AWARD NUMBER: W81XWH-14-1-0163

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

PRINCIPAL INVESTIGATOR: James A. Martin, PhD

CONTRACTING ORGANIZATION: University of Iowa
                          Iowa City, Iowa 52242

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              Fort Detrick, Maryland 21702-5012

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other documentation.
**Title:** Intra-Articular Lubricin Gene Therapy for Post-Traumatic Arthritis

**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 was significantly chondroprotective, while injections of control virus (AAV-GFP) were not. These data indicate that PRG4 gene therapy is a viable option for slowing the progression of PTOA, a finding that warrants progression to a large animal model.
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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 significantly 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. At the same time we recognize that the tendency of AAV to provoke inflammation poses a barrier to translating this work. In response we have proposed in recent grant submissions to develop and test non-viral substitutes for AAV before proceeding to a large animal model.

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-GFPLub (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, 90% 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, 80% 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 delayed 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-Ö 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, 0% 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, 0% 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 (Aim 1) specimens were manually Mankin-scored by three observers and the data were analyzed and prepared for publication. All Major Task 2 (Aim 2) samples for histology were decalcified in preparation for paraffin embedding. PRG4-GFP transgene expression in synovium at 8 weeks post-op was confirmed by rtPCR.

2) Specific Objectives

Complete histologic processing of tissues for Major Task 2. Complete analysis of histology data for Aim 1. Confirm PRG4-GFP expression at 8 weeks post-op.

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

Manual analysis of Mankin scores was completed. Mann-Whitney Rank sum analysis showed that AAV-PRG4/GFP therapy significantly lowered scores compared with AAV-GFP (Figure 1). PRG4/GFP expression in synovial tissue at 2 and 8 weeks after surgery was assessed by rtPCR. Expression in treated joints was 15-fold higher than in non-treated contralateral joints at 2 weeks and over 40-fold higher in treated joints at 8 weeks (Figure 2).
4) Other achievements.

We have confirmed in an internally-funded project that non-viral gene delivery to joint tissues is feasible (Figure 3). This simple delivery method could be used to transfec chondrocytes with lubricin-encoding plasmids, which is much less likely to elicit an inflammatory response than AAV, and more likely to be approved for human use. We recently composed a grant proposal to further develop this approach, that was submitted to the PRMRP as part of a Focused Program Award proposal in October 2017.

![Figure 1. Effect of PRG4-GFP gene therapy in a rabbit PTOA model. Box and whisker plot shows the median (line), quartiles (box boundaries), and minimum and maximum values (error bars) for the indicated treatment groups (n = 10/group). p = 0.04 (#), and 0.03 (*).](image)

![Figure 2. PRG4-GFP expression in synovial tissue in ACLT rabbits. The $2^{\Delta\Delta}Ct$ method was used to calculate fold-increase in treated versus untreated control at 2 weeks (light grey) and 8 weeks (dark grey) (n =3/group).](image)
Figure 3. *In vivo* gene transfer by intra-articular injection of plasmid DNA. rtPCR results are shown for the miR-17 transcript in mice with destabilized medial meniscus (DMM) treated with PMIS-17, empty vector control (EV), or no DNA. Fold change is with respect to EV control.

### Stated Goals not Met

We have not completed histologic analyses due to the unexpected illness and passing of our former histotechnologist in July 2017. This will be addressed by utilizing the services of Histion, a company that has extensive experience with processing osteochondral specimens for Safranin-O histology. We expect they will complete the work in a matter of weeks. Automated image analysis was delayed, but has been started. In the meantime we performed manual Mankin analysis, which we believe will complement automated analysis.

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

Nothing to report

### How were the results disseminated to communities of interest?

An in-progress report was given at Ft. Detrick, Maryland in May 2017

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

Complete automated Mankin analysis for Aim 1. Complete histological processing and analysis of Aim 2 rabbits.

### 4. IMPACT:

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

The premise that lubricin gene therapy offers a means to sustain joint lubrication for months, thereby impeding the development PTOA, has been unequivocally confirmed.

**What was the impact on other disciplines?**

The disease-modifying effects of intra-articular gene therapy will add significantly to the growing body of research supporting the development of gene transfer-based therapies targeting a range of disorders.

**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
We added manual Mankin analysis to complement computer automated analysis.

Actual or anticipated problems or delays and actions or plans to resolve them
All live animal work is completed; thus, veterinary services provided by Douglas Fredericks and his team are no longer required and will not be paid for from grant funds. Dr. Pedersen retired as of 9/1/17: however, he will continue to serve as an image analysis expert and will expedite automated Mankin analyses at no cost to the grant. He is in possession of the computer code and has remote access to scanned images.

Changes that had a significant impact on expenditures
Dr. Douglas Pedersen, Dr. Marc Brouillette, and Ms. Gail Kurriger have departed from the lab. As of 7/12/2017, Dr. Brouillette continues to provide expertise at 10% of his original funding, for remote efforts of data analysis.

Mr. Fredericks will no longer be providing effort, nor will he be paid from the project during No Cost Extension (beginning 8/15/2017), as the animal work has been completed.

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 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.
Presentation:  In-Progress Review Meeting, Ft. Detrick, MD, May 2017

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: 1.2 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: Douglas R. Pedersen, Ph.D. (No longer at University of Iowa starting 9/1/17)
Project Role: Co-Investigator
Researcher Identifier NA
Nearest person month worked: 1.44 person months (Calendar) until 8/31/17
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 for rabbit stifle joint ACL laxity. In the remaining months, he helped with objectively automating analysis of images and histological specimens. As of 8/31/17, he is no longer at the University of Iowa, but will continue to supervise automated image analysis.

Name: Marc Brouillette, Ph.D. (Reduced effort starting 7/12/17 due to leaving Iowa)
Project Role: Postdoctoral Research Scholar
Researcher Identifier NA
Nearest person month worked: ~2.4 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: Gail L. Kurriger, B.S. (0 effort starting 5/1/17, no longer at University of Iowa starting 7/3/17)
Project Role: Research Associate
Researcher Identifier NA
Nearest person month worked: 0.9 person months (Calendar)
Ms. Kurriger performed all rabbit joint dissections and has performed all histological processing and sectioning and safranin-O staining until 5/1/17 when she fell ill. She performed some and immunohistological preparations for GFP and Lubricin as well. She was temporarily replaced by Barbara Laughlin.

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 and has been processing samples to get them ready for histology and immunohistochemistry.
Funding Support: Ms. Laughlin is funded through the Cell Biology Research Lab’s Departmental funding.

Name: Douglas C. Fredericks, B.S. (will be 0 effort starting 8/15/17)
Project Role: Co-Investigator (Research Specialist – Animal Surgeon)
Researcher Identifier NA
Nearest person month worked: 1.2 person months (Calendar)
Contribution to Project: Mr. Fredericks is one of two the animal surgeons and did preliminary animal protocol writing and interacted with the University of Iowa’s Office of Animal Resources for IACUC and ACURO approval, and helped perform the first batches of rabbit surgeries and joint injections. Starting 8/15/17, all animal work has concluded, so Mr. Fredericks will no longer contribute to the project.
Funding Support: Ms. Laughlin is funded through the Cell Biology Research Lab’s Departmental funding.

Name: Cheng Zhou, Ph.D. (no longer working on project as of January 2017)
Project Role: Graduate Research Assistant & Temporary Research Assistant
Researcher Identifier NA
Nearest person month worked: 1 person month (Calendar)
Contribution to Project: Cheng helped with identifying transgene expression in rabbit tissue samples. He has graduated and left the institution.
Funding Support: Ms. Laughlin is funded through the Cell Biology Research Lab’s Departmental funding.

Name: InO Song, M.S. (no longer working on project as of August 2017)
Project Role: Graduate Research Assistant
Researcher Identifier NA
Nearest person month worked: <1 person month (Calendar)
Contribution to Project: InO helped with identifying transgene expression in rabbit tissue samples and in manual Mankin scoring. He has switched to working on other projects as of August 2017.
Funding Support: Ms. Laughlin is funded through the Cell Biology Research Lab’s Departmental funding.

Name: Emily B. Petersen, D.V.M. (ended effort after April 2017)
Project Role: Veterinarian
Researcher Identifier NA
Nearest person month worked: 1 person month (Calendar) – no salary support from the present grant
Contribution to Project: Dr. Petersen is one of two animal surgeons and did preliminary animal protocol writing and interacted with the University of Iowa’s Office of Animal Resources for IACUC and ACURO approval, and helped perform the first batches of rabbit surgeries.
Funding Support: Dr. Petersen is funded through the Bone Healing Research Lab’s Animal Research Surgicenter.
Name: Keli McLaughlin, (ended effort after April 2017)  
Project Role: Veterinary Technician  
Researcher Identifier NA  
Nearest person month worked: 1 person month (Calendar) – no salary support from the present grant  
Contribution to Project: Ms. McLaughlin is one of three veterinary technicians, and helped support the rabbit surgeries and aided with animal welfare checks and data entry.  
Funding Support: Ms. McLaughlin is funded through the Bone Healing Research Lab’s Animal Research Surgicenter.

Name: Amanda Wiebold, (ended effort after April 2017)  
Project Role: Veterinary Technician  
Researcher Identifier NA  
Nearest person month worked: 1 person month (Calendar) – no salary support from the present grant  
Contribution to Project: Ms. Wiebold is one of three veterinary technicians, and helped support the rabbit surgeries and aided with animal welfare checks and data entry.  
Funding Support: Ms. Wiebold is funded through the Bone Healing Research Lab’s Animal Research Surgicenter.

Name: Amie Pluskowski, (ended effort after April 2017)  
Project Role: Veterinary Technician  
Researcher Identifier NA  
Nearest person month worked: 1 person month (Calendar) – no salary support from the present grant  
Contribution to Project: Ms. Pluskowski is one of three veterinary technicians, and helped support the rabbit surgeries and aided with animal welfare checks and data entry.  
Funding Support: Ms. Pluskowski is funded through the Bone Healing Research Lab’s Animal Research Surgicenter.

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 3:  
Newly Awarded during Year 3: **Assessment of AltaPore Lapine Posterolateral Fusion Model**  
Sponsor Agency: Baxter Healthcare Corporation  
25212 W Illinois Route 120, Round Lake, Illinois 60073 Tel. 224-270-4335  
06/08/2017–06/08/2022, total costs  
Douglas C. Fredericks, BS (PI), 1.2 Calendar months

Newly Awarded during Year 3: **Evaluation of a New Ceramic-collagen Implant in a Sheep Intramuscular Osteoinduction Model**  
Sponsor Agency: Medtronic Sofamor Danek, Inc.  
2600 Sofamor Danek Drive, Memphis, Tennessee 38132  
07/28/2017–07/28/2018, total costs  
Douglas C. Fredericks, BS (PI), 0.12 Calendar months

Newly Awarded during Year 3: **Biomechanical Analysis of the Proximal Adjacent Segment Following Thoracolumbar Spine Multilevel Instrumentation**  
Sponsor Agency: Implanet America, Inc.  
8 Faneuil Hall Marketplace, 3rd Floor, Boston, Massachusetts 02109  
01/01/2017–12/31/2017, total project costs
Nicole M. Grosland, PhD (PI)
Douglas C. Fredericks, BS (Co-Investigator), 0.12 Calendar months

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  total costs (total costs K99) Mitchell C. Coleman, PhD (PI)
James A. Martin, PhD (Co-Investigator, Mentor), 0.6 Calendar months (no salary support)

Newly Funded during NCE: **Bioventus Rabbit Posterior Lateral Fusion Model BMP/Carrier Study (Statement of Work #10)**
Sponsor Agency: Bioventus LLC
8 St. Mary’s Street, Boston, Massachusetts 02115
09/05/2017–12/05/2017, total costs
Douglas C. Fredericks, BS (PI), 0.12 Calendar months

Newly Funded during NCE: **Bioventus Rabbit Femur Core Defect dBMP (Statement of Work #9)**
Sponsor Agency: Bioventus LLC
8 St. Mary’s Street, Boston, Massachusetts 02115
09/05/2017–12/05/2017, total costs
Douglas C. Fredericks, BS (PI), 0.12 Calendar months

Effort change for two personnel: **Engineering Endogenous Cartilage Repair**
Sponsor Agency: Arthritis Foundation
Arthritis Foundation National Office
1330 W. Peachtree St., Suite 100
Atlanta, GA 30309
Estimated Start Date 02/01/2016-01/31/2019, Total Direct Costs James A. Martin, PhD (PI), 1.2 Calendar months (no change)
Douglas C. Fredericks, BS, 0.6 Calendar months (effort ended 7/1/17)

Effort change for personnel: **Non-Surgical Treatment of Arthrofibrosis (W81XWH-14-1-0327)**
Sponsor Agency: US Department of Defense, Congressionally Directed Medical Research Program
820 Chandler Street, Fort Detrick, MD 21702
09/01/2014–08/31/2018, total direct costs
James A. Martin, PhD (PI), 1.56 Calendar months (no change)
Douglas C. Fredericks, BS, 1.2 Calendar months (until 9/1/17, then 0 effort due to animal work completed)

Effort change for personnel: **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, Total Direct Costs
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).
Previously listed with new change: **Sirakoss MaxSi TM Graft Putty Formulation Assessment in a Lapine Posterolateral Fusion Model**
Sponsor Agency: SIRAKOSS
02/01/2016–12/01/2017, Total Direct costs
02/01/2016–09/30/2016, Total Direct costs
Douglas C. Fredericks, BS (PI), 0.24 Calendar months

New Master Agreement on Previous listing: **SOW #1: HA-TCP-45S5 Bioactive Glass-collagen Composite Lapine Posterolateral Fusion Model**
**SOW #2: HA-TCP-45S5 Bioactive Glass-collagen Composite Bone Void Filler in a Rabbit Metaphyseal Defect Model**
**SOW #3: DBM-Glycerol Lapine Posterolateral Fusion Model**
**SOW #4: DBM-Glycerol Bone Void Filler in a Rabbit Metaphyseal Defect Model**
Sponsor Agency: Berkeley Advanced Biomaterials
901 Grayson, Suite 101, Berkeley, CA 94710, Tel: 510-883-0500 x12, fgenin@ostetic.com, fgenin@hydroxyapatite.com
03/01/2016–07/31/2016, Total Direct Costs
01/27/2016 – 01/26/2021 (Master Agreement), Total Direct Costs
Douglas C. Fredericks, BS (PI), 0.12 Calendar months each

Projects that closed during Year 3:
**Ended during Year 3: Sirakoss MaxSi TM Graft Granules in a Rabbit Metaphyseal Defect Model**
Sponsor Agency: SIRAKOSS
Sponsor Contact: Dr. Tom Buckland, Chief Strategy Officer, +44-7545-840504, tom.buckland@sirakoss.com
02/01/2016–09/30/2016, Total Direct costs
Douglas C. Fredericks, BS (PI), 0.12 Calendar months

**Ended during Year 3: Sirakoss MaxSi TM Graft Putty Lapine Posterolateral Fusion Model/Rabbit Metaphyseal Defect Model**
Sponsor Agency: SIRAKOSS
Sponsor Contact: Dr. Tom Buckland, Chief Strategy Officer, +44-7545-840504, tom.buckland@sirakoss.com
02/01/2016–09/30/2016,
02/01/2016–09/30/2016,
02/01/2016–06/05/2017,
Douglas C. Fredericks, BS (PI), 0.24 Calendar months

**Ended during Year 3: Evaluation of TriPore Putty in a Sheep Femoral Defect Model**
Sponsor Agency: Orthogem Limited
Sponsor Contact: Paul Markgraf, Phone 0044 115 950 5721, p.markgraf@orthogem.com
BioCity, Pennyfoot Street
Nottingham, NG1 1GF
09/01/2015 – 08/31/2016, Total Direct Costs
Douglas C. Fredericks, BS (PI), 0.12 Calendar months

**Ended during Year 3: Silhouette Lapine Posterolateral Fusion Model**
What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Please see page 15 for the Quad Chart for Year 3.

9. APPENDICES:

Nothing to Report
Intra-Articular Lubricin Gene Therapy for Post-Traumatic Arthritis

OR130365  W81XWH-14-1-0163  Year 3, 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

Multi-observer Mankin scoring for Major Task 1 was performed and the average values analyzed using the Mann-Whitney Rank-sum test. PRG4-GFP expression in synovial tissue at 2 and 8 weeks after surgery was assessed by rtPCR. Osteochondral specimens for major Task 2 were decalcified in preparation for paraffin histology.

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 Goals – Test delayed PRG4 gene therapy on cartilage degeneration at 8 weeks post ACLT
  ✓ Complete ACLT or sham surgeries, apply gene therapy after 4 weeks, euthanize rabbits at 8 weeks
  ✓ Complete drawer test immediately post-euthanasia

Comments/Challenges/Issues/Concerns

Y3 spending was slightly lower than projected, but this is due to incomplete histology at this stage. This will be completed during the NCE year.

Budget Expenditure to Date:

Projected Y3 Expenditure 8/15/16 – 8/14/17: $250,311
Actual Y3 Expenditure: $161,954.92.
Projected total Expenditure: $727,955
Total Spent through Y3: $570,138.57

Timeline and Cost

<table>
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<th>Activities</th>
<th>CY</th>
<th>8/15/14-8/14/15</th>
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Left: PRG4-GFP therapy significantly lowered Mankin scores (#p = 0.04, *p= 0.03) (n = 6). Right: PRG4-GFP Expression in AAV-injected joints was 15-fold and > 40-fold higher than in non-injected controls at 2 and 8 weeks respectively (n = 3).

Accomplishments: Final statistical analysis of Mankin scores for Major Task 1 specimens was completed. PRG4-GFP transgene expression in synovium at 8 weeks post-op was confirmed by rtPCR. Major Task 2 samples were decalcified for histology.

Updated: Y3 (11-7-17), prev. update 5-25-17 purple indicates current location in time with respect to each aim’s timeline.