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TITLE: Intra-Articular Lubricin Gene Therapy for Post-Traumatic Arthritis

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14. ABSTRACT

This project focuses on addressing cartilage lubricant failure in post-traumatic osteoarthritis (PTOA) via intra-articular lubricin/proteoglycan 4 (PRG4) gene therapy. Our findings thus far indicate that a single intra-articular injection of adeno-associated virus (AAV) bearing the PRG4 gene in a rabbit ACL transection (ACLT) model resulted in transgene expression until the end-point of the experiment at 8 weeks post-op, and that the injection treatment was chondroprotective. PRG4 gene therapy appears to be a viable option for slowing the progression of PTOA, a finding that warrants investigation of the strategy in a large animal model. Accordingly we applied for a PRMRP Focused Program Award (FPA), which includes a project to pursue lubricin gene therapy in a Yucatan Minipig intra-articular fracture model. The proposal was recently.

15. SUBJECT TERMS
AAV, PRG4, ACL transection, Mankin score, transgene expression

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1. INTRODUCTION:

This project focuses on addressing cartilage lubricant failure in post-traumatic osteoarthritis (PTOA) via intra-articular lubricin/proteoglycan 4 (PRG4) gene therapy. Our findings thus far indicate that a single intra-articular injection of adeno-associated virus (AAV) bearing the PRG4 gene in a rabbit ACL transection (ACLT) model resulted in transgene expression until the end-point of the experiment at 8 weeks post-op, and that the injection treatment was chondroprotective. PRG4 gene therapy appears to be a viable option for slowing the progression of PTOA, a finding that warrants investigation of the strategy in a large animal model. Accordingly we applied for a PRMRP Focused Program Award (FPA), which includes a project to pursue lubricin gene therapy in a Yucatan Minipig intra-articular fracture model. The proposal was recently funded (*Translating Metabolic Responses to Mechanical Insult into Early Interventions to Prevent PTOA*, award #W81XWH1810658, #PR172087). Because AAV appeared to cause joint effusions in the rabbit ACL transection model, in the FPA non-viral methods of gene delivery will be investigated in the FPA project. Various non-viral formulations will be screened in parallel with AAV for efficacy and toxicity in *in vitro* and rabbit models before choosing a method to be used in the minipig.

2. KEYWORDS:

ACL transection, post-traumatic OA, PRG4, lubricin, gene therapy, adeno-associated virus

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Determine the effects of PRG4 gene therapy on the progression of OA in a rabbit ACL transection model

- Major Task 1: Test the effects of GFPLub transduction on cartilage degeneration at 4 and 12 weeks after ACL transection (ACLT) (months 0-22, 8/15/14 – 6/14/16)
  - Subtask 1: Preparation of AAV-GFP and AAV-GFPLub constructs (completed, late October 2014);
  - Subtask 2: Injection of AAV-GFP or AAV-GFPLub intra-articularly. Perform rabbit ACLT or sham surgery on 20 rabbits each, each split evenly among receiving AAV-GFP or AAV-GFP Lub (months 4-10, 11/15/14 – 6/14/15,completed, June 2016);
  - Subtask 3: Drawer test immediately post-euthanasia at 12 weeks post-op (n = 40) (months 4-10, 11/15/14 – 6/14/1, completed, June 2016);
  - Subtask 4: Confocal microscopy to confirm GFPLub expression (months 4-10, 11/15/14 – 6/14/15, 35% complete. This Subtask was discontinued due to failure to detect GFP in the first 14 rabbits.
  - Subtask 5: Safranin-O histology, Mankin score for OA, Lubricin/GFP immunohistochemistry, Lubricin turnover from synovial fluid harvested at euthanasia (immunoblots) (months 11-16, 6/15/15 – 12/14/15, completed, September 2017); Safranin-O stained sections were scored manually and are currently being scored by computer algorithm.
  - Subtask 6: Statistical Analysis (month 17, 1/15/16 – 2/14/16, 95% complete);
  - Milestone Achieved: The live animal phase of Major Task 1 was completed. Histologic processing of tissues was completed. Results will be gathered and analyzed for statistical significance and write-ups begun on histological, confocal, and joint stability data (months 18-22, 2/15/16 – 7/14/16, 90% complete); Local IACUC Approval (Pre-award, completed April 2014), Marc Brouillette added; and
  - ACURO Approval (Pre-award, completed July 2014)

Specific Aim 2: Determine the effects of PRG4 gene therapy after OA has already begun to develop

- Major Task 2: Test the effects of delayed PRG4 gene therapy on cartilage degeneration at 16 weeks post ACLT; (months 19-36, 3/15/16 – 8/14/17);
  - Subtask 1: Gene therapy (AAV-GFP or AAV-GFPLub, evenly split among ACLT or sham surgeries) will be initiated 8 weeks after ACL transection (20) or sham (20) surgery and its effects on the subsequent progression of OA will be evaluated at 16 weeks post ACLT (months 20-26, 4/15/16 – 11/14/16, completed April 2017);
  - Subtask 2: Drawer test immediately post-euthanasia (months 20-26, 4/15/16 – 11/14/16, completed April 2017);
  - Subtask 3: Confocal microscopy to confirm GFPLub expression (months 20-26; 4/15/16 – 11/14/16, 0% complete); This approach was ineffective and was discontinued.
  - Subtask 4: Safranin-O histology, automated Mankin score for OA, Lubricin/GFP immunohistochemistry, Lubricin turnover from synovial fluid harvested at euthanasia (immunoblots) (months 26-30, 10/15/16 – 3/14/17, 40% complete);
  - Subtask 5: Statistical analysis (month 31, 3/14/17 – 4/14/17, 20% complete);
  - Milestone Achieved: Results will be gathered and analyzed for statistical significance and write-ups begun on histological, confocal, and joint stability data (months 32-36, 4/15/17 – 8/14/17, 40% complete);
  - Local IACUC Approval (Pre-award, completed April 2014); and
  - ACURO Approval (Pre-award, completed July 2014)

What was accomplished under these goals?

1) Major Activities

Major Task 1: Select samples for re-cutting that could not be analyzed due to folded sections. Deliver to Histion for Safranin-O staining.

Major Task 2: Histological processing to decalcification stage and ship to Histion for safranin-O staining and lubricin immunohistochemistry. semi-automated Mankin scoring.

2) Specific Objectives

Major Task 1: Obtain Mankin scores missing due to section folding.

Major Task 2: Obtain safranin-O and immunohistochemically stained sections, scan slides.

3) Significant Results, Key Outcomes, Major Findings, Developments, or Conclusions

All specimens for both Major Tasks have been scanned and are at the image analysis stage for Mankin scoring. Histion is performing immunohistochemistry on Task 2 specimens

4) Other achievements.

As noted above, our PRMRP grant was funded and we are currently preparing to pursue lubricin gene therapy in a large animal model.

Stated Goals Not Met

The project was significantly delayed and is in no-cost extension due to problems with processing specimens for histology. However, we overcame the problems by contracting Histion to do the work. We are now well on our way to finalizing project.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Data from the project were featured in support of our FPA proposal.

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

We expect to complete data collection and analysis. A manuscript that includes *in vitro* validation studies has been drafted and awaits the final rabbit data for completion.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The results of this project taken together with our work on the acute effects of joint injury suggest that combining lubricin gene therapy with amobarbital will add to the chondroprotective effects of both therapies. This amounts to a wholly new treatment concept that was the basic premise of our successful FPA proposal.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

No further delays are anticipated

Changes that had a significant impact on expenditures

Funds will be expended during No Cost Extension in order to finish up histological and analysis work.

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

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

Publications, conference papers, and presentations.

Nothing to report

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

Nothing to report

Other publications, conference papers, and presentations.

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	James A. Martin, PhD (NO CHANGE)
Project Role:	Principal Investigator
Researcher Identifier	NA

Nearest person month worked:	1.18 person month (Calendar)
Contribution to Project:	Dr. Martin has directed all programmatic activities, including decision-making and conducting research group discussions on a weekly basis to assure progress is being made as planned. He has analyzed histological/immunohistological staining.

Name:	Linjun Yang (ADDED)
Project Role:	Graduate Student
Researcher Identifier	NA
Nearest person month worked:	1.44 person months (Calendar) until 11/30/18
Contribution to Project:	Perform automated image analysis for scoring.

Name:	Douglas R. Pedersen, Ph.D. (No longer working on the project)
Project Role:	Co-Investigator
Researcher Identifier	NA
Nearest person month worked:	
Contribution to Project:	Dr. Pedersen was responsible for discussing the design and operation of mechanical testing devices, and helped support the implementation of drawer testing and automated image analysis. Linjun Yang will complete automated image analysis.

Name:	Marc Brouillette, Ph.D. (NO CHANGE)
Project Role:	Postdoctoral Research Scholar
Researcher Identifier	NA
Nearest person month worked:	~0.2 person month (Calendar)
Contribution to Project:	Dr. Brouillette has provided support for the drawer testing device for measuring rabbit knee laxity post-ACLT. He aided in the continuation of the drawer testing (troubleshooting, analyzing drawer testing data along with Dr. Pedersen), and continues to do so at a lower effort (7/12/17 forward). During NCE, Dr. Brouillette will provide ~0.2 calendar months effort.

Name:	Barbara J. Laughlin
Project Role:	Research Assistant
Researcher Identifier	NA
Nearest person month worked:	~1 person month (Calendar)
Contribution to Project:	Helped with joint dissections, processing samples to get them ready for histology and scanning finished slides.
Funding Support:	Ms. Laughlin is funded through the Cell Biology Research Lab's Departmental funding.

Name:	Dong Rim Seol, PhD
Project Role:	Research Assistant
Researcher Identifier	NA
Nearest person month worked:	~0.6 person month (Calendar)
Contribution to Project:	Performed rabbit mechanical testing data analysis, Mankin scoring and analysis.

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

Newly Funded Projects or Changes to Funded Projects during Year 4:
Newly Funded during NCE: **Do Changes in Thiol Metabolism Mediate Osteoarthritis Progression? (1 K99 AR070914-01A1)**
Sponsor Agency: US DHHS, National Institutes of Health/NIAMS
One Democracy Plaza, 6701 Democracy Blvd, Suite 800
Bethesda, Maryland 20892-4872
09/01/2018–08/31/2021 (09/01/2018–08/31/2019 K99), Mitchell C. Coleman, PhD (PI)
James A. Martin, PhD (Co-Investigator, Mentor), 0.6 Calendar months (no salary support), effort ended 8/31/18

Effort change for personnel (and project ending just after Year 4 ends): **NIAMS: CORT Innovations to Assess and Forestall Post-Traumatic Osteoarthritis (P50 AR055533-10)**
Sponsor Agency: US DHHS, National Institutes of Health/NIAMS
One Democracy Plaza, 6701 Democracy Blvd, Suite 800, Bethesda, Maryland 20892-4872
09/01/2012– 08/31/2018,
Joseph A. Buckwalter, MS, MD (PI)
James A. Martin, PhD Reduced efforts starting 9/1/17 during P50 project's NCE (Co-Associate Director, 0.12 Calendar months; Co-Principal Investigator, Project 1: Targeting the Origins of Inflammation in Post-Traumatic Osteoarthritis, 0.96 Calendar months; Advisor, Project 2: Establishing Treatments and Diagnostic Tools for Post-Traumatic OA *In Vivo*, No salary support*;* PI, Joint Trauma Biomarker Core, 1.77 Calendar months).
Douglas R. Pedersen, PhD (PI, Project 2), 0 Calendar months effort starting 9/1/17 during NCE, and due to retirement

Extended: **Joint Distraction Treatments of Intra-articular Fracture-Induced Posttraumatic Osteoarthritis in a Large Animal Model**
Sponsor Agency: US Department of Defense, Congressionally Directed Medical Research Program W81XWH-15-1-0642
820 Chandler Street, Fort Detrick, MD 21702-5014
09/30/2015 – 09/29/2019,
Jessica E. Goetz, PhD (PI)
James A. Martin, PhD (Co-Investigator), 0.6 Calendar months

Notified for award and undergoing award negotiations during Year 4: **Translating Metabolic Responses to Mechanical Insult into Early Interventions to Prevent PTOA (PR172087)**
Supporting Agency: US Department of Defense, CDMRP PRMRP W81XWH-18-1-0658
820 Chandler Street, Fort Detrick, MD 21702-5014
09/01/2018 – 08/31/2022,
Joseph A. Buckwalter, MS, MD (PI)
James A. Martin, PhD (Investigator Project 1, Leader Project 3), 2.4 Calendar months

Projects that closed during Year 4:

Ended during NCE: **Mitochondrial Based Treatments that Prevent Posttraumatic Osteoarthritis in a Translational Large Animal Intraarticular Fracture Survival Model (W81XWH-11-1-0583)**
Sponsor Agency: US Department of Defense, Army Medical Research Acquisition Activity
US Army Medical Research Acquisition Activity
820 Chandler Street, Fort Detrick MD 21702-5014
09/01/11–08/31/2017,
James A. Martin, PhD (PI), 1.73 Calendar months to 0 Calendar months starting 9/1/17 as project has ended

Ending during Year 5: **Non-Surgical Treatment of Arthrofibrosis**
Sponsor Agency: US Department of Defense, CDMRP PRORP W81XWH-14-1-0327
820 Chandler Street, Fort Detrick, MD 21702
09/01/2014 – 08/31/2018,
James A. Martin, PhD (PI), 1.53 Calendar months

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Please see page 11 for the Quad Chart for Year 4.

Intra-Articular Lubricin Gene Therapy for Post-Traumatic Arthritis

OR130365

W81XWH-14-1-0163 Year 4, Technical Progress Report

PI: James A. Martin, PhD **Org:** University of Iowa **Award Amount:** $727,955 Total Award ($493,233.73 Direct)

Study/Product Aim(s)

- Specific Aim 1: Determine the effects of lubricin gene therapy on the progression of OA in a rabbit ACL transection (ACLT) model (months 0-22)
- Subtasks 1-6 (see SOW or annual technical report), milestones
- Specific Aim 2: Determine the effects of lubricin gene therapy after OA has already begun to develop (months 20-36)
  - Subtasks 1-5 (see SOW or annual technical report), milestones

Approach

Major Task 1: Deliver paraffin blocks to Histion for re-cutting and safranin-O staining.

Major Task 2: Deliver decalcified specimens to Histion for paraffin embedding, sectioning and, and safranin-O and immunohistochemical staining.

Received finished safranin-O stained slides from Histion. All have been scanned and are being analyzed by Linjun Yang, a student that is new to the project. Lubricin immunohistochemistry for Major Task 2 is in process at Histion.

Accomplishments: We are set to fully complete the project in the next quarter.

Timeline and Cost

Activities CY	8/15/14 - 8/14/15	8/15/15 - 8/14/16	8/15/16 - 8/14/17	8/15/17 - 8/14/18
Specific Aim 1 (months 0-22)				
SA1 subtask 2-milestone (months 4-22)				
Specific Aim 2 (months 20-36)				
SA2 subtask 4-milestone (months 26-36)				
Estimated Budget ($K)	**$235,334**	**$242,310**	**$250,311**	

Updated: Y4 (9-9-18), prev. update 6-27-18 purple indicates current location in time with respect to each aim's timeline

Goals/Milestones

CY14-15, CY15-16 Goals – **Prepare AAV constructs & ACLT rabbits**

☑ Preparation of AAV-GFP and AAV-GFPLub constructs;
☑ Pilot tests of intra-articular injection of AAV-GFPLub;
☑ Complete survival study animals;

CY16-17 CY 17-18 Goals –Complete survival studies and histologic analyses

☑ Complete safranin-O staining
☑ Complete slide scanning
☐ Complete Mankin analysis
☐ Complete immunohistochemistry

Comments/Challenges/Issues/Concerns

Y4 spending was slightly lower than projected, but due to incomplete histology at this stage. Aim 1 recuts performed during Year 3, Aim 2 IHC being done during Year 4.

Budget Expend. to Date: Y4 Expenditure 8/15/17 – 8/14/18: $157,816.43
Actual Y4 Expenditure: $49,765.44
Projected total Expenditure: $727,955
Total Spent through Y4: $619,904.01