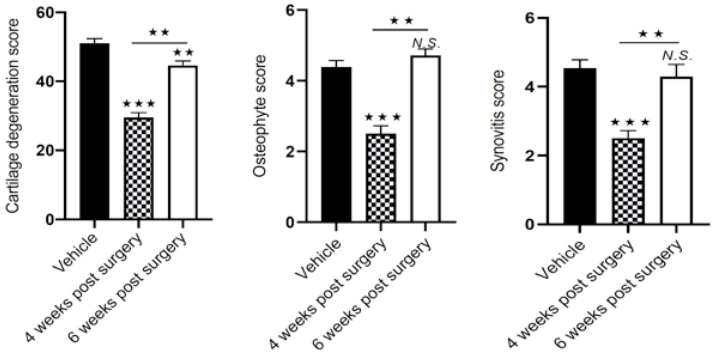


Figure. Testing of differential timing of initiation of tranexamic acid treatment of mice following MMLT. We performed surgical MMLT and initiated daily treatment with TXA either 4 or 6 weeks following surgery, and continued daily treatment until 3 months post MMLT (e.g. for 8 weeks in the group in which 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 was performed to assess the severity of cartilage degeneration, osteophyte score and synovitis. $P > 0.05$; *$P < 0.05$; **$P < 0.01$ by Mann-Whitney U test.

In summary:

- Dyregulated expression of fibrinolysis pathway proteins in OA synovial fluids
- Genetic deficiency or pharmacological blockade of plasmin attenuates OA
- Plasmin contributes to the pathogenesis of OA through multiple mechanisms
- Tranexamic acid blocks plasmin to attenuate the MMLT and DMM model of OA

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.

In the next reporting period we will:

Aim 1:

Major Task 1: Subtask 1: ELISA and Luminex assays on the ACLT and DMT cohorts (months 1-24). In the next reporting period we will perform additional Luminex to profile inflammatory mediators and ELISAs to further validate coagulation mediators and products including plasmin. The resulting datasets will be informatically integrated with the associated clinical data and analyzed to identify associations and further validate correlations between fibrinolysis and coagulation factors with (i) clinical features, and (ii) inflammatory mediators.

Major Task 1: Subtask 2: Continue to validate results using human synovial fluid and serum samples provided by Projects 2 and 4 (months 7-24).

Aim 2: Major Task 1: Subtask 1: MMLT surgery and tranexamic acid treatment on mice (months 1-24). We will continue to perform immunostaining of coagulation factors and inflammatory cell types as well as qPCR analysis of inflammatory mediator and cytokine RNA expression in mouse joint tissue samples from TXA-treated mice to characterize the molecular mechanisms by which TXA protects against the development of PTOA.

Aim 3: Major Task 1 (Regimen 1, months 1-18) and Major Task 2 (Regimen 2, months 7-30): In the next period we will continue to analyze the Regimen 2 TXA treatment experiments to confirm the time frame following injury and the treatment duration needed for successful prevention of OA following joint injury (e.g. following MMLT).

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:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

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

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:** *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)**
  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: *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?
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 2 of 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: October 2021

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 lowgrade 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 echo-time 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
The project is open at Stanford where research activities have been approved by the department and the Dean of Research as long as participants do not fall under a population considered high risk for COVID-19. The TRIA site opened up for clinical research activities on November 9, 2020.

Recruitment materials have been distributed to referring physicians and clinics. Additionally, the study is being marketed by Stanford Healthcare Marketing with materials created to send out to referring physicians and athletic trainers. A study landing page website was created and the study is promoted on google searches.

Enrollment to date: There are 22/50 subjects enrolled who are on track to complete the study.

Stanford: 14/26 subjects are active in the full protocol. 5 subjects were enrolled at Stanford pre-COVID. 15 additional subjects have been enrolled at Stanford since clinical research resumed May 27, 2020. To date, 183 patients have been considered with 31 found to be eligible and 20 subjects enrolled. Of the 20 enrolled Stanford subjects, 14 remain active because 2 failed final screening, 1 was advised by the pharmacist to withdraw from the study, 1 could not complete surgery, and 2 were dropped because they were unable to complete basic study procedures in March 2020 due to the initial COVID shut-down.

TRIA: 3 subjects were enrolled at TRIA pre-COVID: 4 additional subjects have been enrolled at TRIA since clinical research resumed on November 9, 2020. To date, 201 subjects have been considered with 18 found to be eligible and 7 subjects enrolled. All 7 TRIA subjects remain active.

Major Task 3: Clinical Monitoring and Quality Control Procedures

Clinical research coordinators at Stanford and TRIA exchange recruitment and enrollment updates weekly. The DSMB met via Zoom on August 3, 2021. No unanticipated events have occurred. Protocol deviations have been reviewed by the board. No subjects have been withdrawn due to protocol deviations. DSMB recommended the trial to continue as planned. The PI, Dr. Chu, will continue to monitor any protocol deviations. The next DSMB meeting is scheduled for December 7, 2021.

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 at Stanford could not be obtained due to COVID related restrictions preventing the subject from coming onto campus to complete the research scan. And the 6 week post-op x-rays for 3 subjects at TRIA could not be obtained due to COVID related restrictions.

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.

Major Task 6: Analyze and Disseminate Results
Began preliminary analysis.

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.

Specific Aims:

1. Does tranexamic acid administered acutely after ACL injury reduce synovial fluid markers of inflammation and cartilage degradation?
2. Does tranexamic acid administered at injury and at ACLR improve PRO and MRI at 6 weeks, 1 year, and 2 years after ACLR?

Major activities in order to complete aim 1 have been the initial RBC/proteomic data in in 16 subjects. The analysis was performed by the unblinded Biostatistician and have shown no differences in changes of RBC, changes in plasmin, changes, in TNF-alpha, COMP, between the TXA-and placebo-treated group. The plan is to re-evaluate once cohort is complete.

Preliminary biospecimen analyses found associations between pre-operative changes in serum COMP and cartilage composition assessed with qMRI at 6 weeks post-ACLR. Specifically, pre-operative increases 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.

The past year was focused on continued recruitment in compliance with local, institutional, and national COVID guidance. The PI has completed procedures needed to establish an additional Stanford clinical recruitment site at

Emeryville. Marketing efforts were extended to web searches on google ads, Stanford Healthcare websites, and the PI's laboratory website. The PI and research team have also continued to inform primary care and the athletic communities about the study as facilities begin to open. The IRB approved expanded recruitment of subjects to allow for inclusion of subjects who are able to complete the shorter term Aim 1 procedures only. The IRB and HRPO also approved remote electronic consent of subjects. At this point all project procedures (recruitment, randomization, medication, biospecimen retrieval and banking) and outcome assessments (PRO, proteomic, qMRI, data analysis) are well established.

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.

For the next reporting period, to amplify recruitment, we plan to target fall sports, emergency department, student health, urgent care, ski resorts, and club sports through social media and community outreach.

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:*

Project challenge is recruitment. COVID-19 clinical research restrictions were a factor for both recruiting sites. At Stanford, the project was allowed to continue recruitment on May 27, 2020, however from March 16, 2020 to now, aim 2 MRI scans continue to fall under some research limitation. TRIA had clinical research restrictions from March 16, 2020 to September 7, 2020.

Another challenge was the reduction in sports activities which meant fewer ACL injuries. Students were restricted to be on campus. In person learning resumed September 20, 2021 but continued with restricted access. The ski season of 2020-2021 were limited and organized club sports continue to be below pre-pandemic levels.

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.

Actions to enhance recruitment has been improved. Approvals were received to increase allowable age from 30 to 40 years, and eligibility from 4 to 7 days after injury. Additional recruitment sties were established at Stanford Emeryville and Stanford Los Gatos.

Volumes are increasing currently since school and club sports have increased, however restrictions due to COVID variants remain a threat.

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.

The COVID-19 has halted recruitment and research activities of this project.

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

Received approval to recruit ages 18-40.

Significant changes in use or care of vertebrate animals

NA

Significant changes in use of biohazards and/or select agents

NA

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*** and Williams AA. Quantitative MRI Ultrashort Echo Time T2* (UTE-T2*) and T2* Show Progressive and Continued Graft Maturation Over 2 Years in Human Subjects After Anterior Cruciate Ligament Reconstruction. Orthopedic Journal of Sports Medicine. 2019 Aug 2019; 7(8): 2325967119863056. doi: 10.1177/2325967119863056. PMID: 31448301
- **Chu CR***. Concepts Important to Secondary Prevention of Posttraumatic Osteoarthritis. J Athl Train. 2019 Sep; 54(9): 987-988. doi: 10.4085/1062-6050-54.082. PMID: 31437015
- 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. doi: 10.1016/j.ocarto.2020.100026
- Williams AA, Erhart-Hledik JC, Asay JL, Mahtani GB, Titchenal MR, Andriacchi TP, **Chu CR**. Patient Reported Outcomes and Knee Mechanics Correlate to Patellofemoral Deep Cartilage UTE-T2*2 Years After ACL Reconstruction. Am J Sports Med. 2021 Mar; 49(3): 675-683. doi: 10.1177/0363546520982608. Epub 2021 Jan 28. PMID: 33507800
- **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 Mar 31. doi: 10.1002/jor.25045. Epub ahead of print. PMID: 33788306.
- **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).*

- Williams AA, Titchenal MR, Do BH, Guha A, **Chu CR**. Novel Quantitative MRI UTE-T2* Shows Clinically Occult Subsurface Matrix Changes in Patients at High Risk for PTOA. Military Health System Research Symposium (MHSRS) Annual meeting, Aug 19-23, 2019, Orlando, Florida

- Williams AA, **Chu CR**. "MRI UTE-T2* Shows Evidence for Continued Human ACL Graft Maturation Between 1 and 2 Years After Reconstructive Surgery: A Pilot Clinical Study", Orthopedic Research Society Annual Meeting, Feb 2-5, 2019, Austin, Texas

- Williams AA, Eckstein F, Wirth W, **Chu CR***. "Cartilage Thickness Loss Correlates to UTE-T2* Early After ACL Reconstruction", World Congress on Osteoarthritis (OARSI) 2019, May 2-5, 2019 Toronto, Canada

- Williams AA and **ChuCR***, "T2* and UTE-T2* Evaluations of Anterior Cruciate Ligament Graft Maturation", International Society of Magnetic Resonance in Medicine (ISMRM) 27th Annual Meeting, May 11-16, 2019, Montreal, Canada

- **ChuCR*,** Williams AA. "Pre-Osteoarthritis is Seen in Nearly Half of Patients Just One Year After ACL", ACL Study Group Annual Meeting, January 27-30, 2020, Kitzbuhel, Austria

- Williams A, Erhart-Hledik JC, Asay JL, Mahtani G, **ChuCR**, "Tibial Rotation and Knee Flexion Moment Correlate to Patellofemoral Deep Cartilage UTE-T2*2 Years After ACL Reconstruction", Orthopaedic Research Society Annual Meeting, Feb 8-11, 2020, Phoenix, Arizona

- Williams AA, Erhart-Hledik JC, Asay JL, Mahtani G, **ChuCR**, "Tibial Rotation and Knee Flexion Moment Correlate to Patellofemoral Cartilage UTE-T2*2 Years After ACL Reconstruction", International Society for Magnetic Resonance in Medicine Annual Meeting, Aug 8-14, 2020. Virtual Meeting.

- Williams AA, Erhart-Hledik JC, Asay JL, Mahtani GB, Titchenal MR, Andriacchi TP, **ChuCR**, "Knee Mechanics and Patient Reported Outcomes Correlate to Patellofemoral Deep Cartilage UTE-T2*2 Years After ACL Reconstruction", International Workshop on Osteoarthritis Imaging Annual Meeting, Sep 9-13, 2020. Virtual Meeting.

- Williams AA, Erhart-Hledik JC, Asay JL, Mahtani GB, Titchenal MR, Andriacchi TP, **ChuCR**, "Knee Mechanics and Patient Reported Outcomes Correlate to Patellofemoral Deep Cartilage UTE-T2*2 Years After ACL Reconstruction", International Workshop on Osteoarthritis Imaging Annual Meeting, Sep 9-13, 2020. Virtual Meeting.

- Williams AA, Deadwiler BC, Dragoo JL, **ChuCR**. Does Arthroscopic Status At The Time Of ACL Reconstruction Predict Cartilage T2 Change Over The Following Year? Orthopaedic Research Society Annual Meeting, Feb 12-16, 2021. Virtual Meeting.

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.*

Conference Presentations:
*Williams AA, Deadwiler BC, Dragoo JL, Chu CR. Does Arthroscopic Status At The Time Of ACL Reconstruction Predict Cartilage T2 Change Over The Following Year? Poster #601. Virtual Annual Meeting of the Orthopaedic Research Society, Feb 12-16, 2021.

Chu CR: "Is There a. Future for Intra-Articular Injections in Joint Preservation?", Invited Speaker, Osteoarthritis Research Society Internationl (OARSI) Virtual Meetinng, April 29, 2021

Chu CR: "Quantitative MRI Needs & Opportunities: Orthopedic Surgery Perspective", Invited Speaker, ISMRM Member-Initiated Symposium, Virtual Meeting, May 19, 2021

Chu CR: "A Dialogue on the Use of Biologics to Prevent Knee OA", Invited Speaker, COA Annual Meeting, Virtual Meeting, May 21, 2021

Chu. CR: "PRP, BMAC, ACS: What is it, What do we Know, What Matter?", Invited Speaker, International Cartilage Regeneration and Joint Preservation (ICRS) Virtual Convention, June 2, 2021

Chu CR: "Treatment of Early Knee Osteoarthritis with Autologous Platelet Rich Plasma Improves Pain and Function: Is this Good Enough?", Invited Speaker, AOSSM-AANA Joint Meeting, Nashville, TN, July 8, 2021

- **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.)*

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: Yue Xu, PhD
Project Role: Chu Lab Research Scientist (Stanford)
Nearest person month worked: 1
Contribution to project: Dr. Xu assisted with specimen processing and storage.

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: 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.

Name:	Sachi Bansal
Project Role:	Clinical Research Coordinator Associate
Nearest person month worked:	3
Contribution to project:	Sachi assisted with development of study recruitment materials, collaborations with referring clinics and physicians, SOP finalization, and study recruitment.

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.

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.

Since last annual report, study Co-PI Dr. Mark Genovese, MD was no longer a part of the project or Stanford.

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.*

Stanford University School of Medicine, Department of Orthopaedic Surgery
450 Broadway
Redwood City, CA 94063
Coordinating and primary clinical performance site, grant administration

PAVIR-Palo Alto Veterans Institute for Research
3801 Miranda Ave.
Palo Alto, CA 94303
Grant subcontract

University of Minnesota, TRIA Orthopaedic Center
8100 Northland Dr., Bloomington, MN 55431
Clinical Performance Site

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: October 2021

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.*

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.

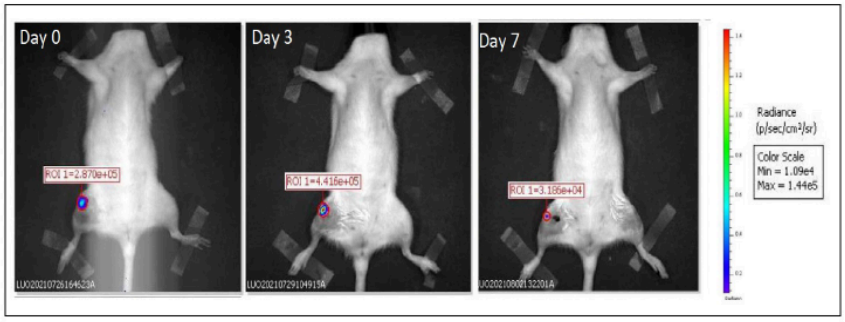
1. Major task 1: Establishing iPSC lines from donors undergoing ACL reconstruction. Human **iPSC lines established** from **blood cells** collected from **2 consented donors, expanded** and quality control in progress.
2. Major Task 2: Growth factor-based differentiation of human iPSC to MSC. Protocol optimized and tested.
3. Major Task 3: Assessment of MSC engraftment and persistence in rats. We have established a model of using rat MSCs labeled with luciferase, which were injected to rat knees in order to observe the engraftment and persistence.

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.

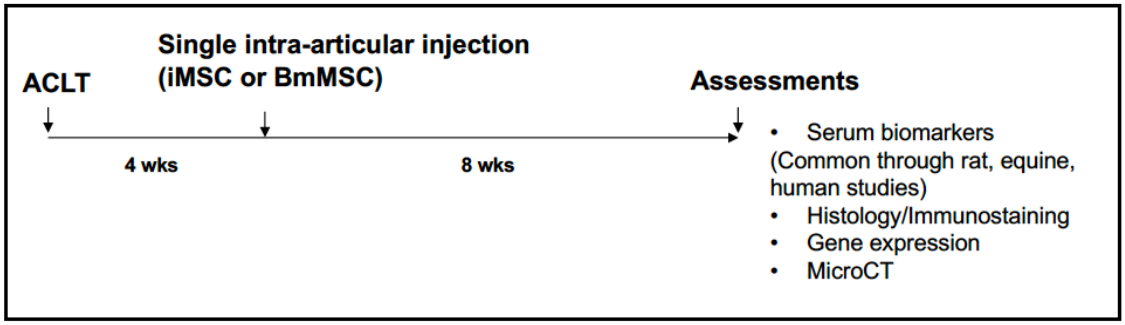
We have procured cells from patients undergoing ACL reconstruction with their consent (n=2) peripheral blood mononuclear cells (PBMC) were isolated, successfully reprogrammed using a sendai-virus protocol to generate iPSC lines that were expanded and banked. The quality control for the established iPSC lines including karyotyping, pluripotency and tri-lineage differentiation assays have been successfully completed. Growth factor-based differentiation of these iPSC to MSC can now be performed for transplantation in rats after the initial pilot experiments.

We have used lentivirus tranduced rat MSCs to express luciferase in vitro; subsequently these cells were intra-articularily injected to rats' knee and were imaged by IVIS machine. By performing this experiment, we were able to detect the transplanted MSCs by imaging and were able to follow up the MSC survival and persistence for about one week in the rats knee by IVIS imaging weekly. These preliminary experiments demonstrated alive cells in vivo survival.

Therapeutic efficacy of iMSC vs BmMSC in a rat model of PTOA



Injections of hMSC (expressing luciferase) in rat right stifle joints and bio-luminescence imaging showed persistence of the injected cells over 7 days, consistent with published literature.



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.

1. Continue *in vitro* studies under Major Task 1-Quality control on the iPSC lines generated from patients completed. We will continue the differentiation of these patient derived iPSC lines to generate mesenchymal stromal cells (MSCs) and testing the differentiation potential of these MSCs in vitro.

2. Continue animal studies to test the engraftment and persistence of MSC following injection into rat stifle joints using the existing VA imaging equipment that will be excessed once the Animal Facility move is completed.

3. Begin human cell study in athymic rats.

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).

Completion of this study will determine whether hiPSC derived MSC are effective in modulating inflammation after ACLT and hence delaying or preventing the development of post traumatic Osteoarthritis.

Accomplishment of these studies propel us closer to application of stem cell-based treatments for early treatment of cartilage and joint injuries to prevent or delay the onset of PTOA, a major unmet clinical need for the VA.

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:*

It will be challenging to standardize the human MSC viral transduction protocol, as the human primary MSCs do not grow as well. During this quarter, we have tried to perform pilot viral transduction protocols and to get better optimal protocol for our cells.

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.

Animal studies planned for the VA animal facility remain pending due to facilities move now planned for the fall of 2021. We have requested commitment of space for 4-5 months in the old facility to the initial study and our request remains pending. Due to COVID related and other delays to the move originally scheduled for the fall of 2020, then rescheduled to the spring of 2021 and now to the fall of 2021, we are working to establish Stanford as an alternative site for the animal studies. Initial review has been completed by ACURO and we are addressing the concerns.

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

NA

Significant changes in use or care of vertebrate animals

Due to COVID related and other delays to the move originally scheduled for the fall of 2020, then rescheduled to the spring of 2021 and now to the fall of 2021, we are working to establish Stanford as an alternative site for the animal studies.

Significant changes in use of biohazards and/or select agents

None

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).*

1. Bruschi M, Sahu N, Singla M, Grandi F, Agarwal P, Chu CR, Bhutani N. (2021) A Quick and Efficient Method for the Generation of Immunomodulatory Mesenchymal Stromal Cells from Human induced-Pluripotent Stem Cells (under review)

2. Sahu N, Agarwal P, Grandi F, Bruschi M, Goodman S, Amanatullah D, Bhutani N. (2021) Encapsulated Mesenchymal Stromal Cell Microbeads Promote Endogenous Regeneration of Osteoarthritic Cartilage Ex Vivo. Adv Healthc Mater. 2021 Apr 10(8)

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.*

1. 'Mesenchymal stromal cells derived paracrine factors elicit regeneration of Osteoarthritic cartilage ex vivo'. Neety Sahu, Michela Bruschi, Pranay Agarwal and Nidhi Bhutani (2020) Orthopedic Research Society 2020 Annual Meeting, Vol 45
2. 'An efficient protocol for early differentiation of mesenchymal stem cells from human induced pluripotent stem cells' Michela Bruschi, Fiorella Grandi, Pranay Agarwal and Nidhi Bhutani (2020) Orthopedic Research Society 2020 Annual Meeting, Vol 45
3.Chu CR: "*Is There a Future for Intra-Articular Injections in Joint Preservation?*", Invited Speaker, Osteoarthritis Research Society International (OARSI) Virtual Meeting, April 29, 2021.
4. Chu CR: "*A Dialogue on the Use of Biologics to Prevent Knee OA*", Invited Speaker, COA Annual Meeting, Virtual Meeting, May 21, 2021

- **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.

None

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

None

- **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.

None

- **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.*

None

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: 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: Yue Xu, PhD
Project Role: Chu Lab Research Scientist (Stanford)
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: Dr. Xu assisted with completion of the IACUC, cultured human and rat MSC, and performed 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: Neety Sahu, PhD
Project Role: Bhutani Lab Post-doctoral Fellow (Stanford)
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3
Contribution to Project: Dr. Sahu assisted with maintenance/expansion of iPSC lines, their quality control and differentiation.

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.

Erika Leonardi, MD and Michaela Bruschi, Phd left their lab's post-doctoral fellowship since last annual 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.*

None

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 of 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: October 2021

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.*

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.

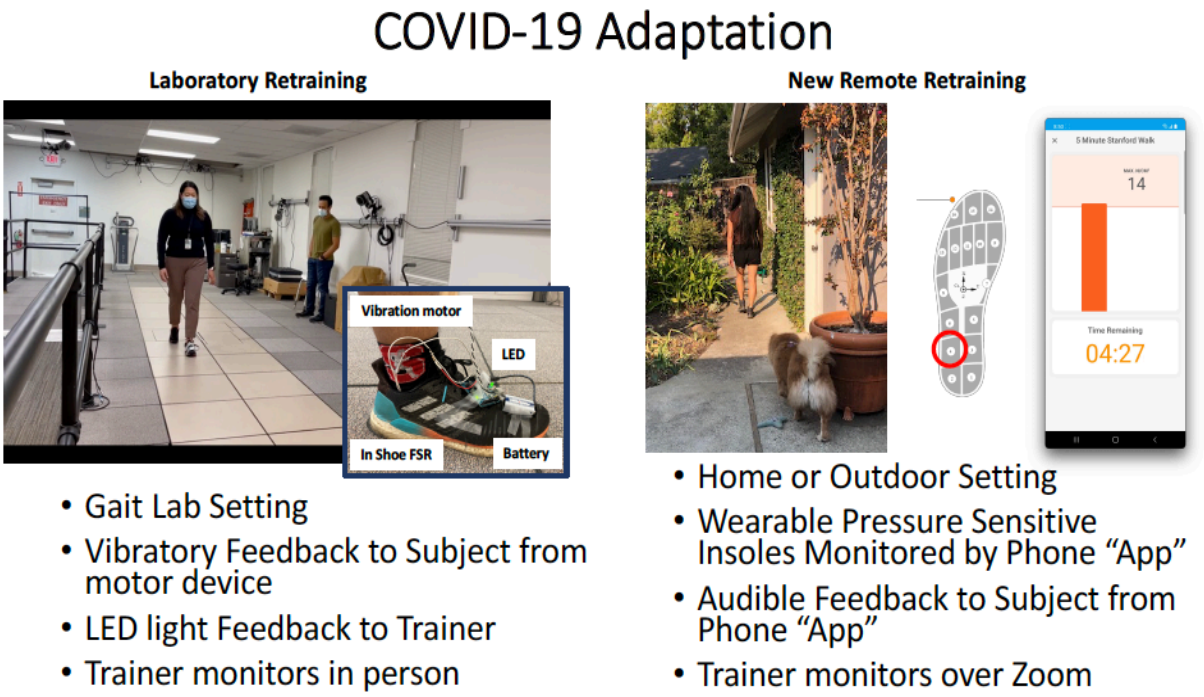
What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of 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 beginning to implement the remote gait retraining protocol.

In-Person Gait Retraining Protocol (pre-COVID)
Six subjects were enrolled prior to the March 2020 COVID-19 research shut-down, completed all in-person gait retraining sessions, and reached the final 6 month study time point. Due to Covid-19, we were unable to perform in-person gait testing at the 3-month visit for 2 subjects, as well as in-person gait testing at the 6 month visit for 3 subjects. All 6 subjects completed patient-reported outcomes at these study time points, and all 6 subjects completed 6-month MRI exams.

Remote Gait Retraining Protocol
At the end of the first quarter of year 3, we received IRB approval (as well as HRPO acknowledgement of the protocol changes, which were considered non-substantive) to begin a remote gait retraining protocol, where participants perform the 8 weeks of gait retraining at home using pressure-sensitive insoles. This remote gait retraining protocol will allow us to reduce potential Covid-19 exposure as well as continue our research efforts should any further research facility closures occur.



We successfully tested new insoles and new software customized for our use in remote gait training and began the new remote gait retraining protocol on February 9, 2021.

Recruitment Status

	Stanford	
Enrolled by September 2021	22	
Dropped	3 screen failures • 1 x-ray • 2 communication lost due to COVID closure	5 withdraws • 2 pregnancy • 2 time commitment or concerns with COVID-19 • 1 out of the window due to the COVID-19 closure
Missing study activities due to COVID-19 regulations	Gait • 2 three month post re-training gait visit • 3 six month post re-training gait visit	Cartilage Stress test • 9 baseline mPanel • 8 post re-training mPanel
Active	15/26 (6 in person, 9 remote retraining)	6 completed (all completed PRO/MRI; 3 completed gait due to COVID closure)

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.

During the next reporting period, we plan to continue screening and enrolling study participants for remote gait retraining, as well as perform follow-up visits. We will continue our recruitment efforts through both recruitment letters and study flyers.

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.*

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

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:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Implementing the remote retraining protocol has been a significant change in response to the COVID-19 clinical research restrictions at the VA Palo Alto (March 16, 2020-June 4, 2021) and Stanford Research MRI(some restrictions still remain). Regulatory approvals were completed, and customization of insoles and "App" development by insole manufacturer were done. February 9, 2021 was the initiation of the remote protocol have since enrolled 9 active subjects.

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.

Restrictions due to COVID variants remain a threat for gait, biomarker, and MRI outcome assessment but it is anticipated that enrollment will increase during the upcoming in person academic year.

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.

The development of the remote protocol and working with the insole companies took time along sign regulatory approvals.

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

NA

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).*

• 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).*

• 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

• 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 et al. "Gait Retraining Induced Changes in Center of Pressure Associated with Reductions in Knee Adduction Moment Following ACL Reconstruction", presented at the 2021 Orthopedic Research Society annual meeting.

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.*

- **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:	Jessica Asay, MS (Stanford)
Project Role:	Gait Mechanics Engineer
Researcher Identifier (ORCHID ID):	_____
Nearest person month worked:	2
Contribution to project:	Jessica Asay has worked on preparation and testing of the gait retraining device, data collection and processing, and subject recruitment efforts, and development of candidate COVID-19 alternative approaches.
Name:	Christine Hoang
Project Role:	Clinical Coordinator
Researcher Identifier (ORCHID ID):	_____
Nearest person month worked:	2
Contribution to project:	Christine Hoang 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.

Jennifer Erhart-Hledik, Phd who was the study Co-I and led preparation of study protocol planning and activates left the research group since the last annual reporting period.

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: October 2021

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.*

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.

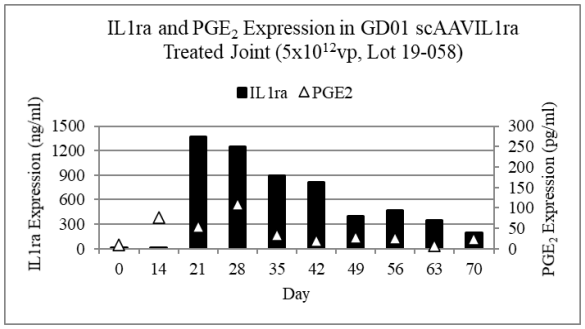
1. Develop a safe and effective scAAV-based gene therapeutic approach to treat PTOA in the equine model.
2. Validate biomarkers in a time-sensitive manner as it relates to exercise in the equine model to reflect PTOA disease status and response to therapy.

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.

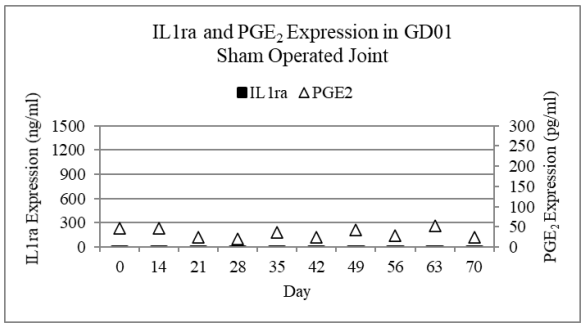
We have completed our pilot study with 5 horses in which we set out to determine the appropriate dose of virus needed for the primary study. ELISA results comparing IL1ra and PGE_2 expression indicate that there is a strong correlation between an increase in the amount of IL1ra produced within the joint and a decrease in the amount of PGE_2. Biologically significant levels of IL1ra were detected in the synovial fluid of the $5x10^{12}$vp, $5x10^{11}$vp and $5x10^{10}$vp scAAVIL1ra dosed joints, with IL1ra levels of 1200 ng/ml, 900n g/ml and 600 ng/ml peaking at D21 (1week post treatment). We saw a decrease of around 2 fold in PGE_2 concentrations between D14 and D21 in the $5x10^{12}$vp and $5x10^{11}$vp dosed horses; however these levels remained elevated in the $5x10^{10}$vp dosed horse (Figure 1).

Figure 1

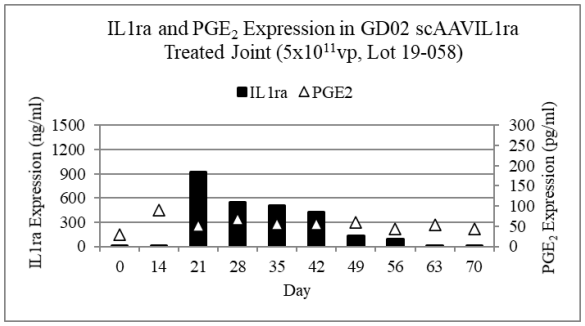
A



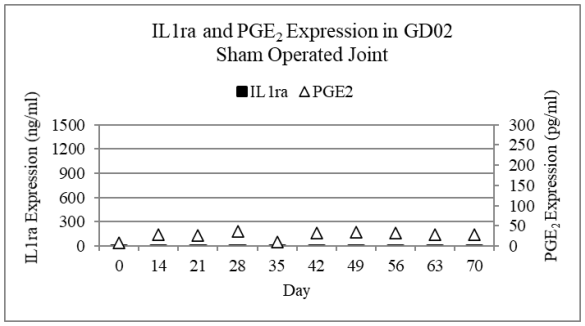
B



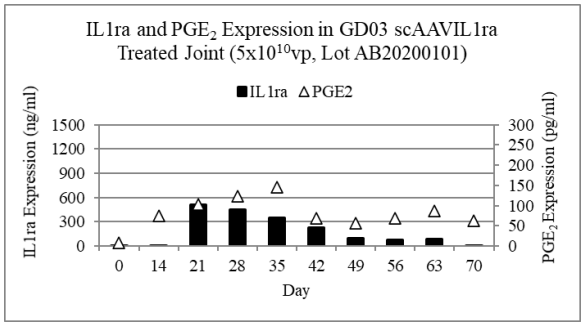
C



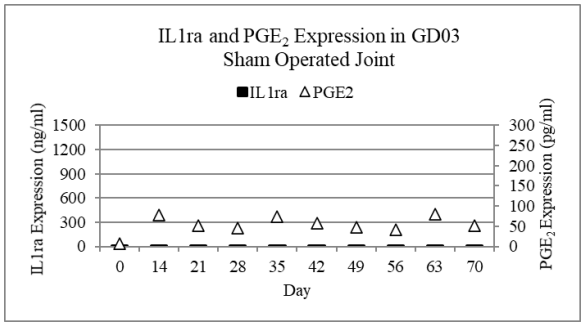
D



E



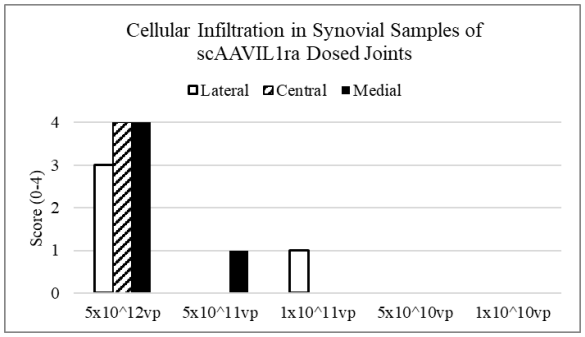
F



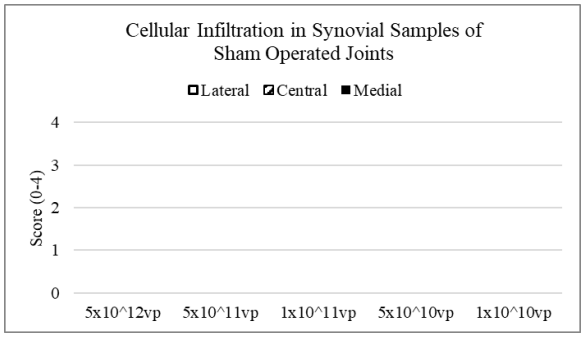
Histological analysis of lateral, central and medial synovial membrane samples indicate a significant increase for cellular infiltration (perivascular cuffing) and subintimal fibrosis (worsening) in the $5x10^{12}$vp dosed joint (Figure 2), and are supported with the images in Figure 3.

Figure 2

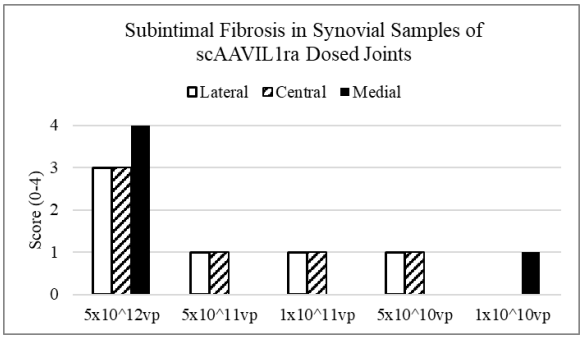
A



B



C



D

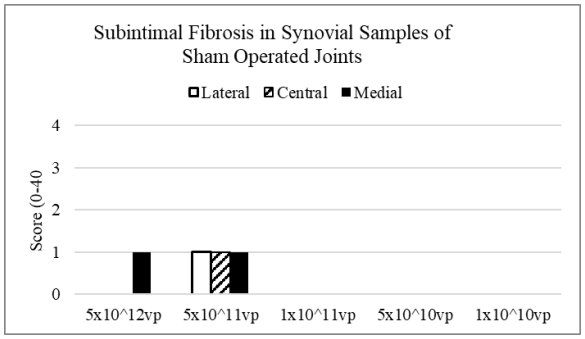
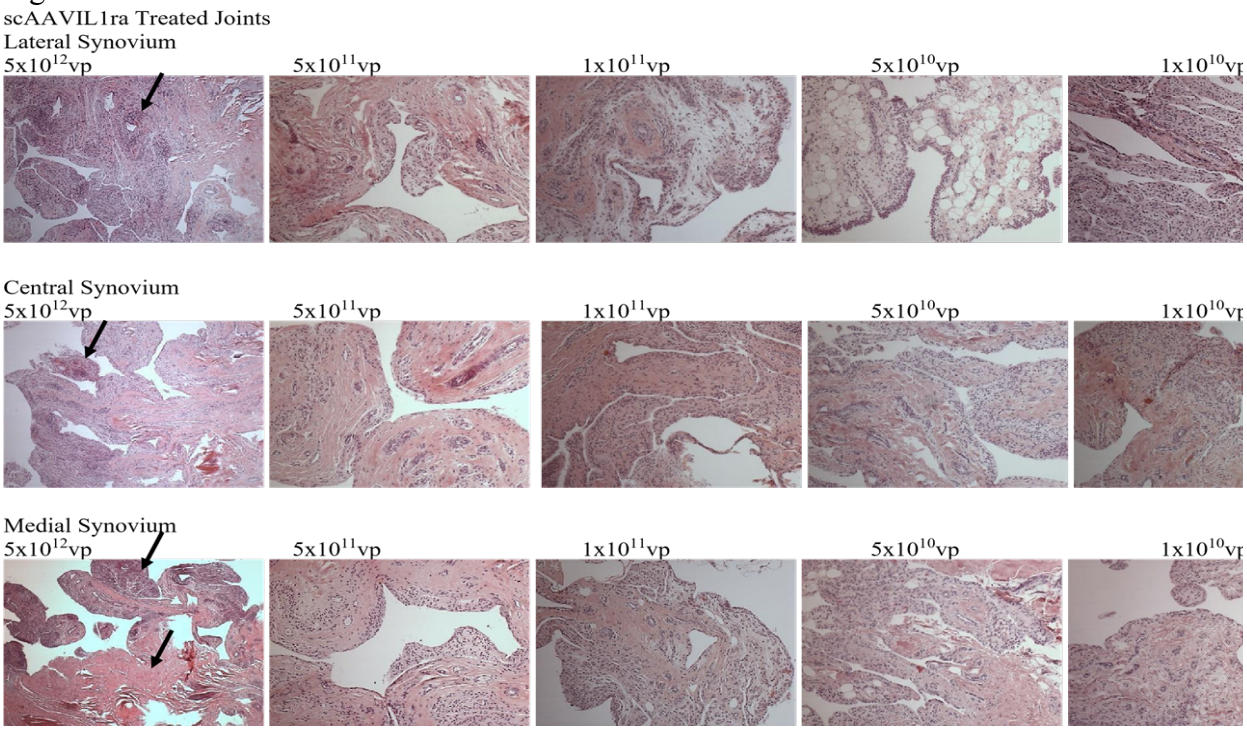


Figure 3



*Arrows indicate areas of perivascular cuffing and subintimal fibrosis in the highest dosed joint.

These results indicate that, while the $5x10^{12}$vp dose elicits a biologically significant amount of IL1ra able to reduce the level of PGE_2 in a surgically induced osteoarthritis joint, it also causes a significant amount of perivascular cuffing and subintimal fibrosis. The $5x10^{11}$vp dose elicits slightly lower IL1ra levels, but is still able to reduce the level of PGE_2 to near baseline within 2 weeks of administration. This dose does not produce elevated levels of perivascular cuffing and subintimal fibrosis. We, therefore, plan to proceed to the primary study using a $5x10^{11}$vp dose of scAAVIL1ra.

At this time we have prepared a new batch of IL1ra expression vector for viral production and have confirmed its sequence and that it will express our protein of interest. This vector will be used for the 32 horse, 1year study.

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.

We will begin synthesizing the vectors needed for the animal study, and screen animals to be enrolled in the study. Viral constructs have been generated and tested *in vitro* for confirmation of efficacy in equine joint tissues. A separately funded pilot study is currently underway to determine the effective dose required for the viral constructs in an *in vivo* equine joint.

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:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

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.

We have delayed the start of all animal studies due to the Coronavirus pandemic. A start date has not yet been established.

While we are still under restrictions, we are able to work on animal studies at a reduced percentage. To date we have been working on a separately funded pilot study to determine the appropriate dosage of viral constructs for the main animal study.

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.

We are unsure of an official start date for the study until restrictions due to the COVID pandemic have been lifted.

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

NA

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

NA

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)**
  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: 0.2
Contribution to project: Over saw the submissions of the CSU/DoD/ACURO project approvals; perform 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: 1.25
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: 0.75
Contribution to project: assist with the chip fragment induction in horses; lamness exams and sample collections.

Name: Jennifer Phillips
Project role: research associate
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 and IGF). Weekly sample analysis & preparation of samples; collection and analysis of weekly lameness exams and final testing parameters; histology for endpoint samples.

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.*