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PRINCIPAL INVESTIGATOR: Constance Chu

CONTRACTING ORGANIZATION: The Leland Stanford

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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.

15. SUBJECT TERMS

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STATEMENT OF WORK
W81XWH-18-1-0590
Novel Strategies to Combat Post-Traumatic Osteoarthritis
PI: Constance R. Chu, MD
PROPOSED START DATE Dec 1, 2019

The grant transfer is not expected to negatively impact accomplishment of the statement of work (SOW). Approval of pre-award spending supported progress on the SOW during grant transfer. For reasons previously reported in the Year 1 quarterly and annual reports, the program has essentially experienced close to a one year delay. After completing a one day review of all 5 projects attended by all Project PI and their respective team members on October 29, 2019 (agenda attached), the team is prepared and committed to accomplishing the SOW on an accelerated pathway. To further support completion of the SOW, a 1-year no cost extension is requested.

Project 1: The Role of Plasmin in Post-traumatic OA

Site 1: VA Palo Alto Health Care System
3801 Miranda Avenue
Palo Alto, CA 94304
PI: Dr. William Robinson (WR)
Co-I: Dr. Constance Chu (CC)

Site 2: Stanford University
269 Campus Drive
Stanford, CA 94305
Partnering PI: Dr.
Constance Chu (CC)

Site 3: University of Minnesota/TRIA
Partnering PI: Dr. Brad Nelson (BN)

Specific Aim 1: To develop synovial fluid- and/or serum-based fibrinolysis biomarkers for the prediction of recovery outcomes following ACL injuries and the sequential development of post-traumatic OA.	**Timeline**	**VA**	**Stanford**	**UMinn /TRIA**
Major Task 1: ELISA and Luminex biomarker assays on existing human synovial fluid and serum samples Human Anatomical Substances (HAS) used: deidentified synovial fluid and serum samples [ChuLab Repository]	Months			
Subtask 1: ELISA and Luminex assays on the ACLT and DMT cohorts 50%[1]	1-18	WR, CC		
Subtask 2: ELISA and Luminex assays on the Mechanically Stimulated Study cohort 0%[2]	7-20	WR, CC		
Milestone #1: Integrated datasets from ELISA and Luminex assays that suggest biomarker candidates 50%[1]	25-30	WR, CC		
Major Task 2: Validation of results from Major Tasks 1-2 by using human synovial fluid and serum samples provided by Projects 2 and 4.				
Subtask 1: Collect samples in Projects 2 and 4 Human Anatomical Substances (HAS) used: de-identified synovial fluid and serum samples [ChuLab Repository] 50%[1,2]	1-32	CC, WR	CC	BN
Subtask 2: ELISA and Luminex assays on samples provided by Projects 2 and 4 0%[2]	20-32	WR, CC		
Milestone #2: Validated synovial fluid- and/or serum-based fibrinolysis biomarkers for the development of PTOA following ACLT 50%[1]	24-32	WR, CC		
Subtask 3: ▏Decision on biomarker selection and clinical applicability. ▏Co-author manuscript on biomarker development. 50%[1]	24-32	WR, CC		
Milestone #3: Manuscript on the development of fibrinolysis biomarkers for the development of PTOA following ACLT 50%[1]	24-32	WR, CC	CC	BN
Specific Aim 2: To determine the mechanisms by				

which fibrinolysis contributes to recovery outcomes following ACL injury, and to development of post-traumatic OA.				
Major Task 1: Histological analysis of mouse joints following MMLT or MMLT&ACLT and tranexamic acid treatment Animals used: 12- to 16-week-old male B6 mice (n=120)				
Subtask 1: MMLT or MMLT&ACLT surgery and tranexamic acid treatment on mice 100%	1-18	WR		
Subtask 2: Histological analysis of the mouse joints 100%	7-20	WR, CC		
Major Task 2: Immunohistochemical analysis of mouse joints following MMLT or MMLT&ACLT and tranexamic acid treatment				
Subtask 1: Immunohistochemistry on the mouse joints 75%	6-27	WR		
Major Task 3: Cytokine/chemokine analysis of mouse joints following MMLT or MMLT&ACLT and tranexamic acid treatment				
Subtask 1: Cytokine/chemokine analysis of mouse joint Tissue 75%	25-32	WR		
Major Task 4: MMP expression and activation analysis of mouse joints following MMLT or MMLT&ACLT and tranexamic acid treatment				
Subtask 1: MMP expression and activation analysis in the mouse joints 75%	28-32	WR		
Milestone #4: Manuscript on the mechanisms by which fibrinolysis mediates the development of PTOA following ACLT 75%	32+	WR, CC		
Specific Aim 3: To optimize the tranexamic acid dosing regimen for the prevention of PTOA in mice by examination of (i) delayed treatment and (ii) duration of dosing.				
Major Task 1: Regimen 1 Animals used: 12- to 16-week-old male B6 mice (n=180)				
Subtask 1: Design and perform Regimen 1 tranexamic acid treatment on mice subjected to MMLT or MMLT&ACLT 100%	1-18	WR, CC		
Subtask 2: Evaluate the effects of treatment on PTOA development by histologic analysis. 100%	11-20	WR, CC		
Major Task 2: Regimen 2 Animals used: 12- to 16-week-old male B6 mice (n=180)				
Subtask 1: Design and perform Regimen 2 tranexamic acid treatment on mice subjected to MMLT or MMLT&ACLT 100%	16-24	WR, CC		
Subtask 2: Evaluate the effects of treatment on PTOA development by histologic analysis. 75%	27-30	WR, CC		
Major Task 3: Contingent experiments Animals used: 12- to 16-week-old male B6 mice (n=120)				
Subtask 1: Design and perform contingent experiments as outlined in alternative strategies. 50%	30-32+	WR, CC		
Milestone #5: Optimized tranexamic acid treatment regimen for preventing or slowing PTOA following ACLT 50%	31-32+	WR, CC		
Subtask 2: | Decision on clinical applicability of the mouse treatment regimen | Co-author manuscript on therapeutics development.	24-32+	WR, CC	CC	BN
Milestone #6: Manuscript on the development of disease-modifying therapeutics for PTOA following ACLT	24-32+	WR, CC	CC	

1. ELISA and Luminex Assays have been completed on banked ACLT and DMT samples from the ChuLab Repository. These data have been analyzed and a manuscript is in progress.

2. The Projects 2 and 4 cohorts are incomplete and samples are still being collected as subjects are enrolled. They will be batched for analyses upon completion of cohorts and subcohorts.

A manuscript demonstrating a central role for plasmin (employing TXA as a pharmacological inhibitor to plasmin) is in the final stages of preparation for submission to JCI Insight.

Qian Wang, Guoqiang Shao, Heidi H. Wong, Richard RL Cao, Nick Hu, Michelle S. Bloom, Rong Mao, Nicholas Giori, Stuart Goodman, Pier F. Indelli, Zhen Cheng, Constance R. Chu, William H. Robinson, **Plasmin Promotes Inflammation and Cartilage Degeneration in Osteoarthritis**, in preparation.

Project 2: The Effects of Tranexamic Acid on Joint Inflammation and Cartilage Health in Anterior Cruciate Ligament–Injured Patients

Site 1: Stanford University School of Medicine
Department of Orthopedics
Stanford, CA 94305
PI: Dr. Constance Chu (CC)
Co-PI: Dr. Mark Genovese (MG)
Co-I: Dr. James Quinn

Site 2: University of Minnesota
TRIA Orthopaedic Center
Partnering PI: Dr. Bradley Nelson (BN)

Site 3: VA Palo Alto Health Care System
3801 Miranda Avenue
Palo Alto, CA 94304
PI: Dr. Constance Chu (CC)
Co-I: Dr. William Robinson (WR)

Specific Aim 1: Acute TXA, 1 week synovial fluid aspirates	**Timeline**	**VA Palo Alto**	**TRIA/U Minn**	**Stanford**
Major Task 1: Study Start-up 100%	Months			
Subtask 1: Prepare Clinical Coordinating Center Regulatory Documents and Regulatory Documents including IRB/HRPO	completed		BN	CC
Subtask 2: Execute Subcontract Agreements	1-3		BN	CC
Milestone #1: Execute Subcontracts	1-3		BN	CC
Milestone #2: Execute Data Use Agreements	completed		BN	CC
Milestone #3: Establish MRI Agreements and Protocols	completed		CC, BN	existing
Milestone #4: Finalize Randomization Schema – Specific Aims 1 & 2	completed			
Subtask 6: Investigator Training	completed		BN	CC, MG, JQ
Milestone #5: Site Initiation Visit	completed		BN	CC
Major Task 2: Subject Recruitment 19% at Stanford[1-3]; 12.5% at TRIA[1,2]				
Subtask 1: Distribution of Recruitment Materials	1-3		BN	CC, MG, JQ
Subtask 2: Subject Recruitment & Enrollment	1-27		BN	CC, MG, JQ
Subtask 3: Monthly Monitoring of Recruitment	1-27		BN	CC, MG, JQ
Milestone #6: Subject Enrollment Complete	27		BN	CC, MG
Specific Aim 2: Post-op follow-up of PRO and MRI through 2 years				
Major Task 3: Clinical Monitoring & Quality Control Procedures 50%				
Subtask 1: Conduct Remote Interim Site Visit	4-12			CC
Subtask 2: Conduct Interim Site Visits	13-32			CC, MG
Subtask 3: Conduct Review of Monthly Quality Report, Prepare Materials for Quarterly DSMB Meetings	1-32			CC, MG
Subtask 4: Monitor Data for AEs and SAEs, Monitor and	1-32			CC, MG

Address Protocol Deviations, Monitor and Address Adherence and Fidelity to Randomization Assignment				
Milestone #7: Study Monitoring Complete	32			CC, MG
Major Task 4: Subject Follow-up				
Subtask 1: Collect baseline PRO and Synovial Fluids at Day 1 (pre-treatment) for measurement of synovial fluid IL-1 and COMP. These samples will also be de-identified for use in Project 1. Human Anatomical Substances (HAS) used: deidentified synovial fluid samples [ChuLab Repository] 19% at Stanford[3]; 12.5% at TRIA	1-28	CC, WR	BN	CC, MG
Subtask 2: Collect Synovial Fluids at Day 8 (1 week follow-up) for measurement of synovial fluid IL-1 and COMP. Thse samples will be also banked and used for Project 1. Human Anatomical Substances (HAS) used: deidentified synovial fluid samples [Chulab Repository]19% at Stanford[3]; 12.5% at TRIA (See Table)	1-28	CC, WR	BN	CC, MG
Subtask 3: Collect MRI Scans and Patient-Reported Outcomes at 6 weeks, 1 year and 2 years after ACL Injury and subsequent ACLR for MRI UTE-T2* mapping of articular cartilage and KOOS pain and function scoring. 11.5% at Stanford[3,4]; 12.5% at TRIA (See Table)	2-32+		BN	CC, MG
Subtask 4: Collect Radiographs at 6 weeks and 2 years after ACLR for assessment of OA by Kellgren and Lawrence grade. 11.5% at Stanford[3,4]; 0% at TRIA[5]	2-32+	CC	BN	CC, MG
Milestone #8: Subject Follow-up Complete	32+	CC	BN	CC, MG
Major Task 5: Study Governance 50% complete				
Subtask 1: Monthly Meeting for ESC	1-32+			CC, MG
Subtask 2: Quarterly Meeting for ESC, IAB to Discuss Study Progress; Quarterly Conference Calls for Study Governance Sub-committees	1-32+		BN	CC, MG, JQ
Subtask 3: Conference Call for External Adverse Events Adjudication Committee Twice Per Year	1-32+		BN	CC, MG
Subtask 4: Annual Investigator Meetings	1-32+	CC, WR	BN	CC, MG
Milestone #9: Study Governance Complete	32+			CC, MG
Major Task 6: Analyze and Disseminate Results 50%[6]				
Subtask 1: Analysis of Data For Primary and Secondary Aims	1-32+	CC, WR	BN	CC, MG, JQ
Subtask 2: Preparation and Submission of Abstracts & Manuscripts for Primary and Secondary Aims	1-32+	CC, WR	BN	CC, MG, JQ
Subtask 3: Final Data Cleaning & Verification	32+			CC, MG, JQ
Milestone #10: Report results from data analyses including submission of peer-reviewed papers and presentation of project data	32+	CC, WR	BN	CC, MG, JQ

Projected Quarterly Enrollment

	Year 1				Year 2				Year 3				Total
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3		
Stanford	0/3[1]	0/4[1]	0/4[1]	0/4[1]	1/4[1]	3/4	1/3[2]	0/0[2]	2/0	0	0		**7[3]/26**
TRIA/UMinn	-	0/4[1]	0/4[1]	0/4[1]	0/4[1]	3/4	0/4[2]	0/0[2]	0/0[2]	0	0		**3/24**

1. Initiation of recruitment commenced in Year 2 due to delays in receipt of Year 1 subcontracts followed by administrative transfer of the grant which was completed in the 2nd quarter of Year 2.

2. Recruitments at both sites were ramping up in the 2nd quarter of Year 2 until shut-down of research activities the following quarter (3rd quarter) due to COVID-19. Prohibition of in person research activities continued into the 4th quarter at both sites, and into the 1st quarter of Year 3 at TRIA.

3. Two enrolled subjects who were Stanford students were ordered to vacate campus in March 2020 due to COVID-19 and relocated out of state prior to completion of basic study procedures. They have been removed from the study.

4. The two most recent enrolled subjects have not reached 6 weeks post-ACLR follow-up.

5. Due to COVID-19 restrictions on in person research activities, study radiographs could not be obtained at TRIA for the 6-week follow-ups.

To address the delays in recruitment responsible for the shortfalls in accomplishing Major Tasks 2, 4, and 6, we are pursuing the following alternative approaches:

- To accelerate completion of Major Task 4 subtasks 1 and 2, we have obtained IRB approval to increase initial enrollment so that we can enroll subjects who are able to complete the shorter term Aim 1 procedures independent of whether they can complete the longitudinal 2-year study comprising Aim 2. HRPO has reviewed and determined these are not substantive changes to the protocol.
- To further accelerate completion of Major Task 4 subtasks 1 and 2, we have submitted an IRB modification to the inclusion criteria that will allow recruitment of any healthy subject with a hemarthrosis after a joint injury. Once approved, we will submit this modification to HRPO for review.
- To increase subject enrollment in the coming year, we propose to establish additional recruitment sites.
    a. Within Stanford, we are establishing new recruitment sites at the main campus Emergency Department, the Los Gatos Orthopedic clinic, and the Emeryville Orthopedic clinic. The Emeryville clinic is 1 ½ hours (without traffic) away from Stanford campus and thus will need purchase of a desktop centrifuge and a -80 freezer to process and properly store biospecimens in a timely fashion.
    b. We have reached out to Dr. Jason Dragoo, a CoInvestigator on the original project, to potentially establish a new performance site at his new institution of the University of Colorado at Denver.
- TRIA has been approved to restart project recruitment in the 2nd quarter of Year 2.

6. Funded personnel have analyzed and disseminated data and results related to the Aims of this Project:

PUBLICATIONS

1. Williams AA, Erhart-Hledik JC, Asay JL, Mahtani GB, Titchenal MR, Lutz AM, Andriacchi TP, **Chu CR**. Patient Reported Outcomes and Knee Mechanics Correlate to Patellofemoral Deep Cartilage UTE-T2* 2 years after ACL Reconstruction. American Journal of Sports Medicine, in press.

2. Chappell KE, Williams AA, **Chu CR**. Quantitative Magnet Resonance Imaging of Articular Cartilage Structure and Biology. In Cartilage Injuries of the Knee: State-of-the-Art Treatment and Controversies. (Krych A, Biant L, Gomoll A, Nakamura N, Eds.) Springer, in press.

3. **Chu CR**, Williams AA, Erhart-Hledik JC, Titchenal MR, Qian Y, Andriacchi TP. Visualizing PreOsteoarthritis: Integrating MRI UTE-T2* with Mechanics and Biology to Combat Osteoarthritis: The 2019 Elizabeth Winston Lanier Kappa Delta Award. Invited manuscript, under review by the Journal of Orthopaedic Research.

4. Williams AA, Deadwiler BC, Dragoo JL, **Chu CR**. Does arthroscopic status at the time of ACL reconstruction predict cartilage T2 change over the following year? Under review by the Journal of Orthopaedic Research.

ABSTRACTS

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

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

3. Williams AA, Erhart-Hledik JC, Asay JL, Mahtani GB, **Chu CR**, Tibial Rotation and Knee Flexion Moment Correlate to Patellofemoral Deep Cartilage UTE-T2* 2 Years After ACL Reconstruction. Poster #2751. International Society for Magnetic Resonance in Medicine, Virtual Conference and Exhibition, Aug 8-15, 2020.

4. Williams AA, Erhart-Hledik JC, Asay JL, Mahtani GB, Titchenal MR, Andriacchi TP, **Chu CR**, Knee Mechanics and Patient Reported Outcomes Correlate to Patellofemoral Deep Cartilage UTE-T2* 2 Years After ACL Reconstruction, Poster, 2020 Annual International Workshop on Osteoarthritis Imaging (IWOAI), Salzburg, Austria, Sept 9-11.

5. **Chu CR**, Erhart-Hledik JC, Torres H, Williams AA, Xu Y, Robinson W, Mahtani GB, Asay JL. Pain Relief Following Treatment Of Early Knee Osteoarthritis With Autologous Platelet Rich Plasma Correlate With Improved Gait Mechanics And Cytokine Profile: Is This Good Enough? Orthopaedic Research Society Annual Meeting, Feb 12-16, 2021. Virtual Meeting.

Project 3: Cellular Rejuvenation to Combat Posttraumatic OA

Site 1: Stanford University School of Medicine
Department of Orthopedics
Stanford, CA 94305
PI: Dr. Nidhi Bhutani (NB)
Co-PI: Dr. Constance Chu (CC)

Site 2: VA Palo Alto Health Care System
3801 Miranda Avenue
Palo Alto, CA 94304
Partnering PI: Dr. Constance Chu (CC)
Co-I Dr. William Robinson (WR)

Specific Aim 1: To test the hypothesis that iPSC derived MSC will show comparable anti-inflammatory activity as BM-MSC. (Experiments will be performed using cells from 3 patient donors)	**Timeline**	**Stanford**	**VA Palo Alto**
Major Task 1: Establishing iPSC lines from donors undergoing ACL reconstruction	Months		
Milestone #1: IRB/SCRO Approval 100%	completed	NB, CC	
Subtask 1: Procurement of blood and tissues from donors undergoing ACL reconstruction and generation of MSC 80%	1-6	CC	CC
Subtask 2: Cellular reprogramming of donor-derived native cells to iPSC cell lines and validation (pluripotency assays, teratoma formation in mice) 80%	1-18	NB	
Milestone #2: Generation of MSC and iPSC cell lines 80%	1-18	NB, CC	CC
Major Task 2: Characterization and optimization of the immunomodulation potential of iPSC-derived MeSC			
Subtask 1: Growth factor based differentiation of iPSC to MSC 60%	1-20	NB	
Subtask 2: In vitro assays to assess immunomodulation 60%	1-24	NB	WR
Milestone #3: Generation of IPSC-MSC and characterization of their immunomodulation 60%	1-24	NB	WR
Specific Aim 2: To test the hypothesis that ACL injured joints receiving intra-articular injections of human iPSC-derived MSC will show reduced signs of joint degeneration and osteoarthritis. (Cells will be from a total of 3 patient donors)			
Major Task 3: Assessment of MSC engraftment and persistence			
Milestone #4: IACUC/ACURO Approval 100%	completed		CC
Subtask 2: Perform ACL transection surgeries in athymic rats (n=60)	7-28		CC
Subtask 3: Experimental cells from Donor #1 generated and labeled for in vivo localization 80%	7-28	NB, CC	CC
Subtask 4: Intra-articular injections with in vivo labeled cell imaging at 1, 4 and 12 weeks 0%[1] Animals used: Athymic rats (n=60) Subtask 3a: Acute Intervention Study-Injection of labeled iPSC-MSC, labeled MSC, or Saline Control groups (10 rats per group) 7 days after ACL injury (n=30) Subtask 3b: Early Intervention Study-Injection of labeled iPSC-MSC, labeled BM-MSC, or Saline Control Groups (10 rats per group) 4 weeks after ACL injury (n=30)	8-30		CC
Milestone #5: MSC dosage, engraftment and persistence validated 20%[1]	8-30	CC, NB	CC
Major Task 4: ACLT surgeries, MSC injections and assessment of disease progression			
Subtask 1: Performing ACLT surgeries Animals used: Athymic rats (n=120) 0%[1,2]	13-32		CC

Subtask 3: Generation of experimental cells from Donors 2 and 3 80%	13-32+	NB, CC	CC
Subtask 2: Intra-articular injections (2 additional replicates using cells from Patient Donor #2 and Patient Donor #3) 0%[1,2] Animals used: Athymic rats (n=120) Subtask 2a: Acute Intervention Study-Injection of iPSC-MSC, luciferase labeled MSC, or Saline Control groups (10 rats per group) 7 days after ACL injury (n=30 per donor, total n=60) Subtask 2b: Early Intervention Study-Injection of iPSC-MSC, luciferase labeled MSC, or Saline Control Groups (10 rats per group) 4 weeks after ACL injury (n=30 per donor, total n=60)	14-32+		CC
Subtask 3: Assessment of disease progression-gross histology, immunostaining, microCT, serum biomarkers at sacrifice 12 weeks after surgery for rats from Patient Donors #1, #2, and #3 (n=180). 0%[1,2]	25-32+	CC, NB	CC
Milestone #6: Completion of all disease progression analyses and compilation of results for publication. At least 2 major publications anticipated reporting on 1) functional comparison of MSC and iPSC-MSC and 2) Effects of stem cell mediated immunomodulation on PTOA disease progression and treatment 0%[3]	24-32+	CC, NB	CC, WR

1. *In vivo* studies have been delayed due to COVID-19 research shut-down followed by equipment issues at the VA Palo Alto. The rat IVIS imaging machine at the VA Palo Alto has been determined to be unserviceable and will not be replaced until after the move into a new facility originally scheduled for summer/fall 2020. This move is now delayed due to COVID-19. We are working to establish Stanford University as an alternative site.
2. Stanford University has small animal conventional and PET-MRI expertise and capabilities that would allow us to accelerate and enhance completion of the SOW through performing longitudinal study of animals and to potentially image the development of pain pathways during PTOA development. We would need to purchase small animal MRI coils and include new collaborators.

3. Funded personnel have analyzed and disseminated data and results related to the Aims of this Project:

PUBLICATION

1. Grandi FC, Baskar R, Smeriglio P, Murkerjee S, Indelli PF, Amanatullah DF, Goodman S, Chu CR, Bendall S, **Bhutani NA.** Single-Cell Mass Cytometry Reveals Cross-Talk Between Inflammation-Dampening and Inflammation-Amplifying Cells in Osteoarthritic Cartilage. Sci Adv. 2020 Mar 13; 6(11):eaay5352. doi: 10.1126/sciadv.aay5352. PMID: 32201724

ABSTRACTS

1. Burschi M, Grandi FC, Agarwal P, Sahu N, Chu CR, **Bhutani NA** Quick Protocol For Efficient Differentiation Of Mesenchymal Stromal Cells From Human Induced Pluripotent Stem Cells'. Orthopedic Research Society 2020 Annual Meeting, Vol 45 (2020)

2. **Chu CR** et al, "Enzymatic and Non-Enzymatic Separation Methods Comparison for Isolation and Characterization of the Adipose-Derived Stromal Stem Cells: Preliminary Results", European Society for Sports Traumatology, Knee Surgery and Arthroscopy Annual Congress (ESSKA), Accepted but not presented due to COVID-19

Project 4: Gait Retraining to Reduce Inflammation, Joint Loading, and PTOA Risk

Site 1: VA Palo Alto Health Care System
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Co-I: Dr. William Robinson (WR)

Site 2: VA Palo Alto Health Care System 3801 Miranda Avenue
Palo Alto, CA 94304
PI: Dr. Constance Chu (CC)

Co-I: Dr. Jennifer Erhart-Hledik (JEH)

Specific Aim 1: To test the hypothesis that 8 weeks of active-feedback gait retraining will significantly reduce the KAM (medial compartment joint loading) immediately after the end of training as well as at 3 and 6 months after the end of training	**Timeline**	**VA Palo Alto**	**Stanford**
Major Task 1: Prepare for prospective "pre-post" study 100%	Months		
Milestone #1: Local IRB approval received	completed	CC	CC/JEH
Milestone #2: HRPO approval received	completed	CC	CC/JEH
Major Task 2: Participant Recruitment, Baseline Assessment, Gait Retraining Program, and Gait Analysis Follow-up Assessments			
Subtask 1: Recruit subjects meeting inclusion/exclusion criteria and perform baseline gait analysis. 18%*	1-28	CC	CC
Subtask 2: Complete laboratory gait retraining sessions for enrolled participants14%*	1-30	CC	CC/JEH
Subtask 3: Subtask 3: Complete immediately post-training, 3 month, and 6 month post-training gait analysis evaluations. 18%.*	1-32	CC	CC/JEH
Milestone #3: Complete gait retraining sessions and post-training follow-up gait sessions for all participants 18%*	1-32	CC	CC/JEH
Specific Aim 2: To test the hypothesis that changes in joint loading (KAM) following the gait retraining program will be associated with changes to serum inflammatory markers immediately after the end of training.			
Major Task 3: Assess all participants at baseline and immediately post-training with the 'Cartilage Stress test' protocol. Perform measurement of concentrations of serum biomarkers pre- and post-mechanical stimulus.			
Subtask 1: Complete 'Cartilage Biomarker Stress Test' at baseline and immediately post-training for all enrolled participants Human Anatomical Substances (HAS) used: deidentified serum samples [ChuLab Repository] 14%[1]	1-30	CC	CC/JEH
Subtask 2: Perform measurement of concentrations of serum IL-1, TNF-α, and COMP pre- and post-mechanical stimulus 0% (not due yet)	30-32+	CC, WR	
Milestone #4: 'Cartilage Stress Test' data collected and analyzed	1-32+	CC	CC/JEH

Specific Aim 3: To test the hypothesis that changes in KAM from baseline to 6 months after completion of training will correlate with changes in medial compartment QMRI over the same time frame.			
Major Task 3: Assess all participants at baseline and 6 months with quantitative MRI. Perform calculation of T2 and UTE-T2* values for specified ROIs from MRI data.			
Subtask 1: Complete MRI at baseline and 6 months post-training for all enrolled participants 14%[1]	1-32+	CC	CC
Subtask 2: Perform calculation of T2 and UTE-T2* values 14%[1]	1-32+	CC	CC
Milestone #5: MRI data collected and analyzed	1-32+	CC	CC
Major Task 4: Complete statistical analyses for Aims 1-3.			
Subtask 1: Complete statistical analyses for effects of gait retraining on joint loading, IL-1, TNF-α, COMP, and QMRI. Not yet due. Initial analyses on joint loading in first 6 participants have been completed.	28-32+	CC, WR	CC/JEH
Subtask 2: Preparation and submission of abstracts and manuscripts for study aims Not yet due. Initial analyses on joint loading in first 6 participants have been completed.	28-32+	CC, WR	CC/JEH
Milestone #6: Report results from data analyses including submission of peer-reviewed papers and presentation of project data. 50%[2]	28-32+	CC, WR	CC/JEH

Projected Quarterly Enrollment

	Year 1				Year 2				Year 3			Total
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	
Target Enrollment (per quarter)	4	5	4	5	4	5	4	5	4	5		**45**
Target Enrollment (cumulative)	4	9	13	18	22	27	31	36	40	45		**45**

1. From March 2020 to September 2020 clinical research was halted due to COVID-19. Clinical research resumed in a staged fashion at the VA Palo Alto on September 28, 2020. Due to COVID-19 related restrictions on entry and use of patient care facilities, we remain unable to complete the biomarker protocol requiring hourly blood draws and some MRI. Our ability to bring subjects in for 8 weeks of gait retraining and for gait analyses also remain inconsistent.

Major Task 2:

- **Subtask 1:** 8 participants (18%) of the targeted 45 have completed baseline gait testing. Of these 1 withdrew (developed exclusion criteria) and 1 has not yet started gait retraining due to Covid-19
- **Subtask 2:** 7 participants (16%) of the targeted 45 began gait retraining and 6 (14%) completed all gait

retraining sessions (1 withdrew due to pregnancy).

- **Subtask 3**: 6 participants (17%) of the targeted final cohort of 36 (enrollment of 45 subjects allows for 20% dropout) have completed follow-up testing. Due to Covid-19, we were been unable to perform in-person gait testing at the 3 month visit for 2 subjects, as well as in-person gait testing at the 6 month visit for 4 subjects (we are in the process of scheduling 6 month gait exams as the gait laboratory is now open). However, all subjects have completed patient-reported outcomes at each study time point.
-

Major Task 3:

- **Subtask 1:** 5 participants (14%) of the targeted final cohort of 36 completed cartilage biomarker stress tests at both baseline and follow-up. 1 additional subject completed the baseline biomarker stress test, but was unable to complete the follow-up biomarker stress test due to Covid-19.

Major Task 4:

- **Subtask 1:** 5 participants (14%) of the targeted final cohort of 36 completed cartilage biomarker stress tests at both baseline and 6 month follow-up. 1 additional subject completed the baseline MRI, but has been unable to complete the 6 month follow-up MRI due to Covid-19. We will continue recruiting/testing utilizing the remote gait retraining protocol as described above to reach our target sample size.

Proposed Alternative Approach: COVID-19 restrictions and considerations pose continued substantial barriers to accomplishing the in person visit intensive nature of the original protocol. We are therefore pursuing a remote gait retraining protocol as an alternative approach. We have received IRB approval (as well as HRPO acknowledgement of the protocol changes, which were considered non-substantive) to begin a remote gait retraining protocol, where participants will perform 8 weeks of gait retraining at home using pressure-sensitive insoles, and we are in the process of finalizing the remote gait retraining method. With approval, we plan to add Dr. Markus Wimmer (Rush University Medical Center) as a consultant to this project, as Dr. Wimmer's group has utilized pressure-sensitive insoles to provide gait retraining in healthy and OA populations, and his expertise will be very valuable in this endeavor. This remote gait retraining protocol will allow us to reduce potential Covid-19 exposure as well as to continue the Project should there be any further research restrictions or facilities closure. We have a list of potential subjects who will complete in-person screening and baseline testing as soon as the remote gait retraining protocol is ready for use (anticipated in December 2020), and will continue our recruitment efforts to reach our target of 45 recruited subjects.

2. Funded personnel have analyzed and disseminated data and results related to the Aims of this Project:

1. **Andriacchi TP**, Griffin TM, Loeser RF, Chu CR, Roos EM, Hawker GA, Erhart-Hledik JC, Fischer AG. Bridging Disciplines as a Pathway to Finding New Solutions for Osteoarthritis a Collaborative Program Presented at the 2019 Orthopaedic Research Society and the Osteoarthritis Research Society International. Osteoarthritis and Cartilage Open. 2020 Mar; Volume 2, Issue 1. doi: 10.1016/j.ocarto.2020.100026

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

3. **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. *Under review by the Journal of Orthopaedic Research.*

4. 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. *Under review by the Journal of Orthopaedic Research.*

1. Titchenal M.R, Williams A.A., Asay J.L., Migliore E., Erhart-Hledik J.C., Andriacchi T.P., **Chu C.R**. Mechanically Stimulated Serum CS846 Correlates with Ultrashort Echo Time Enhanced T2* MRI and Gait Mechanics 2 Years after Anterior Cruciate Ligament Reconstruction. Under review by Osteoarthritis and Cartilage.

ABSTRACTS

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

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

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

4. Fischer AG, Titchenal M, Williams A, Migliore E, Asay JL, Erhart-Hledik JC, Andriacchi TP, **Chu CR**, "Elevated TNF-α, Reduced Knee Loading and Increased UTE-T2* Signal 2 Years Post ACLR: A Signal for Knee OA in a Subset of Patients", Orthopaedic Research Society Annual Meeting, Feb 8-11, 2020, Phoenix, Arizona

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

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

7. Mahtani GB, Erhart-Hledik JC, Asay JL, Andriacchi TP, **Chu CR**. Gait Retraining Induced Changes In Center Of Pressure Associated With Reductions In Knee Adduction Moment Following ACL Reconstruction. Orthopaedic Research Society Annual Meeting, Feb 12-16, 2021. Virtual Meeting.

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

Project 5: Localized Gene Therapy for Prolonged Anti-inflammatory Treatment to Prevent or Delay PTOA in an Equine Model*

Site1: Colorado State University
Orthopedic Research Center
College of Veterinary Medicine
300 W Drake Rd, Ft. Collins, CO
PI: Dr. Laurie Goodrich (LG)
Co-I's: Wayne McIlwraith (WM), Jude Samulski (JS), David Frisbie (DF), Christopher Kawcak (CK)

Site2: Stanford University School of Medicine
Department of Orthopedics
Stanford, CA 94305
Partnering PI: Dr. Constance Chu (CC)

Site2: VA Palo Alto
Partnering PI: Dr. Constance Chu (CC)

Specific Aim1: ***Develop a safe and effective scAAV-based gene therapeutic approach to treat PTOA in the equine model.***	**Timeline**	**CSU**	**Stanford**	**VA**
Major Task1: Treadmill exercise of all 16 horses to monitor biomarkers on horse prior to beginning the gene therapy preclinical trial	Months (0%)			
Milestone #1: ACURO Approval	Complete	LG		
Subtask 2: Begin "normal period" of gene therapy study. All horses will be exercised on treadmill and blood and serum will be taken on arrival before acclimatization to treadmill, baseline before surgery, D14 and monthly for 12 months.	2 (0%)	LG/WM/ JS/DF/CK		
Subtask 3: Begin gene therapy study comparing AAVIL-1ra to control in horses with PTOA	18 (0%)	LG/WM/ JS/DF/CK		
Subtask 4: Complete biomarker protocol and blood draws	18 (0%)	LG/WM/ JS/DF/CK		
Milestone #2: Completion of all horses in the one year preclinical gene therapy trial.	18 (0%)	LG/WM/ JS/DF/CK		
Major Task2: Outcome analysis of all physical and necropsy data from the preclinical equine study and MRI analysis and scoring of all T2 mapping				
Subtask 1: Analysis of all physical outcome parameters of synovial effusion, lameness and joint flexion and necropsy outcomes of histological evaluation of synovial membrane and articular cartilage.	20 (0%)	LG/WM/ JS/DF/CK	CC	
Subtask 2: Transport of all joints to Stanford for acquisition and analysis of MRI and quantitative MRI data	24 (0%)	LG	CC	
Milestone #3: Completion of assessments/analysis with publication of scAAVIL-1ra gene therapy effects in PTOA.	32+ (0%)	LG/WM/ JS/DF/CK	CC	
Specific Aim 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.***				
Major Task3: Biomarker analysis				
Subtask 1: Analyze all synovial fluid samples	24 (0%)	LG	CC	
Subtask 2: Analyze all serum samples	24 (0%)	LG	CC	
Milestone #4: Complete analyses and publish biomarker correlations to PTOA disease progression and treatment	32+ (0%)	LG/WM/ JS/DF/CK		

* Project initiation was targeted for Year 2 due to delay in receipt of the Year 1 subcontract followed by grant transfer which was completed in the 2nd quarter of Year 2. Plans to schedule ACURO site visit and project initiation were disrupted by COVID-19 in the 3rd quarter of Year 2.

Study Completion Plan:

We plan to begin the acquisition of live animals at the start of 2021, and are able to perform studies at 30% during this time; with this in mind, we anticipate that we will be able to add animals biannually during Year 3 and all remaining animals by Q1 of Year 4. Major Tasks 3 and 4 will be completed during Years 4 and 5(NCE).

	Year 3				Year 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Target Enrollment (quarterly)	4	0	4	0	8	0	0	0
Target Enrollment (Cumulative)	4	4	8	8	16	16	16	16