

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 2022

TYPE OF REPORT: Annual

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

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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.					
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Summary of Progress

From 1 September 2021 to 30 August 2022, progress accelerated as the nation emerged from the COVID-19 pandemic. Recruitment into the human clinical trials of Projects 2 and 4 have increased. We have continued the in vivo mouse studies of Project 1, completed the first group of athymic rats comprising the induced pluripotent stem cell and mesenchymal stromal cell studies in Project 3, and begun the comprehensive 1-year localized gene therapy clinical trial in horses comprising Project 5. We have also increased synergy between our projects by linking Project 5 with Projects 1 and 2 through evaluating fibrinolysis in equine samples and by working to link Project 3 with Projects 1 and 2 by introducing a tranexamic acid treatment arm into the rat studies pending local IACUC and DOD ACURO approvals. We plan to maintain momentum, further accelerate progress and seek a no cost extension to support completion of the projects in the coming year. The individual project summaries follow.

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: September 16, 2022

TYPE OF REPORT: Annual

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

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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-traumatic OA
3. Anterior Cruciate Ligament (ACL)
4. ACL reconstruction (ACLR)
5. Inflammation
6. Plasmin
7. Fibrinolysis
8. Tranexamic Acid (TXA)
9. 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.** To develop synovial fluid- and/or serum-based fibrinolysis biomarkers for the prediction of recovery outcomes following ACL injuries and the sequent development of post-traumatic OA.
- Aim 2:** To determine the mechanisms by which fibrinolysis contributes to recovery outcomes following ACL injury, and to development of sequent post-traumatic OA.
- Aim 3:** To optimize the tranexamic acid dosing regimen for the prevention of PTOA in mice by examination of (i) delayed treatment and (ii) duration of dosing.

What was accomplished under these goals?

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.

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

associated clinical data and analyzed to identify associations between fibrinolysis and coagulation factors with (i) clinical features, and (ii) inflammatory mediators, with the goal of identifying biomarkers that predict progression to PTOA. We extended these analyses to serum and synovial fluid samples from an additional 165 OA patients, and have performed cytokine analysis on these samples are now performing ELISA analysis of complement, mast cell, coagulation and other mediators. We also performed bulk RNA-Seq analysis of paired synovial lining samples, and are in the process of analyzing the datasets to characterize dysregulation of the fibrinolysis and coagulation pathways. We have a paper in press describing our bulk RNA-Sequencing and inflammatory biomarker results (Zhao et al, Clinical Immunology, in press). We are now performing integrated analysis of the proteomic findings on the additional 165 OA synovial fluid and serum samples with our RNA-Seq results on matched synovial lining samples.

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

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

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

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

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

In summary:

- Human OA synovial fluid and joint tissue exhibits dysregulated expression of fibrinolysis pathway proteins
- In mice, genetic deficiency or pharmacological blockade of plasmin attenuates multiple mouse models of OA
- Plasmin contributes to the pathogenesis of OA through multiple mechanisms
- Treatment of mice with tranexamic acid started 2 – 4 weeks following surgical-induction reduces the development of OA in both the MMLT and DMM models.

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

Aim 2: MMLT surgery and tranexamic acid treatment on mice. 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 cellular and molecular mechanisms by which TXA protects against the development of PTOA.

Aim 3: Continue to develop and analyze TXA treatment regimens to optimize TXA treatment and to confirm the time frame following injury and the treatment duration needed for successful prevention of OA following joint injury. Continue to perform an 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.

4. **IMPACT:** Describe 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

No significant changes.

ACURO PR171647.e001 renewed May 22, 2021, expires 04/29/2024

- APLAC ROW1748 expires 04/29/2024

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).*

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

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

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: September 2022

TYPE OF REPORT: Annual

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

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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-traumatic OA
3. Anterior Cruciate Ligament (ACL)
4. ACL reconstruction (ACLR)
5. Inflammation
6. Plasmin
7. Fibrinolysis
8. Randomized Clinical Trial (RCT)
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.

An abbreviated (short) cohort was created for patients who sustained an ACL tear within the last 6 months who will be randomized to receive TXA or Placebo during surgery with 6 months of follow up to assist with longitudinal data collection towards Specific Aim 2. We are to recruit 50 subjects for this cohort.

Enrollment to date: There are 44/50 subjects enrolled who are on track to complete the study.

Stanford: 23/26 subjects are active in the full protocol. 5 subjects were enrolled at Stanford pre-COVID. 25 additional subjects have been enrolled at Stanford since clinical research resumed May 27, 2020. To date, 259 patients have been considered with 40 found to be eligible and 30 subjects enrolled. Of the 30 enrolled Stanford subjects, 23 remain active because 2 failed final screening, 1 was advised by the pharmacist to withdraw from the study, 2 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: 21/24 subjects are active in the full protocol. 3 subjects were enrolled at TRIA pre-COVID: 20 additional subjects have been enrolled at TRIA since clinical research resumed on November 9, 2020. To date, 287 subjects have been considered with 34 found to be eligible and 23 subjects enrolled. 21 subjects are currently active in the study. 1 subject was withdrawn from the study due to prior medical history, and 1 could not complete study follow-up visits.

Stanford (augmentation cohort). 9 subjects have been enrolled in the augmentation cohort of subjects recruited greater than 7 days from injury who will undergo TXA/placebo treatment only at the time of ACL surgery. 8 subjects are active in the study and 1 subject was withdrawn because the patient was found to have sufficiently healed their ACL and did not need the ACL reconstruction.

Major Task 3: Clinical Monitoring and Quality Control Procedures

Two SAEs were reviewed on 05/03/2022 and 08/02/2022 by the monitoring entity (DSMB) and determined to not be study related. A report for each SAE has been filed in the study regulatory folder and will be reported to the IRB during continuing renewal.

Major Task 4: Subject Follow-up

Subtasks 1&2, collection of PROs and biospecimens are proceeding according to approved protocols. Subtask 3, collection of MRI scans and patient reported outcomes at 6 weeks, 1 year and 2 years after ACL injury are also proceeding according to approved protocols. The 1-year MRI for one subject could not be obtained due to COVID related restrictions preventing the subject from coming onto campus to complete the research scan.

Major Task 5: Study Governance

The Yr 2 Annual Investigator Meeting was held on Oct. 29, 2019. The Yr 3 Annual Investigator Meeting was held on Oct. 9, 2020. The PRMRP Mid-point Review Meeting was held with the DOD on Oct. 4, 2021. In addition, the PI are meeting on a monthly basis and the research teams are meeting quarterly for a scientific project review.

Major Task 6: Analyze and Disseminate Results

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

What was accomplished under these goals?

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

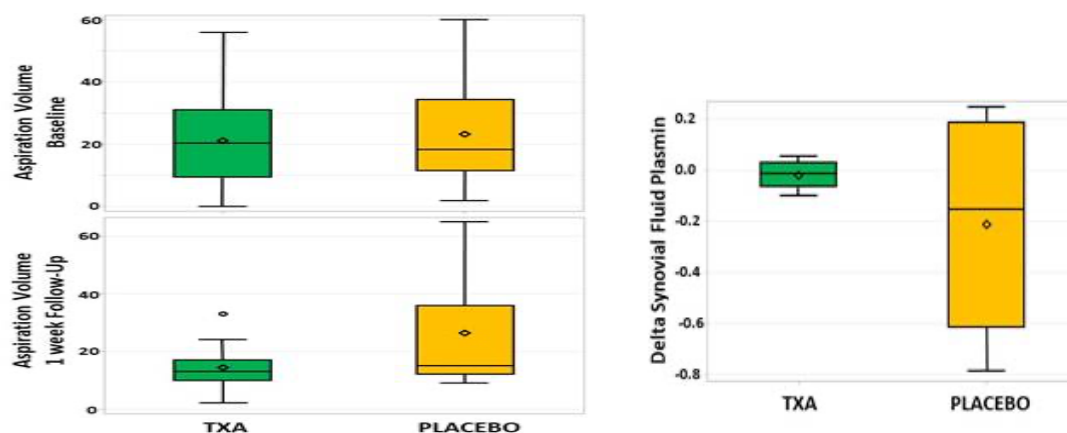
Specific Aims:

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 towards completion of aim 1 include continued recruitment efforts at all sites and completion of an initial RBC/proteomic analyses from the first 16 subjects. Preliminary analysis performed by the unblinded Biostatistician showed no differences in changes of RBC, changes in plasmin, changes, in TNF-alpha or COMP, between the TXA- and placebo-treated group. The plan is to re-evaluate once the 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 to 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 may be associated with greater cartilage degeneration as early as 6 weeks post-ACL reconstruction.

Synovial fluid aspiration volume obtained from the subjects' one week follow-up visit was smaller for those who received TXA treatment, although the aspiration volume obtained at baseline did not show a strong difference between the TXA- and placebo-treated groups. This was in accordance with significantly smaller changes in synovial fluid plasmin activity for TXA-treated subjects compared to placebo-treated subjects.



The past year was focused on continued recruitment in compliance with local, institutional, and national COVID guidance. 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. Following discussion with DOD program, the IRB approved and HRPO signed off on an expanded recruitment of subjects to allow for an 'augmentation cohort' to include subjects who are able to complete only the Aim 2 procedures. 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.

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

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, we plan to complete recruitment by targeting fall sports, emergency department, student health, urgent care, ski resorts, and club sports through social media and community outreach.
Once we complete recruitment of the last 6 subjects, we plan comprehensive analyses of the synovial fluids and blood samples to complete Aim 1.

- 4. IMPACT:** *Describe 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.

As recruitment picked up in 2022, we anticipate completing recruitment for the originally proposed cohort of 50 by April of 2023. Due to the challenge and delay in recruiting this population within 7 days of injury at both sites, we will be unable to complete 2-year follow-up on recent recruitments to this cohort. Consequently, we obtained IRB and HRPO approval to allow recruitment of up to an additional 50 subjects who will have ACL surgery within 3 months of injury (amended to 6 months from injury) to receive TXA/placebo at the time of surgery to determine whether TXA intervention at this later timepoint that more closely matches current practice patterns will improve patient reported and MRI outcomes. We plan to seek additional funding to complete longitudinal follow-up on subjects recruited into this study.

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

Actions to enhance recruitment include increasing allowable age from 30 to 40 years, and the window of eligibility was increased from 0-4 days after injury to 0-7 days after injury. Additional recruitment sites were established at Stanford Emeryville and Stanford Los Gatos.

Volumes are increasing and we anticipate completing the originally proposed recruitment of 50 subjects within 7 days of ACL injury, as well as an ‘augmentation cohort’ of up to an additional 50 subjects undergoing ACL surgery within 6 months after injury.

Changes that had a significant impact on expenditures

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

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

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

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

Significant changes in use or care of human subjects

Received approval to recruit within 6 months of injury for the ‘augmentation’ cohort for the Stanford Site. IRB 46092 approval was obtained and HRPO E00178.1a approved the changes on 8/8/2022.

No significant changes to the TRIA’s protocol. HRPO E00178.1b renewal approved 08/18/2022.

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.** Can we afford to ignore the biology of joint healing and graft incorporation after ACL reconstruction? J Orthop Res. 2022; 40(1):55-64. PMID: 34314066.
- Williams AA, Deadwiler BC, DragooJL, **Chu CR.** Cartilage Matrix Degeneration Occurs within the First Year after ACLR and is Associated with Impaired Clinical Outcome. Cartilage. 2021 Dec11: 19476035211063856. Doi:10.1177/19476035211063856. Epub ahead of print. PMID: 34894770.

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).*

None

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, Qian Y, Chu CR. Bi-exponential UTE-T2* Evaluation of Longitudinal Changed in Knee Cartilage Over 2 Years Following Anterior Cruciate Ligament Reconstruction. Annual Meeting of the International Society for Magnetic Resonance in Medicine, Podium Presentation #0347, May 6-13, 2022, London, UK

Williams AA, Chu CR. Bone & Cartilage Changes After ACL Reconstruction Resemble Early OA. R Lane Smith Visiting Professor Scientific Session, Stanford CA April 27, 2022.

Chu CR. Interlimb Bone Shape Differences 2-Years After ACLR Show Relationships Between Higher Activity and More OA-like Morphology. 2022 Herodius Society Annual Meeting, 6 May 2022, Podium Presentation.

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

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

- **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

-

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: 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:	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:	Adam Wadsworth
Project Role:	Clinical Research Coordinator Associate
Nearest person month worked:	3
Contribution to project:	Adam assists with study recruitment, and study visit follow-up by independently acquiring MRI.
Name:	Emmanuel Chavez
Project Role:	REACH Research Assistant
Nearest person month worked:	3
Contribution to project:	Emmanuel assists with study recruitment, and specimen processing and storage.
<u>TRIA Minnesota Site:</u>	
Name:	Brad Nelson, MD
Project Role:	Study Co-PI (TRIA, Minnesota)
Nearest person month worked:	1
Contribution to project:	Dr. Nelson oversaw the Minnesota site activities.
Name:	Megan Reams, MS
Project Role:	Research Manager (TRIA/Minnesota Site)
Nearest person month worked:	2
Contribution to project:	Megan assisted with study recruitment and coordination.
Name:	Andrea Lange, MS
Project Role:	Research Coordinator (TRIA/Minnesota Site)
Nearest person month worked:	3
Contribution to project:	Andrea is the primary contact for subjects, IRB reporting. She assists with study recruitment and coordination.

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

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

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.*

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 2022

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

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.

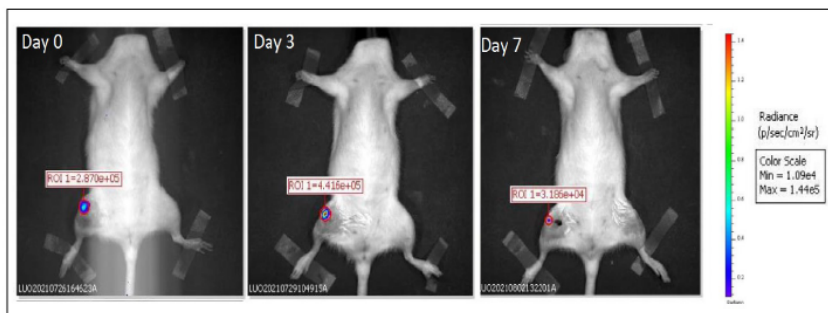
What was accomplished under these goals?

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.

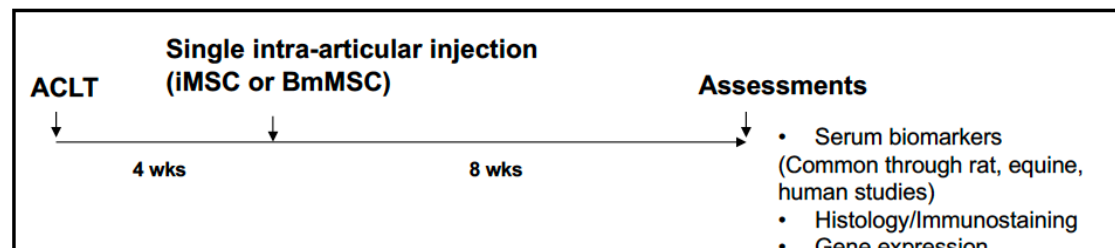
We have procured cells from patients undergoing ACL reconstruction with their consent (n=2) peripheral blood mononuclear cells (PBMC) were isolated, successfully reprogrammed using a sendai-virus protocol to generate iPSC lines that were expanded and banked. The quality control for the established iPSC lines including karyotyping, pluripotency and tri-lineage differentiation assays have been successfully completed. In the last year, we have successfully performed growth factor-based differentiation of patient derived iPSC to MSC and performed quality control experimentst including FACS analyses and trilineage differentiation assays. Transplantation experiments to test their efficacy in OA have been initiated in rats and will be continued in the coming months and analyzed.

We have used lentivirus tranduced rat MSCs to express luciferase in vitro; subsequently these cells were intra-articularly 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."

Constance Chu, MD trained Ken Lin, MD and Ran Atzman, MD on the performance of the ACLT procedure. She also oversaw Jules Kouki, BS in learning mesenchymal stem cell culture and characterization. Yue Xu, MD-PhD received training from the imaging core on performance of rat stifle CT scans.

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 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.
2. Continue comparison studies through intra-articular injections of control, MSC and iPSC-derived MSC in athymic OA rats to test their relative efficacy in modulation of OA.

- 4. IMPACT:** *Describe 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 has been challenging to obtain sufficient numbers high quality iPSC-derived MSC for the iPSC derived from patients. We have however now optimized the FACS sorting protocols to achieve a high viability of these cells such they can be quickly expanded to obtain the necessary quantities of 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

Animal studies originally planned for the VA animal facility were delayed due to a facilities move planned for 2020 that was completed in 2022. We established Stanford as an alternative site for the animal studies and completed an initial set of 20 rats (10 acute injection protocol and 10 delayed injection protocol) at Stanford under IACUC 33924 with ACURO PR171647.e004 approval October 5, 2021. The VA animal facility was certified for rat surgery in August 2022 and we plan to accelerate progress to complete the study by using both the Stanford and the VA facilities in the upcoming final 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.

Nothing to report

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

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

Significant changes in use or care of human subjects

No significant changes. IRB and HRPO approved renewal on December 8, 2021.

Significant changes in use or care of vertebrate animals

We will use both Stanford and the VA as performance site to accelerate progress this coming year. To enhance synergy with Projects 1 and 2, we have submitted protocol modifications to add tranexamic acid (TXA) treatment to the one group of animals for comparison with the results from groups that did not receive TXA. This development is pending IACUC and ACURO approval.

VA Palo Alto ACURO PR171647.e006 (previously PR171647.e002) was approved on 11/23/2021.

- ACORP Chu1768 approved on 12/7/2018, expired on 09/11/2021
- ACORP Chu1768 approved on 09/10/2021, expires on 09/10/2024

Stanford ACURO PR171647.e004 approved on 10/05/2021

- IACUC 33924 expires on 12/08/2023

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).*

Bruschi M, Sahu N, Singla M, Grandi F, Agarwal P, Chu CR, Bhutani N (2022) A Quick and Efficient Method for the Generation of Immunomodulatory Mesenchymal Stromal Cell from Human Induced Pluripotent Stem Cell. Tissue Eng Part A. 2022 May;28(9-10)

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.*

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.

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: “Biological Injections in Osteoarthritis, State of the Art and Future Strategies”, Invited Speaker, BOA Annual Congress 2021, Prerecorded Talk, September 23, 2021

Chu CR: “From Horses to Humans: Studying OA and Cartilage Repair in Horses and People”, Invited Speaker, 2021 NAVRMA Conference, Fort Collins, CO, September 23, 2021

Chu CR: “Pros and Cons on the Use of PRP for OA: Future Formulations May be Better?” Invited Speaker, California Orthopaedic Association 2021, Prerecorded Talk, April 10, 2022

Chu CR: “Debate: OA Treatment Yes/No – Pro, BMAC”, Invited Speaker, Biologic Association 3rd Annual Summit, San Francisco, CA, May 17, 2022

- **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:

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.

Name: Ran Atzmon, MD
Project Role: Chu Lab Post-doctoral Fellow (Stanford)
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: Dr. Atzmon assisted with IACUC, and ACLT procedure

Name: Kenneth Lin, MD
Project Role: Chu Lab Sports Medicine Fellow (Stanford)
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Dr. Lin assisted with ACLT procedure

Name: Jules Kouki
Project Role: Chu Lab Research Assistant (PAVIR)
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: Ms. Kouki assisted with human specimen processing and culture of human and rat MSC, and performance of animal studies.

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

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

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

None.

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: 30 September 2022

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

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

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

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

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

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

What were the major goals of the project?

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

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

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

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

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

What was accomplished under these goals?

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

progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

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

Currently 38 subjects have consented to participate in the study and 21 are active (completed or pending to complete).

A total of 17 participants withdrew or discontinued, including 9 failed additional radiograph screening, 2 withdrew due to loss of contact after Covid-19, 2 withdrew due to concerns over Covid-19, 2 withdrew due to becoming pregnant which is an exclusion criterion, and 2 discontinued due to unable to commit further.

Of the 21 active participants, 14 completed the study and 7 are pending to complete.

- Of the 14 completed the study, all 14 completed MRI, gait, and patient reported outcomes (PROs) data at baseline and 6-month. There are missing data due to COVID19 regulations. Of the same 14 completed the study, 1 missed 3- and 6-month gait, 2 missed 6-month gait, 1 missed post-training cartilage stress test and 3-month gait, and 7 did not undergo the cartilage stress test.
- Of the 7 pending to complete, 1 is pending for baseline visit, 1 is undergoing re-training, 2 are undergoing 3-month follow-up, 3 are undergoing 6-month follow-up.

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.

This project in 2022 provided Jade He, a postdoctoral scholar, a training and professional development opportunity to attend the Motor Control Summer School XIX organized by the International Society of Motor Control. From the small group interactive lecture series, Jade whose training was in biomechanics gained knowledge in motor control and motor learning from a historical perspective and the most up-to-date research. The topics are relevant to the project and Jade's postdoctoral research goals, as gait retraining involves a motor learning process and requires appropriate approaches to facilitate (i.e., augmented feedback) and monitor the process. Jade presented her preliminary work titled "Different intersegmental coordination patterns after anterior cruciate ligament reconstruction as compared to healthy controls." Jade also made contacts and had conversations with participating faculties and graduate students, expanding her professional network.

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

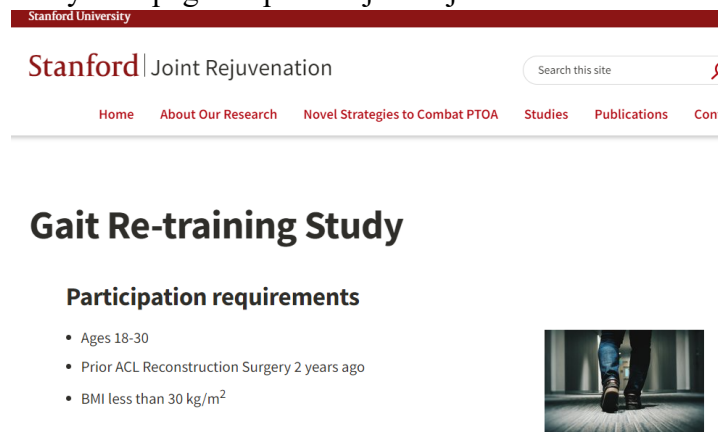
What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state "Nothing to Report."

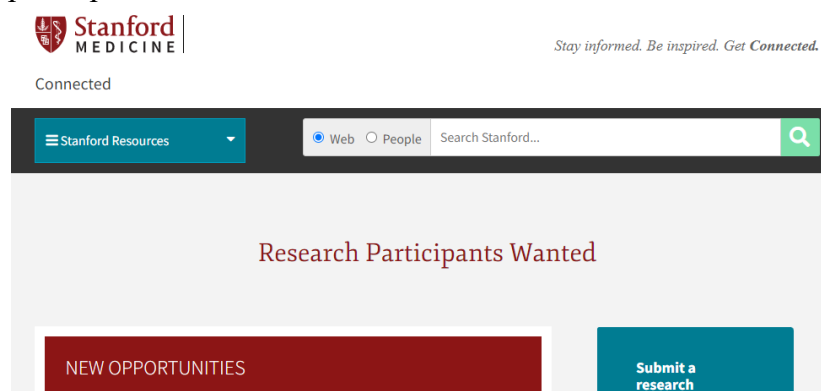
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period, we plan to continue screening and enrolling study participants for remote gait retraining to reach the target recruitment number, as well as perform follow-up visits. In addition to recruitment letters and study flyers, we will amplify our recruitment efforts by posting and sharing our study website on social media and online platforms. We will also implement an online screening form via RedCap when sharing about our study online, making it more convenient for potential participants to contact the research team and find out their eligibility.

Study web page: <https://chujointrejuvenation.stanford.edu/gait-re-training-study>



Stanford Medicine Research Participants Wanted page: <https://med.stanford.edu/connected/research-participants-wanted.html>



- 4. IMPACT:** Describe 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.

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.

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.

Nothing to report.

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

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

Significant changes in use or care of human subjects

No significant changes. IRB 46025 and HRPO E00178.2a (VA) & E00178.2b (Stanford) approved on 08/04/2022 for the continuing renewal.

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).*

• 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. 2022; 40(4): 791-798. PMID: 34185322.

• **Chu CR**. Can we afford to ignore the biology of joint healing and graft incorporation after ACL reconstruction? J Orthop Res. 2022; 40(1):55-64. PMID: 34314066.

• Erhart-Hledik JC, Chu CR, Asay JL, Mahtani GB, Andriacchi TP. Vertical ground reaction force 2 years after anterior cruciate ligament reconstruction predicts 10-year patient reported outcomes. J Orthop Res. 2022; 40 (1), 117-128. Awarded Editor’s pick of the month.

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.*

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

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

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

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

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

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

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

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

Nothing to report

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

Nothing to report

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

Nothing to report

- **Other Products**
Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
 - *data or databases;*
 - *physical collections;*
 - *audio or video products;*
 - *software;*
 - *models;*
 - *educational aids or curricula;*
 - *instruments or equipment;*
 - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
 - *clinical interventions;*
 - *new business creation; and*
 - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of

compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

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

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 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 2022

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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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-1 β receptor with the administration of and IL-1ra gene therapeutic treatment may result in decreased joint catabolism. Short term clinical trials have indicated that the gene delivery of IL1ra to affected joints have resulted in significant improvements in both clinical and histological outcomes. The achievement of the long-term production of these gene therapies may have significant and extended symptom and disease modifying benefits. Moreover, the identification of reliable biomarkers that accurately represent the stage and progression of PTOA, as well as the extent of response to treatment, are of crucial importance.

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

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

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

What were the major goals of the project?

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

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

What was accomplished under these goals?

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

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

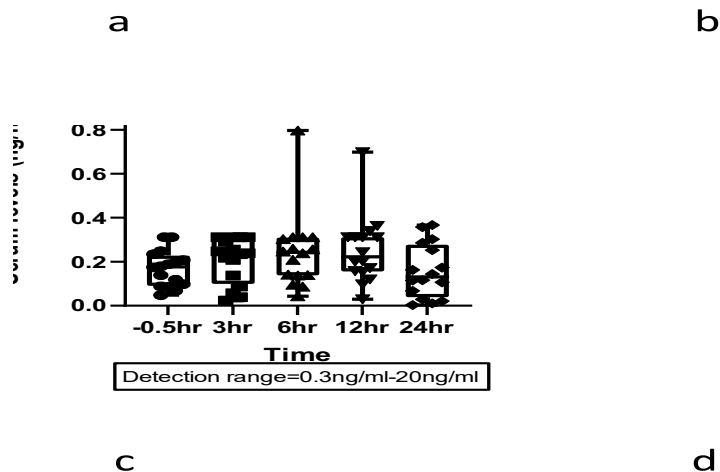
We have completed our pilot study with 5 horses in which we set out to determine the appropriate dose of virus needed for the primary study. A dose of 5×10^{11} vg dose of scAAVIL1ra was identified as the optimal dose, and this will be used for the 16-horse, 1year study.

We have officially started the 1-year study with all 16 horses. We have collected Day -30 baseline serum samples and analyzed them for relevant biomarkers. All horses underwent surgery to create an osteochondral defect in their carpal joint, 30 days after baseline blood draws. Sample collections for the day of treatment (when the horses received the viral vectors 14 days after surgery) have also been completed. The treadmill activity training, as well as baseline blood samples according to the mechanically stimulated biomarker protocol timepoints of -0.5hr, 3hrs, 6hrs, 12hrs, and 24hrs after treadmill activity were completed prior to surgery.

Analysis of these samples is ongoing.

The levels of relevant biomarkers tested are minimal in all baseline serum samples; thus, we have established a baseline reference value for all biomarkers to be tested in this study. Below are representative graphs for the biomarker analysis from the baseline serum samples from all 16 horses.

As expected, IL1ra levels in serum samples from baseline healthy horses are close to the minimum detection level of 0.3 ng/ml. We would expect to see biologically significant levels of IL1ra in the serum and synovial fluid samples from the horses that received the scAAVIL1ra treatment. Levels of MMP3 (a key mediator of cartilage degradation) were minimal and close to the minimum detection level of 0.03 ng/ml. IL1 α and IL1 β (both key molecules involved in joint inflammation) were below detection in all the samples tested. There were no significant differences between blood draw timepoints (-0.5hr, 3hrs, 6hrs, 12hrs, 24hrs) in any of the samples tested. We plan to further evaluate these biomarkers in serum samples from the day of treatment and 14 days post-treatment to establish optimal timing parameters of serum draws for the remainder of the 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:** *Describe 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 had delayed the start of all animal studies due to the Coronavirus pandemic. We have officially started the study and horses have undergone surgery for creation of defect and dosing with scAAVIL1ra vectors. Sample collections are ongoing.

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

Nothing to report. We have successfully obtained ACURO (PR171647.e003) approval in May 2019 and CSU IACUC (18-8255A) approval in December 2018 for this Grant. Stanford University was added and approved as an additional site for animal research in Nov 2020.

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.*

The results of the pilot study using 5 horses will be presented as a poster at the 4th Orthopedic Research Symposium at the University of Colorado.

- **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: 3.0

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; lameness exams and sample collections.

Name: Jennifer Phillips

Project role: research associate

Research identifier: N/A

Nearest person month worked: 4.0

Contribution to project: Generation, validation and testing of experimental expression vectors planned for use in this project (ie, IL1ra). Scheduled sample analysis & preparation of samples; histology for endpoint samples; contributions to reports and final manuscripts for publication.

Name: Dr. Parvathy Thampi

Project Role: research scientist

Research identifier: N/A

Nearest person month worked: 2.0

Contribution to project: Generation, validation and testing of experimental expression vectors planned for use in this project (ie, IL1ra). Scheduled sample analysis & preparation of samples; generation of reports and final manuscript for publication.

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

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

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.*

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- 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.*