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TITLE: Combating Posttraumatic Osteoarthritis via Enhanced Intra-Articular Recruitment of Stem Cells and Targeting Delivery of a Chondrogenic Agent

PRINCIPAL INVESTIGATOR: Kevin Baker, Ph.D.

RECIPIENT: William Beaumont Hospital Research Institute
Royal Oak, MI 48073

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6. AUTHOR(S)

Kevin Baker, Ph.D.

E-Mail: kevin.baker@beaumont.edu

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5e. TASK NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

William Beaumont Hospital
3601 W. 13 Mile Rd.
Royal Oak, MI 48073-6712

8. PERFORMING ORGANIZATION REPORT NUMBER

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U.S. Army Medical Research and Materiel Command
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14. ABSTRACT: The focus of this project is to utilize an in situ regenerative medicine-based approach to mitigate the onset and progression of post-traumatic osteoarthritis (PTOA) following rupture of the anterior cruciate ligament (ACL). Though a preliminary finding, our group has validated that rupture of the anterior cruciate ligament (ACL) in the knee results in a significant increase in the number of marrow-derived mesenchymal stem cell circulating in peripheral blood. We have also determined that these cells in circulation migrate preferentially to the injured knee and engraft within the synovium. This potentially validates our hypothesis that additional molecular signals (chemokines) are needed to increase the recruitment of stem cells inside the injured joint to mitigate the onset and progression of post-traumatic osteoarthritis. Work is no underway using chimeric animals with marrow cells that express green fluorescent protein. This chimeric model will be useful in assessing the efficacy of our treatment methods with respect to interrupting the acute phase biologic cascade that leads to degenerative changes to articular cartilage, as well as subchondral and epiphyseal bone

15. SUBJECT TERMS: Post-traumatic osteoarthritis; mesenchymal stem cells; stem cell mobilization; chemokines; regenerative medicine

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

Post-traumatic osteoarthritis (PTOA) is a specific variant of joint degeneration that stems from a traumatic event, such as a fracture, cartilage injury, or ligamentous disruption. Rupture of the anterior cruciate ligament (ACL) in the knee is strongly associated with the development of PTOA, despite significant advancements in ACL reconstruction surgery. We propose a method of *in situ* regenerative medicine (ISRM) whereby stem and progenitor cells from bone marrow are mobilized into peripheral blood via an FDA-approved CXCR4 antagonist (AMD3100), and recruited to the injured knee via nanoparticle-encapsulated chemokine (SDF-1β) and subsequently exposed to a chondrogenic agent (dexamethasone) conjugated to a dendrimer. We hypothesize that this ISRM method will increase recruitment of marrow-derived stem and progenitor cells to the ACL-injured knee (***Aim 1***) and will mitigate the onset and progression of PTOA after ACL injury, while improving healing after ACL reconstruction (***Aim 2***).

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

Post-traumatic osteoarthritis; ACL rupture; mesenchymal stem cells; chemokines; nanoparticle-based drug delivery; disease-modifying osteoarthritic drugs

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.

- **Local IACUC Approval**
-
- **ACURO Approval**
-
- **Aim 1:**
    - **Creation of Chimeric Rat Models**
    - **ACL Rupture Procedure and Treatments**
    - **Characterize Stem Cell Recruitment to Knee**
- **Aim 2:**
    - **Preparation of Test Substances**
    - **ACL Rupture Procedure and Treatments**
    - **Characterization of Knee Osteoarthritis**
    - **Data Analysis and Manuscript Preparation**

What was accomplished under these goals?

Major Goal 1: Local IACUC Approval

- Local IACUC approval (Protocol AL-15-11) was obtained on 10/02/15.

Major Goal 2: ACURO Approval

- ACURO approval (PR141926) was obtained on 12/18/15.

Aim 1:

- **Major Goal 3: Creation of Chimeric Rat Models**
  - Creation of chimeric rats is presently underway. A modification to the original technique for total body irradiation and subsequent marrow transplantation was necessary to both increase the success rate and kinetics of GFP+ cell transplantation, and to reduce the rate of mortality. The final procedure involves subjected rats to 10 Gy of X-irradiation using a small animal radiation research platform. Approximately two hours post-irradiation, the animals are transfused with $3x10^7$ whole bone marrow cells from a transgenic rat (Lewis background) bearing the GFP transgene. Experimentation (ACL rupture) is now underway with the GFP-chimeric Lewis rats.

- **Major Goal 4: ACL Rupture Procedure and Treatments**
  - ACL rupture procedures are underway for Aim 1 with chimeric Lewis rats with GFP+ bone marrow. All procedures are performed in accordance with Protocol AL-15-11, which was approved by the WBH IACUC on 10/02/15 and the ACURO (PR141926) on 12/18/15. Our group has accumulated a significant amount of experience with the ACL rupture procedure. Prior to initiating the present experimentation, we extensively characterized the biomechanics of the injury model (Maerz T, et al. *Biomechanical characterization of a model of noninvasive, traumatic anterior cruciate ligament injury in the rat*. Ann Biomed Eng. 2015. 43(10): 2467-76. PMID: 25777293). We subsequently characterized the onset and progression of post-traumatic osteoarthritis (PTOA) using high-resolution μCT scanning and validated histologic measures. The first study focused on comparing the remodeline of subchondral and epiphyseal bone following ACL rupture versus the more traditional surgical transection of the ligament (Maerz T, et al. *Subchondral and epiphyseal bone remodeling following surgical transection and noninvasive rupture of the anterior cruciate ligament as models of post-traumatic osteoarthritis.* Osteoarthritis Cartilage. 2016. 24(4): 698-708. PMID: 26620090). Articular cartilage changes were also characterized using contrast-enhanced μCT (EPIC-μCT) and histologic…

methods (Maerz T, et al. *Articular cartilage degeneration following anterior cruciate ligament injury: a comparison of surgical transection and noninvasive rupture as preclinical models of post-traumatic osteoarthritis*. Osteoarthritis Cartilage. 2016. Article In Press. PMID: 27349462). This preliminary work also led to the development of a μCT-based method to measure the surface properties and three-dimensional structure of articular cartilage (Maerz T, et al. *Surface roughness and thickness analysis of contrast-enhanced articular cartilage using mesh parameterization.* Osteoarthritis Cartilage. 2016. 24(2): 290-8. PMID: 26455998).

The infrastructure and workflow for characterizing the onset and progression of PTOA has been significantly advanced by our preliminary work cited above. With complete three-dimensional characterization of cartilage structure and biochemistry, the efficacy of stem cell mobilization, and the intraarticular delivery of both liposomal SDF-1β and dendrimer-conjugated dexamethasone will be examined. This infrastructure is also important with respect to providing context for the recruitment of marrow-derived stem cells to periarticular structures around the injured knee. Work is now underway with chimeric Lewis rats, which will provide important information regarding both native recruitment of marrow-derived stem cell populations, as well as the ability to actively recruit them to the injured joint.

Major Goal 5: Characterize Stem Cell Recruitment to Knee

- This goal has not yet been completed. As discussed elsewhere in the progress report, there were delays associated with production of the chimeric Lewis rats. This impacted the start date for ACL rupture procedures. Those procedures are no underway and we anticipate endpoints beginning in September ’16 for Aim 1 animals.

  While awaiting the production of chimeric Lewis rats, our group undertook experimentation (internally funded) to characterize the recruitment of intravenously-administered marrow-derived stem cells in the post-ACL injury setting. Wild-type Lewis rats underwent the ACL rupture procedure and were subsequently transfused with allogenic (litter mates) marrow-derived stem cells labeled with an optical probe (CellVue Burgundy, Licor Inc.). Live *in vivo* near-infrared imaging (NIR) was used to track the migration of the labeled cells to the injured joint. As suspected, the stem cells migrated preferentially to the injured knee, with a near 50% increase in cell concentration in the knee by 48 hours post-injury. Articular structures were then harvested post-mortem and it was demonstrated that the overwhelming bulk of cells traveled to the synovium of the injured knee, but no labeled cells could be found within the joint. This reinforces our hypothesis that additional signals (chemokines, such as SDF-1β) are necessary to recruit stem cells inside the joint to mitigate the onset and progression of degenerative changes.

Aim 2:

Major Goal 6: Preparation of Test Substances

- Prof. Kannan at Johns Hopkins has synthesized PAMAM dendrimer-dexamethasone conjugates using a four-step synthesis process as outline in previous publications by his group [ref]. Briefly, 4th generation, OH-functionalized polyamidoamine (PAMAM) were modified with reactive amine groups. The amine-functionalized dendrimers were then reacted with carboxylated dexamethasone to yield PAMAM dendrimer-dexamethasone conjugates (D-Dex). The D-Dex conjugates were characterized via 1H-NMR and MALDI-TOF-MS to verify the extent of drug loading. The purity and size of the conjugates were evaluated with HPLC/GPC and zeta-potential measurements, respectively.

- Using dehydration-rehydration reactions, our group has successfully encapsulated stromal cell-derived factor-1beta (SDF-1β) in liposomes. The liposomes, which consist of dipalmitoilphosphatidylcholine (DPPC), form multilamellar vesicles upon rehydration with an aqueous solution containing the drug to be encapsulated. Briefly, carrier-free SDF-1β is reconstituted with sterile phosphate-buffered saline to a concentration of 100 μg/mL. The solution is then passed through 0.2 μm polycarbone syringe filters 2-3 times. Lyophilized DPPC is allowed to equilibrate at25^{o}C before adding 1.0 mL of the aqueous SDF-1β. The solution is then gently inverted-reverted several times to facilitate rehydration of the entire DPPC cake. DPPC spontaneously forms liposomes, which encapsulate the SDF-1β protein with a high degree of efficiency. Because of the small volume of the rat knee, we have aimed to provide a 100 ng dose of SDF-1β in a 50 μL volume, which is then injected intra-articularly to promote stem cell homing. The encapsulation efficiency, as well as release kinetics have been characterized using fluorescently-tagged SDF-1β and a combination of flow cytometry and fluorescence spectroscopy.

Major Goal 7: ACL Rupture Procedure and Treatments

This Goal not only involves rupturing the ACL of wild-type Lewis rats using our previously-described methods, but also surgically reconstructing the ACL using an allogenic flexor tendon graft. This Goal is not yet complete, but work is underway with respect to optimizing the surgical protocol. Our Co-Investigator, Asheesh Bedi, M.D., first developed the surgical technique during his orthopaedic fellowship at the Hospital for Special Surgery. The original technique involves harvesting an autologous flexor tendon. Because we are interested in characterizing cell recruitment as a function of injury to the joint, we moved to an allogenic graft which should minimize the additional recruitment associated with surgical trauma. Numerous preliminary surgeries have been conducted to date. Experimental animals will undergo procedures beginning this September and October.

Major Goal 8: Characterization of Knee Osteoarthritis

- This Goal is not y et met, as animals will begin undergoing ACL rupture and ACL reconstruction procedures beginning in September and October ’16.

Major Goal 9: Data Analysis and Manuscript Preparation

- This Goal is not y et met, as animals will begin undergoing ACL rupture and ACL reconstruction procedures beginning in September and October ’16.

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."

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."

Nothing to Report.

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

Increased Production of Chimeric Lewis Rats: Progress on Aim 1 was slower than anticipated due primarily to unforeseen complications with the total body irradiation and bone marrow transplant procedure used to generate chimeric Lewis rats. To facilitate the execution of experimentation related to the goals of Aim 1, production of chimeric Lewis rats has been increased. We have implemented a new protocol for total body irradiation and bone marrow transplant that has increased survivability in the post-transplant period. This protocol includes total body irradiation performed on a small animal radiation research platform (SARRP, Xstrahl Inc.), which facilitates a more even delivery of X-radiation over the entire body of the animal. The X-radiation dose has also been increased from 8.5 Gy to 10 Gy to increase the ablation of the marrow cavity which will improve engraftment kinetics in the post-transplant period. The number of green fluorescent protein (GFP) positive bone marrow cells transplanted into the irradiated wild-type rats via intravenous injection was increased to 3.0×10^7. Finally, to increase survivability following irradiation and marrow transplant, the animals are now given a broad spectrum antibiotic that is effective in suppressing overgrowth of gram-negative organisms from the animals' intestinal tract. With this new protocol in protocol in place, as well as increased breeding of transgenic (GFP^+) animals by our vendor, the production of Chimeric Lewis rats has been increased. These chimeric animals are integral to our ability to assess the intra-articular recruitment of marrow-derived stem and progenitor cells to the ACL-injured knee both as a function of joint trauma and the intra-articular administration of SDF-1β, and systemic administration of AMD3100.

Validated Characterization Methods: Despite unanticipated delays in Aim 1 experimentation, our group has validated and improved the work flow of histology and micro-computed-tomography (μCT) techniques. Histology- and μCT-based techniques will be implemented to characterize the effect of stem cell mobilization, and chemokine delivery. The significant work performed prior to the onset of this project will lead to increased throughput, efficiency, and thoroughness. Our group has now performed ACL rupture and subsequent characterization of acute and chronic changes on well over 100 animals. Aim 1 work is well underway with chimeric Lewis rats.

Ligament Reconstruction Training: Dr. Bedi and our team have performed preliminary reconstruction surgeries with good results. We are in the process of training an Orthopaedic Sports Medicine Fellow (M.D. completed orthopaedic surgery residency, currently in a Sports Medicine fellowship program at William Beaumont Hospital). Having this Fellow available to assist Dr. Bedi will greatly enhance throughput associated with this surgery.

Summary: Despite initial delays associated with production of chimeric Lewis rats for Aim 1, we have greatly improved throughput for all current and future experimentation. With the added time of a one year no-cost extension, we anticipate that both Aims 1 and 2, and the final analyses will be completed. The current priority is experimentation associated with Aim 1, which we anticipate will be completed by 12/31/16. Surgical procedures for Aim 2 are scheduled to begin in September and October, with a goal of completing all experimentation and characterization by 04/01/17. Final data and statistical analyses, as well as manuscript(s) preparation will be undertaken from 04/01/17 – 07/01/17.

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.

Though not the direct goal of the funded research, the technique for generating chimeric Lewis rats may have an impact on other disciplines. The development of chimeric rats is important for disciplines that focus on the response of marrow-derived cells to trauma, surgical procedures, medical treatments, or native pathology. This new workflow for chimeric animal generation reduces animal mortality, while potentially increasing the rate of chimerism.

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."

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:

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.

Our group experienced a significant delay associated with the production of chimeric Lewis rats needed for Aim 1 experimentation. These days related initially to a reduction in the supply of transgenic (green fluorescent protein-positive) animals from a vendor. After resolving those issues, we experienced unexpectedly high mortality rate associated with the total body irradiation and bone marrow transplant procedure. We hypothesized that this mortality was related to overgrowth of gram-negative gut flora. Subsequent experimentation with a higher X-irradiation dose and the addition of a broad-spectrum antibiotic with gram-negative activity has improved the generation of these incredibly important animals. This new protocol has improved engraftment efficiency (more efficient chimeric animal generation), improved recovery, and reduced complications and mortality.

While the chimeric animal generation has been adequately addressed, our group has sought to minimize the effects of this setback on other areas of the project. Production of dendrimer-dexamethasone (PAMAM, D-Dex) conjugates and subsequent molecular characterization has been significantly ramped up to ensure timely completion of Aim 2 experimentation. Additionally, Dr. Bedi (Co-I) has worked with Dr. Baker to validate the characterization workflow, and surgical technique (ACL reconstruction) from Aim 2 experiments. Dr. Bedi has also worked directly with Dr. Baker's group to improve μCT and histologic characterization of post-traumatic osteoarthritis and the effect of stem cell-based treatments.

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.

We have altered the technique for generating chimeric Lewis rats to improve the rate of engraftment, while reducing mortality. Briefly, the total body irradiation dose was changed from 8.5 Gy to 10 Gy of X-irradiation. Additionally, this dose of radiation is not administered by a small animal radiation research platform, which ensures a uniform, full body dose of radiation in each animal. After irradiation, the rats are transfused with a larger number ($3x10^7$) of GFP+ bone marrow cells to improve engraftment while reducing morbidity. We have also incorporated the use of an antibiotic to suppress the overgrowth of gram-negative gut flora, which compromised earlier groups of chimeric animals.

These changes are part of a protocol that is approved by the local IACUC. This protocol is specifically for the generation of chimeric animals that are subsequently distributed to other protocols to investigate the contribution of bone marrow cells to healing of musculoskeletal injuries. One such protocol is AL-15-11, which is both local IACUC-approved and ACURO-approved. After successful engraftment of the GFP+ cells, the animals are transferred to AL-15-11 to be used for Aim 1 experimentation.

We anticipate that the change in the technique for chimeric animal production will greatly improve the rate at which Aim 1 experimentation is completed. No other significant changes have been made to the planned experimentation.

Changes that had a significant impact on expenditures

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

The delays associated with the generation of chimeric animals for Aim 1 experimentation significantly reduced the anticipated expenditures in Year 1 of this grant. This will not affect total expenditures for the project. Preliminary work required to improve the efficacy of irradiation/transplantation procedures was funded internally. Additionally, the development of a more thorough, and efficient workflow for the characterization of osteoarthritis was also funded by a combination of a peer-reviewed graft from the American Orthopaedic Society for Sports Medicine, as well as internal funds. All of this work will directly benefit the current and future work funded by this grant. Our group does plan on requesting a no-cost one year extension.

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

Nothing to report.

Significant changes in use or care of vertebrate animals

Changes to the total body irradiation and stem cell transplantation procedure are covered by a separate local protocol that is focused on the production of chimeric animals which are distributed to numerous protocols.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  Nothing to report.

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

  Nothing to report.

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

  Nothing to report.

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

  Nothing to report.

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

  Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: *Kevin Baker, Ph.D. (Beaumont)*
Project Role: *PI*
Researcher Identifiers: *ORCID: 0000-0001-8257-5673 Research Gate: (https://www.researchgate.net/profile/Kevin_Baker5)*
Nearest Person Month Worked: *6*
Contribution to Project: *Dr. Baker is supervising all aspects of this project. He also actively participates in the production of chimeric Lewis rats, performance of ACL rupture procedures, and characterization of joint degeneration.*
Funding Support: *Dr. Baker is supported by an internal operating budget, as well as peer-reviewed grants from the CDMRP (current), the American Orthopaedic Society for Sports Medicine, the Cervical Spine Research Society, and the American Orthopaedic Foot and Ankle Society. Dr. Baker also receives research support from major orthopaedic device manufacturers for both contract research and investigator initiated research projects. All funding flows through the William Beaumont Hospital Research Institute.*

Name: *Tristan Maerz, Ph.D. (Beaumont)*
Project Role: *Key Personnel*
Researcher Identifier: *Research Gate: (https://www.researchgate.net/profile/Tristan_Maerz)*
Nearest person month worked: *3*
Contribution to Project: *Dr. Maerz works under the direction of the PI (Baker) at the Orthopaedic Research Laboratories of Beaumont Hospital. He has worked to improved the production of chimeric Lewis rats, and has also been hands-on with ACL rupture procedures. Dr. Maerz has also spear-headed the effort to improve the throughput and efficiency of our characterization of acute and chronic joint changes following ACL injury.*
Funding Support: *Dr. Maerz is supported primarily by operating budget through Beaumont's Orthopaedic Research Laboratory. The research he is involved in is funded by both peer-reviewed research grants (Baker as PI) and industry partners.*

Name: *Mackenzie Fleischer, B.S. (Beaumont)*
Project Role: *Key Personnel*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *3*
Contribution to Project: *Mackenzie works under the direction of the PI, and in close collaboration with other key personnel, including Dr. Maerz. She has worked primarily with harvesting and processing bone marrow from transgenic animals to aid in the production of chimeric animals. Mackenzie also assists with microCT imaging, histologic processing, and microscopy.*
Funding Support: *Mackenzie is funded by the Orthopaedic Research Laboratory's operating budget. Her time is also built into several peer-reviewed and industry grants.*

Name: *Asheesh Bedi, M.D. (University of Michigan)*
Project Role: *Co-Investigator*
Researcher Identifier: *ORCID: 0000-0001-8926-7139; ResearchGate: (www.researchgate.net/profile/Asheesh_Bedi)*
Nearest person month worked: *4*
Contribution to Project: *Dr. Bedi has worked with Dr. Baker to oversee production of chimeric animals and has participated in the characterization of acute injury following our ACL rupture procedures. Dr. Bedi is currently optimizing the ACL reconstruction surgical technique to be used in Aim 2. He is also training a sports medicine fellow to assist him with the ACL reconstruction procedures.*
Funding Support: *Dr. Bedi has funding from numerous federal, private and industrial entities, including the NIH, AOSSM, Smith & Nephew, and Arthrex.*

Name: *Rangaramanumjam M. Kannan, Ph.D.*
Project Role: *Key Personnel - Subcontractor*
Researcher Identifier: Research website:
http://www.hopkinsmedicine.org/profiles/results/directory/profile/1184619/kannan-*rangaramanujam*
Nearest person month worked: *4*
Contribution to Project: *Prof. Kannan has overseen the production of denrimer-dexamethasone conjugates that are to be used in Aim 2 to stimulate chondrogenic differentiation of recruited stem cells. In addition to synthesis, Prof. Kannan's lab has utilized a multi-modal approach to characterize the drug loading rate, size, and purity of the conjugates. He will further assist with Aim 2 work to determine if the conjugates are being actively uptaken by chondrocytes and other cells within the joint.*
Funding Support: *Prof. Kannan is Co-Director for the Center for Nanomedicine at the Wilmer Eye Institute of Johns Hopkins Medical Institute. He is funded by the operating budget of that facility, as well as grants from the NIH and DoD, as well as industry partners such as AstraZeneca.*

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)*

William Beaumont Hospital Research Institute: ***Kevin Baker, Ph.D. (PI)***

- Royal Oak, MI USA
- Principal Investigator; Animal procedures; Advanced Imaging; Histology

University of Michigan: ***Asheesh Bedi, M.D. (Co-I)***

- Ann Arbor, MI USA
- Co-Investigator; Animal procedures (at Beaumont); histology grading; microCT grading

Johns Hopkins University: ***Rangaramanujam M. Kannan, Ph.D. (Consultant)***

- Baltimore, MD USA
- Consultant; Manufacture and characterization of PAMAM-Dexamethasone conjugates

Imperial College London: ***Sara Rankin, Ph.D. (Unpaid Consultant)***

- London, England
- Unpaid Consultant; Review of flow cytometry and stem cell homing data

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://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

8. **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.