AWARD NUMBER: W81XWH-21-1-0385

TITLE: Accelerated Healing of Traumatic Fractures and Nonunion

PRINCIPAL INVESTIGATOR: Dr. Steven Teitelbaum

CONTRACTING ORGANIZATION: Washington University, St. Louis, MO

REPORT DATE: July 2022

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| July 2022 | Annual | 01Jun2021-31May2022 |

**4. TITLE AND SUBTITLE**

Accelerated Healing of Traumatic Fractures and Nonunion

**5a. CONTRACT NUMBER**
W81XWH-21-1-0385

**5b. GRANT NUMBER**
n/a

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
Dr. Steven Teitelbaum and Dr. Matthew Silva

E-Mail: teitelbs@wustl.edu; silvam@wustl.edu

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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

Washington University
One Brookings Drive
Saint Louis, MO 63130-4862

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

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

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

Our goals are to determine the mechanisms for rapid bone formation observed in DT/DTR$^{ADQ}$ mice wherein adiponectin expressing cells are ablated, and to harness this knowledge to test new strategies for promoting osteogenesis to treat fracture nonunion. Contrary to our initial hypothesis, we find that osteoblasts stimulated by DT/DTR$^{ADQ}$ are not derived from adiponectin-lineage cells. Rather, adiponectin-expressing CAR cells in the bone marrow express BMP receptor inhibitors, and ablation of these cells in DT/DTR$^{ADQ}$ promotes BMP signaling and osteogenesis by activating Col1a1-3.6 kB expressing osteoprogenitors lining the marrow cavity, which then differentiate into non-proliferative, mature Col1a1-2.3 kB osteoblasts. We are testing whether delivery of these primed DT/DTR$^{ADQ}$ bone cells via a polymer scaffold accelerates segmental defect non-union healing in WT mice. Relevant to the identification of 3.6Col1a1 cells as essential to DT/DTR$^{ADQ}$ intramedullary osteogenesis, we find that the proliferation of these cells is also essential to periosteal callus formation after fracture. Single-cell RNAseq analysis of periosteal callus from healing and non-healing bones has been completed, and examination of the 3.6Col1a1 expressing cells is ongoing with the goal of determining which subpopulation of osteoprogenitors is responsible for callus formation. Finally, we have completed a study showing that driving BMP2 expression in cells delivered in a polymer scaffold can enhance osteogenesis in non-healing fractures.

**15. SUBJECT TERMS**
non-union, BMPR, diphtheria toxin activated adiponectin Cre targeted diphtheria toxin receptor (DT/ DTR$^{ADQ}$), bone formation

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRDC |
| Unclassified | Unclassified | Unclassified | Unclassified | 21 | 19b. TELEPHONE NUMBER *(include area code)* |

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**1. INTRODUCTION:** Traumatic fractures, as experienced by our combat military, not infrequently, promote nonunion. We developed a murine model exhibiting the most rapid and profound systemic increase in bone mass yet observed which holds promise to provide effective strategies to promote non-union healing. Generation of this model entailed mating mice expressing the primate diphtheria toxin receptor (DTR), to those bearing adiponectin (ADQ) Cre (ADQ) ($DTR^{ADQ}$ mice). This robust bone formation, which reflects markedly enhanced osteoblast activity, is accompanied by induction of uniquely vigorous osteogenic precursor cells. $DTR^{ADQ}$ induction of osteogenesis is caused by bone morphogenetic protein receptor (BMPR) activation likely due to skeletal depletion of its inhibitors gremlin1 (GREM1) and chordin like 1 (CHRDL1). In addition, our data indicate that the poorly defined cells responsible for fracture repair are characterized by expression of the 3.6 kB Col1a promoter. If the mechanistic data in hand are supported by the proposed experiments, there is a reasonable possibility it will eventuate in development of a potent osteogenic drug(s), based upon combined suppression of GREM1 and CHRDL1 and extremely robust osteoprogenitor cells to be transplanted into nonunions. The potent osteogenic drug and osteoprogenitors alone, and particularly in combination, may rapidly promote nonunion healing. Thus, the overarching goal of this proposal is to confirm our mechanistic observations and assess their potential to promote nonunion repair.

**2. KEYWORDS:** non-union, BMPR, diphtheria toxin activated adiponectin Cre targeted diphtheria toxin receptor (DT/ $DTR^{ADQ}$), bone formation.

**3. ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Aim 1: Determine the mechanism of $DTR^{ADQ}$ bone formation. Aim 2: Determine the effect of $DTR^{ADQ}$ activation on defect non-union healing. Aim 3A: Characterize the periosteal osteoprogenitor cells in fracture callus and their deficit in atrophic nonunion. Aim 3B: Test the potential of 1) implanted DTRADQ MSCs and 2) local delivery of factors to rescue atrophic nonunion.

- **What was accomplished under these goals?**

Major task 1 of Specific Aim 1 states "Characterize the osteoblast precursors modified by $DTR^{ADQ}$ activation."
**Teitelbaum lab:** We have established, using thymidine kinase and GFP reporter mice, respectively, that the targeted osteoblast progenitor expresses Col1a driven by its 3.6kB promoter which, in turn differentiates into the non-proliferative mature osteoblast in which Col1a is induced by its 2.3kB promoter (Fig. 1). We have also confirmed that despite evidence of being mature osteoblasts, Col1a 2.3kB promoter-driven osteoblastic cells are not precursors to form new bone in DT/ $DTR^{ADQ}$ mice (Fig 2). Surprisingly, the same holds true regarding classical marrow residing MSCs. We found DT administration decreases MSC CFU-fibroblast formation and osteoblast differentiation in $DTR^{ADQ}$ mice (Fig 3). Also contrary to other circumstances, with lineage tracing analysis we found the osteoblasts stimulated by DT/$DTR^{ADQ}$ are not derived from adiponectin positive lineage cells, which is consistent with our finding that marrow residing MSCs are not osteogenic precursors in DT/$DTR^{ADQ}$ mice. (Fig 4). Alternatively, DT administration to $DTR^{ADQ}$ mice increases osteoblast

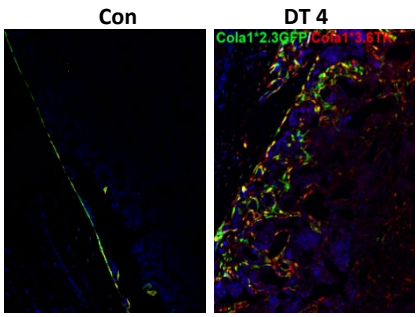


**Fig 1**. Thymidine Kinase (TK) fluorescent immunohistochemical staining (red) of femoral diaphysis of $DTR^{ADQ}$/Col1a1*2.3-GFP/Col1a1*3.6-TK mice treated with PBS (con) or DT for 4 days.

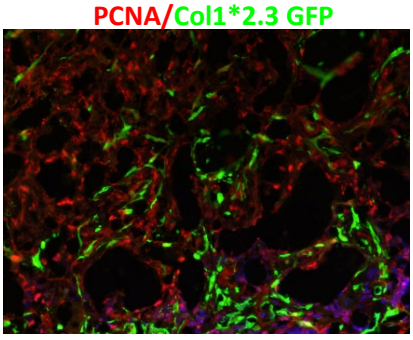


**Fig 2**. PCNA (red) fluorescent immunohistochemical staining of femoral diaphysis of $DTR^{ADQ}$/Col1a1*2.3 GFP mice treated with DT for 4 days. Arrows indicate occasional cells (yellow) expressing both PCNA and GFP.

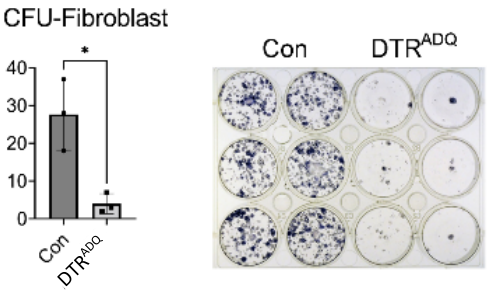


**Fig 3.** Con and $DTR^{ADQ}$ mice were treated with DT for 2 days. Equal number of marrow cells were cultured for Colony Forming Units-Fibroblast (Left) and CFU-OB (Right).

differentiation from collagenase digested whole bone cells (Fig 5), suggesting osteoblast precursors, in this circumstance, locate at the bone surface. Col1a*3.6-TK+ cells are in proliferating stage after DT treatment (Fig 6), indicating their potential as osteogenic progenitors in DTR$^{ADQ}$ mice. Thus, our data now indicate that cellular events promoting DTR$^{ADQ}$ osteogenesis involves BMP mediated activation of early committed Col1a3.6 kB progenitors which differentiate into non-proliferative, mature Col1a 2.3 kB osteoblasts. These observations have led us to obtain Col1a3.6 kB-GFPtpz reporter mice that will enable us to directly confirm our hypothesis and identify the responsible subset by scRNA-seq.

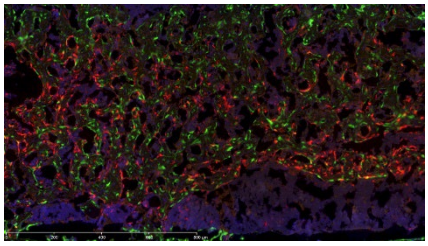

**Fig 4.** DTR$^{ADQ}$/Cola1*2.3GFP/TdTomato mice were treated with DT for 7 days. Representative image of femur diaphysis. Note that 2.3GFP+ osteoblasts are not overlapped with TdTomato+ cells (adiponectin positive derived cells).

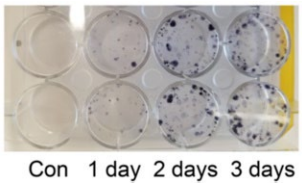


**Fig 5.** CFU-OBs generated from collagenase digested whole femur of DTR$^{ADQ}$ mice treated with DT with time. No DT treatment serves as Con.

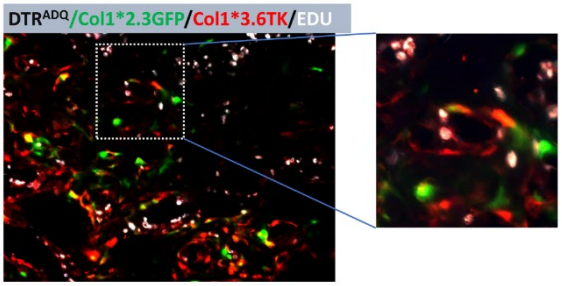


**Fig 6.** Thymidine Kinase (TK) (Red) and EDU (white) fluorescent immunohistochemical staining of femoral diaphysis of DTR$^{ADQ}$/Col1a1*2.3-GFP/Col1a1*3.6-TK mice treated with PBS (con) or DT for 4 days. EDU were injected into mice 6 hours before sacrifice to label proliferating cells.

<u>Major task 2 of Specific Aim 1 states "Explore the role of the BMPR inhibitors CHRDL1 and GREM1 in DT/DTR$^{ADQ}$ bone formation"</u>. **Teitelbaum lab:** Our efforts focused on identifying the bone residing cells responsible for producing CHRDL1 and GREM1. We found that DTR$^{ADQ}$ activation immediately and completely eliminates marrow Cxcl12 and LepR (Fig 7). Because these molecules are the principal markers of CAR cells, we hypothesized they may be the key producers of the BMPR inhibitors. Using reporter mice, we established that CAR cells universally express ADQ (Fig 8) and are depleted by DT/DTR$^{ADQ}$ (Fig 9). To confirm they are the source of CHRDL1 and GREM1, we isolated stromal cells by FACS from CAR reporter mice and find the GFP+, but not the GFP- population express the inhibitors (Fig 10). Thus, CAR cells reduce bone formation by expressing BMPR inhibitors and present as a therapeutic target.

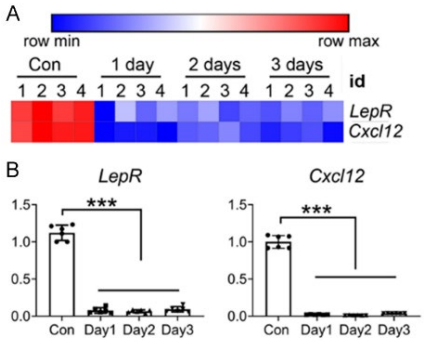


**Fig 7. A)** Heatmap of RNA sequencing of bone marrow mRNAs of DTR$^{ADQ}$ mice treated with or without (con) DT with time. **B)** qPCR analysis of LepR and Cxcl12 mRNA in femoral bone marrow of 2 months old DTR$^{ADQ}$ mice treated with DT with time.

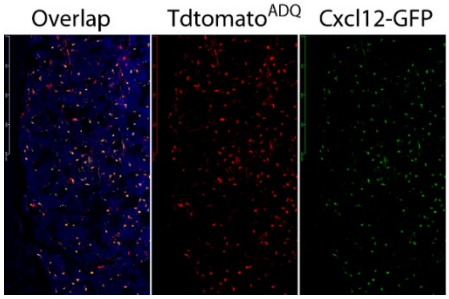


**Fig 8**. Representative image of 2 months old Cxcl12-GFP/TdTomato$^{ADQ}$ femur diaphysis. Note that all Cxcl12GFP+ cells are overlapped with ADQ-TdTomato+ cells.

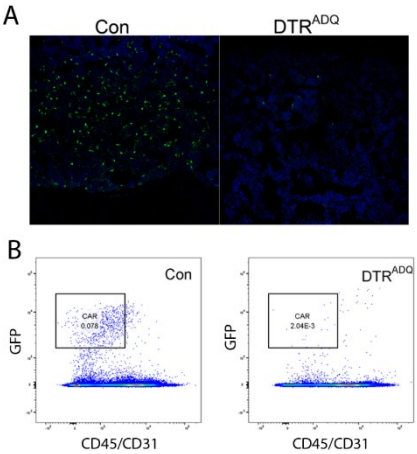


**Fig 9**. 2 months old Cxcl12-GFP (Con) or DTR$^{ADQ}$/Cxcl12-GFP mice were treated with DT for 2 days. **A)** Representative image of femur diaphysis; **B)** Marrow Cxcl12-GFP+ MSCs were quantitated by FACS.

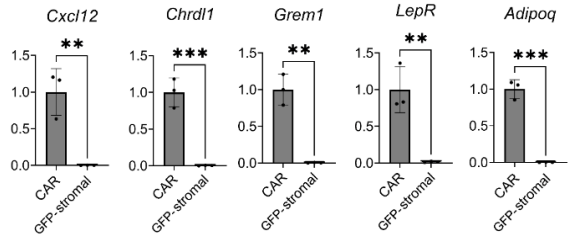


**Fig 10**. Marrow GFP+ MSCs and GFP- stromal cells from 2 months old Cxcl12-GFP mice were sorted by FACS. Expression of Cxcl12, Chrdl1, Grem1, LepR and Adipoq genes were analyzed by qPCR.

Major task 2 of Specific Aim 2 states "Explore cellular induction of non-union defect healing". **Teitelbaum and Silva labs:** To this end, we first created a 3.5 mm segmental defect in DTR$^{ADQ}$ or WT femurs and administered DT daily. After 10 weeks, x-ray and µCT revealed enhanced bone formation in the non-unions of DTR$^{ADQ}$ mice relative to control (Fig 11).This observation suggested osteoprogenitors of DT/DTR$^{ADQ}$ mice may be more effective at inducing bone formation and thus healing when implanted in segmental non-unions. We therefore implanted hydrogels containing equal numbers of cells isolated from collagenase digested whole bone of WT or DT/DTR$^{ADQ}$ mice subcutaneously into WT mice. After 14 weeks, there was no evidence of bone formation in implants containing WT cells whereas it was extensive in those containing cells derived from DT/DTR$^{ADQ}$ mice (Fig 12). These observations provide a foundation for determining if hydrogel containing DT/DTR$^{ADQ}$ bone cells accelerate segmental non-union healing in WT mice.

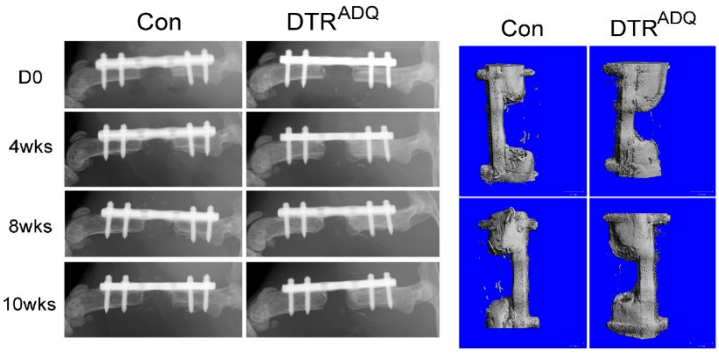


**Fig 11**. A 3.5 mm segmental defect were created in DTR$^{ADQ}$ or WT femurs and then administered DT daily. X-ray images at different time after surgery (left); µCT images 10 weeks after surgery (right).

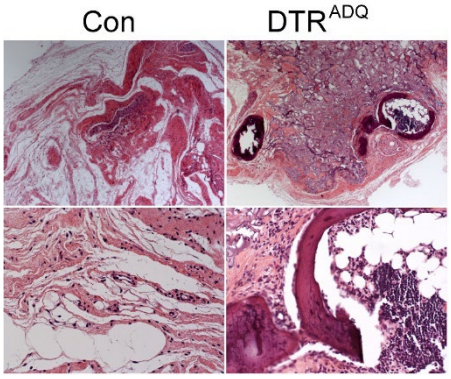


**Fig 12**. Hydrogels containing equal numbers of cells isolated from collagenase digested whole bone of WT or DT/DTR$^{ADQ}$ mice 3 days after DT administration were subcutaneously implanted into WT mice. After 14 weeks, the implants were stained with HE staining.

Major task 1 of Specific Aim 3A states "Perform scRNA-seq on fracture callus from 3.6Col1-TK mice." **Silva lab:** We recently used 3.6Col1a1-TK mice to show that proliferation of cells expressing Col1a1 (driven by the 3.6kB promoter) are essential for periosteal callus formation and healing of a mid-diaphyseal femur fracture (Hixon et al., 2021, https://doi.org/10.1002/jbmr.4424). Thus, we reasoned that by comparing 3.6Col1-TK mice treated with water (control) to 3.6Col1-TK mice treated with ganciclovir (nonunion), we could elucidate differences between healing and nonunion callus, and identify cell populations that are absent in the latter. Mid-shaft femur fractures were created in 12-wk old 3.6Col1-TK mice, followed by treatment for 5 or 10 days with either water (control) or ganciclovir (nonunion). Callus tissues (n=3/timepoint/treatment) were collected post mortem and single-cell isolates prepared, followed by RNAseq (NovaSeq S4) using the 10x Chromium 3' single-cell platform. A total of 104,000 cells were sequenced (8700 cells per sample). After quality control, cluster analyses were performed accounting for treatment (Control, GCV) and time (5, 10 days), along with cell cycle analysis (Seurat). Twenty clusters were identified in Control samples, including a closely related set of 7 clusters of mesenchymal/skeletal cells (Fig 13). Day 5 callus cells are predominantly undifferentiated, matrix (ECM)-expressing cells; a small number of cells in clusters 0 and 1 express multiple markers of skeletal stem cells (*Pdgfra, Acta2, Ctsk, Cxcl12, Cd200*). Callus cells on Day 10 are more differentiated, with most of the mesenchymal/skeletal cells comprising chondrocytes, hypertrophic chondrocytes, and osteoblasts. Proliferating cells are abundant in clusters 0, 3 and 5, especially at Day 5. Notably, a subset of cells highly express *Sp7* (Osterix) and are replicating (S-phase) at days 5 (clusters 0, 5, 7) and 10 (clusters 2, 7, 8, 9), indicating proliferation of (pre)osteoblasts. An even smaller subset of cells express *Bglap* (Ocn) and are replicating at days 5 and 10, indicating proliferation of mature osteoblasts. Comparing GCV (non-healer) vs. Control (healer) groups reveals depletion of hypertrophic chondrocytes and early osteoblasts in the GCV group at day 5 (Fig. 14), and depletion of mature osteoblasts and hypertrophic chondrocytes at day 10, consistent with the eventual outcome of a poorly mineralized callus. Analysis of this data is ongoing but is expected to yield major insights into the cellular composition of early fracture callus and how it is altered in a nonunion condition. We further anticipate identifying a rare subpopulation of 3.6Col1a1 expressing cells that will contain putative skeletal stem cells that are necessary for fracture healing. These analyses will be completed, along with validation, in Year 2.

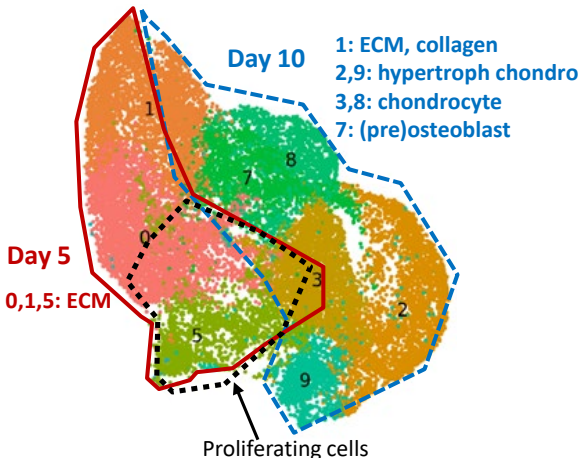


**Fig 13**. Mesenchymal cell clusters from scRNA-seq of Control callus 5 and 10 days after fracture in Col1-TK mice. From days 5 to 10, cells become more differentiated and the number of proliferating cells declines.

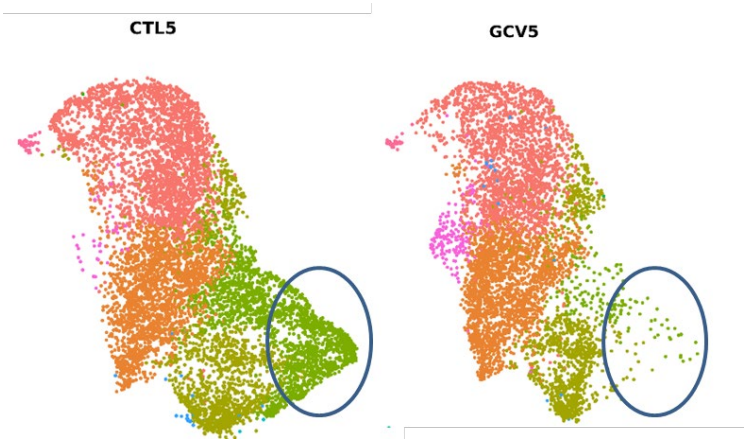


**Fig 14**. Mesenchymal cell clusters from scRNA-seq of Control and GCV groups at Day 5. The GCV callus shows depletion of chondrocytes and early osteoblasts in cluster 5 (circles), consistent with impaired callus formation.

To complement the scRNA-seq experiment, we worked to elucidate the critical time windows for osteoblast lineage cell proliferation. Our published work established that inhibiting proliferation of 3.6Col1a1-expressing cells for 2 weeks after fracture results in persistent nonunion (Hixon et al., 2021). Here, we hypothesized that shorter durations of inhibition delays but does not prevent healing. Mid-shaft femur fractures were created in 12-wk old WT (wildtype/TK$^-$) and 3.6Col1-TK (TK$^+$) mice, followed by treatment for 3, 7 or 14 days with ganciclovir (GCV) to block osteoblast proliferation in TK+ mice (Fig. 15). Healing was evaluated 5, 10 and 21 days after fracture. Three days of GCV treatment delayed callus formation in TK+ mice compared to WT mice, but by day 21 the callus appeared normal and was not different between groups (Fig. 15A,B). Seven days of GCV treatment blocked the early formation of cartilage and bone callus in TK+ mice, and at day 21 the callus was comprised of mostly cartilage, in contrast to the bony callus in WT mice (Fig. 15C,D), indicating delayed healing in TK+ mice. As expected, 14 days of GCV treatment blunted normal callus formation, leading to a small, fibrous callus at day 21 (Fig. 15 E,F). These findings indicate that inhibiting proliferation of 3.6Col1a1-expressing cells for as little as 3 days disrupts fracture healing, although healing may recover if the block on proliferation is less than 14 days.

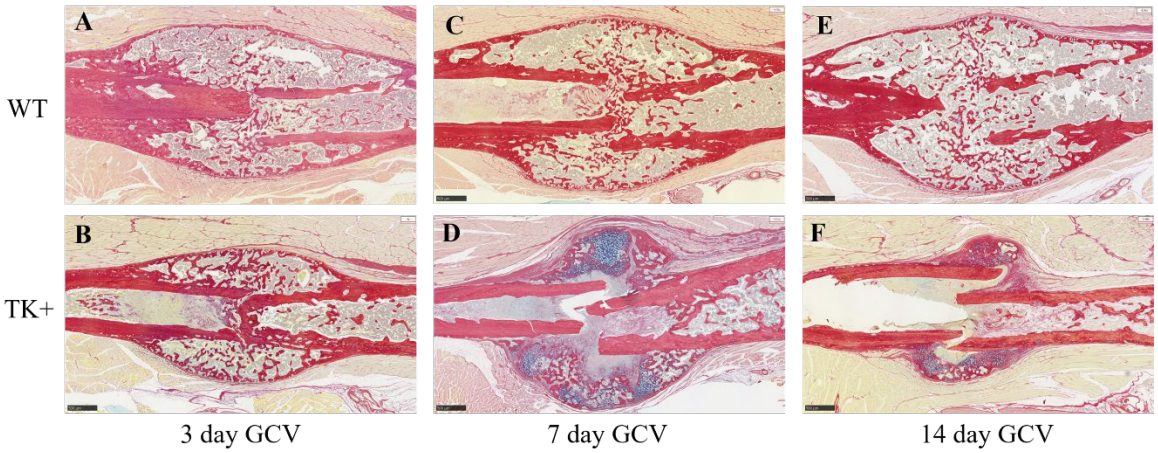


**Fig 15**. Histological sections at day 21 after fracture in mice treated with GCV for 3, 7 and 14 days show that healing is progressively impaired in TK+ mice with increased GCV duration. (A,C,E) Normal healing in WT mice results in a fully bridged bone callus. (B,D,F) Callus composition is normal in TK+ mice treated with GCV for only 3 days, but is impaired with longer duration of GCV treatment.

Major task 1 of Specific Aim 3B states "Test potential of implanted $DTR^{ADQ}$ MSCs to rescue atrophic nonunion in Col1-tk mice." **Silva lab:** As discussed above (*Major task 2 of Specific Aim 1*) the induction of robust bone formation in the $DTR^{ADQ}$ model is related to loss of BMP inhibitors leading to activation of BMP signaling. The ligand BMP2 is a known, potent osteoinductive factor. For our initial study to rescue atrophic nonunion in Col1-TK mice, we opted for a BMP2-based tissue engineering strategy with a cryogel delivery scaffold. We hypothesized that the scaffold-mediated lentiviral delivery of doxycycline-inducible a BMP-2 transgene would induce osteogenesis at the fracture site. Cryogel scaffolds were biofabricated through the cross-linking of a chitosan–gelatin polymer solution at subzero temperatures, which results in a macroporous, spongy structure that may be advantageous for a bone regeneration application. Murine adipose-derived stem cells were seeded onto the cryogel scaffolds, where they underwent lentiviral transduction. Following the establishment of atrophic non-unions in the femurs of Col1-tk mice (4 weeks post-fracture), transduced, seeded scaffolds were surgically placed around the site of non-union, and the animals were given doxycycline water to induce BMP-2 production. Controls included GFP+ cells on the cryogel scaffolds, acellular scaffolds, and sham (no scaffold). Weekly radiographs were taken, and endpoint analysis included micro-CT and histological staining. After 2 weeks of implantation, the BMP-2+ scaffolds were infiltrated with cartilage and woven bone at the non-union site, while GFP+ scaffolds had woven bone formation. After 8 weeks, there was woven bone and vessel formation within the BMP-2+ and GFP + scaffolds with cortical bridging of the original fracture site in both groups. Overall, the cell-seeded cryogels promoted osseous healing (Fig. 16). However, while the addition of BMP-2 promoted the endochondral ossification, it may provide a slower route to healing. This proof-of-concept study demonstrates the potential for cellularized cryogel scaffolds to enhance the healing of non-unions and indicates that activation of BMP signaling can promote periosteal endochondral healing in the setting of an established nonunion.

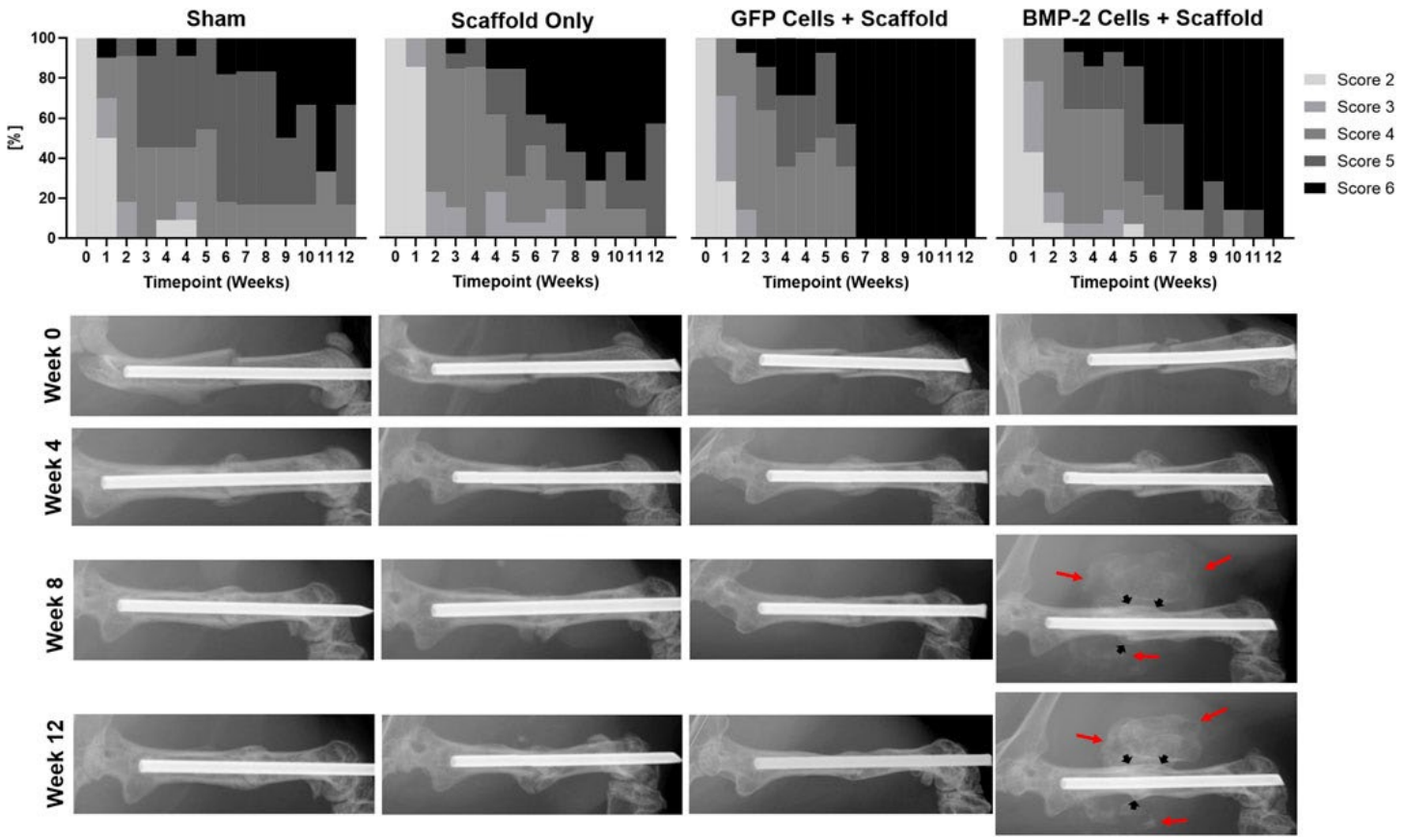


**Fig 16**. Radiographic scoring of atrophic non-unions treated with sham (no scaffold or cells) control, cryogel (acellular) scaffold only, scaffolds seeded with GFP+ cells, and scaffolds seeded with cells expressing BMP-2. Due to the treatment with GCV and insufficient rescue, less than 50% of the Col1-tk scaffold only or sham groups had complete bridging at 12 weeks. Comparatively, the GFP+ and BMP-2+ cells were the only groups to achieve full bridging by week 12, with GFP+ cells displaying a score of 6 by week 6. Scoring of the radiographs demonstrated no significant difference between any groups at 6 weeks. However, there were significant differences between both the sham and scaffold groups as compared to both the GFP and BMP-2 groups at 12 weeks ($\chi^2$, $p < 0.05$). Representative radiographs are shown from the time of fracture (Week 0) and at 4, 8, and 12 weeks. The BMP-2+ cellularized scaffolds possessed visually mineralized scaffolds surrounding the non-union site beginning 1–3 weeks following implantation (red arrows) in addition to the endogenous fracture callus (black arrows).

• **What opportunities for training and professional development has the project provided?** The past year provided training opportunities for both senior faculty and a post-doctoral fellow. Professor Wei Zou, who supervises the segmental nonunion study as well as Dr. Aersilan Alimasi were trained by members of Dr. Silva's group namely Dr. Anna Miller and Dr. Katherine Hixon who provided instruction on generation of segmental non-unions in mice and appropriate hydrogels to serve as scaffolding for $DTR^{ADQ}$ cells. Combined meetings of the Silva and Teitelbaum labs provided cross-training for their respective lab members. Two PhD candidates, Mr. Andre Coello (Biomedical Engineering) and Ms. Leyi Chen (Mechanical Engineering & Materials Science), assisted in these studies and learned techniques for femur fracture, Col1-TK husbandry, and cryogel fabrication from senior lab members (Drs. McKenzie and Hixon).

• **How were results disseminated to the communities of interest?** The investigators have been invited by a number of organizations to present the data. Specifically, Dr. Wei Zou was an invited speaker at the Avioli Bone and Mineral Conference and the Washington University Musculoskeletal Biology and Regeneration Meeting (May 2022). Drs. Teitelbaum and Silva are invited speakers at the 2022 Bones and Teeth Gordon Research Conference (Sept.). Dr. Silva et al. submitted an abstract to the 2022 International Society for Fracture Repair meeting, Mr. Coello et al. submitted an abstract to the 2022 American Society for Bone and Mineral Research Meeting. We published a paper on the cryogel scaffold-mediated BMP2 delivery to promote nonunion healing (Hixon et al., 2022).

• **What do you plan to do during the next reporting period to accomplish your goals?** During the next period we will focus on determining if hydrogels containing $DTR^{ADQ}$ bone cells are more efficient at healing segmental non-unions than are those containing WT cells. These studies will require further characterization of the BMP target cells responsible for the induced osteosclerosis and will likely involve scRNA-seq analysis of cells derived from Col1a3.6 kB-GFP reporter mice. They will require further optimization of methods for cryogel scaffold fabrication and cell seeding for application to segmental defect and atrophic non-union fracture healing. We will also initiate studies determining the effects of genetic deletion of GREM1 and CHRDL1 on bone formation. We will complete the analysis of scRNA-seq data from periosteal fracture callus from control and 3.6Col1-TK non-healer mice.

**4. IMPACT:**

• **What was the impact on the development of the principal discipline(s) of the project?** Our studies to date fortify the concept that osteogenic precursor cells derived from $DTR^{ADQ}$ mice will accelerate delayed fracture healing. They also establish the mechanism by which the BMPR inhibitors are eliminated upon adiponectin-Cre mediated DTR targeting of bone cells.

• **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.** Nothing to report.

• **Changes that had a significant impact on expenditures.** Nothing to report.

• **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.** Nothing to report.

**6. PRODUCTS;**

• Dr. Wei Zou Has delivered an invited lecture at the Avioli Musculoskeletal Research Conference and the 2022 Musculoskeletal Biology and Regeneration Meeting. Dr. Steven Teitelbaum was a keynote speaker at the 2021 Korean Bone and Mineral Society Meeting. Drs. Teitelbaum and Silva are invited speakers at the 2022 Gordon Bone and Tooth Conference. All presentations focus on the current project.

• Dr. Silva et al. submitted an abstract to the 2022 International Society for Fracture Repair meeting, Mr. Coello et al. submitted an abstract to the 2022 American Society for Bone and Mineral Research Meeting.

• We published a paper on the use of cryogel scaffold-mediated BMP2 delivery to promote nonunion healing: Hixon KR, Katz DB, McKenzie JA, Miller AN, Guilak F, **Silva MJ**. *Cryogel Scaffold-Mediated Delivery of Adipose-Derived Stem Cells Promotes Healing in Murine Model of Atrophic Non-Union*. Front Bioeng Biotechnol. 2022;10:851904. PMCID: PMC9117654.

**Website or other internet sites**.

Abstract for Dr. Wei Zou's 2022 Musculoskeletal Biology and Regeneration Meeting:
https://sites.wustl.edu/mrccrm2022meeting/files/2022/05/MRMB_Meeting_2022_Book_FINAL.pdf

1. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Dr. Steven Teitelbaum lab.

| | |
|---|---|
| Name: | Steven Teitelbaum, MD |
| Project Role: | Principal Investigator |
| Researcher Identifier (e.g. ORCID ID): | 0000-0002-4054-6679 |
| Nearest person month worked: | 2 |
| Contribution to Project: | Dr. Teitelbaum is responsible for all programmatic and executive aspects of the project related to Specific Aims 1 and 2. As head of the principal laboratory, he oversees the project and assures its progress. He also directly supervises aspects of the project related to determining the mechanisms of $DTR^{ADQ}$ induced osteogenesis |
| Funding Support: | |
| | |
| Name: | Wei Zou, MD, PhD |
| Project Role: | Co-Investigator |
| Researcher Identifier (e.g. ORCID ID): | 0000-0001-8081-268X |
| Nearest person month worked: | 5 |
| Contribution to Project: | Dr. Zou is responsible for all experiments related to defect nonunion. |
| Funding Support: | |
| | |
| Name: | Aersilan Alimasi, PhD |
| Project Role: | Postdoctoral Research Associate |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 5 |
| Contribution to Project: | Dr. Alimasi maintains the relevant mouse colonies (including genotyping), and performing microCT scanning and histomorphometry. |
| Funding Support: | |

Dr. Matthew Silva lab.

| | |
|---|---|
| Name: | Matthew Silva, PhD |
| Project Role: | Principal Investigator |
| Researcher Identifier (e.g. ORCID ID): | 0000-0001-7375-4522 |
| Nearest person month worked: | 2 |
| Contribution to Project: | Dr. Silva partners with Dr. Teitelbaum to direct the overall project. Dr. Silva directs the work in Specific Aim 3 as well as oversee multiple aspects of Aims 1 and 2, including cryogel fabrication and microCT imaging. He shares responsibility with Dr. Teitelbaum for study design and management, results interpretation, progress reporting and authoring abstracts and manuscripts. He supervises the work of the Staff Scientist, Dr. McKenzie, and the Bioinformatics Scientist, Dr Tiandao Li. |
| Funding Support: | |
| | |
| Name: | Jenny McKenzie, PhD |
| Project Role: | Staff Scientist |
| Researcher Identifier (e.g. ORCID ID): | 0000-0002-5723-4057 |
| Nearest person month worked: | 4 |
| Contribution to Project: | Dr. McKenzie has worked on Aim 3 of the project. She was responsible for mouse colony management. She conducted the single-cell RNAseq specimen preparation and coordinated with the WU sequencing center (GTAC) to execute the work. She has performed initial analysis of scRNAseq data. |
| Funding Support: | |
| | |
| Name: | Tiandao Li, PhD |
| Project Role: | Bioinformatics Scientist |
| Researcher Identifier (e.g. ORCID ID): | 0000-0003-1650-0555 |
| Nearest person month worked: | 2 |
| Contribution to Project: | Dr. Li was recruited to join the project to provide bioinformatics analysis of the scRNAseq data generated in Aim 3A. He is a Bioinformatics Scientist working in the Center for Regenerative Medicine, Bioinformatics Core. |
| Funding Support: | |

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

Dr. Teitelbaum has had a new R01 funded along with 4 Foundation for Barnes Jewish Hospital grants. All changes
are marked with “New” at the top of the entry.

Dr. Zou – nothing to report.

Dr. Silva has had a new NIH R21 funded (PI) and a new NIH R01 funded (Co-I). All changes are marked with “New” at the top of the entry.

- **What other organizations were involved as partners?** Nothing to report.

**Previous/Current/Pending Support for Steven Teitelbaum**

**Previous (Past 5 years)**

**Title of the project**: Mechanisms Of αvβ3 Integrin Mediated Bone Resorption
**Funding agency:** National Institutes of Health
**Goals of the project:** The major goals of this project are to determine 1) the mechanisms by which M-CSF, interacting with its receptor c-Fms, transmits intracellular signals which activate the αγβ3 integrin in OCs; 2) the role of talin and its recognition sequences in the β3 integrin cytoplasmic domain in mediating M-CSF-induced OC cytoskeletal organization and function, and 3) the impact of inhibiting αγβ3 activation, in vivo, on pathological bone resorption
**Specific aims/tasks:**
1. Determine the mechanism by which M-CSF, interacting with its receptor, c-Fms, transmits intracellular signals which activate the αvβ3 integrin in osteoclasts.
2. Determine the role of talin and its recognition sequence in the β3 cytoplasmic domain in mediating M-CSF-induced osteoclast cytoskeletal organization and function.
3. Determine the impact of inhibiting αvβ3activation, in vivo, on pathological bone resorption.
**Estimated start and end dates (month/day/year - month/day/year):** 2/1/2000-1/31/2021
**Level of effort (%) in the project:** 1% (0.12 person months)
**Total Award Amount**:
**Point of contact at the funding agency:** Steve Austin; 301-594-3504; steve.austin@nih.gov

**Title of the project:** Regulating TNF family activity and receptor oligmerization to treat skeletal diseases
**Funding agency:** Shriner's Hospitals for Children
**Goals of the project:** Our goals are to assess the efficacy of a single chain TNF trimer which selectively inhibits TNFR1 receptor1 while sparing TFNR2 in preventing inflammatory osteolysis and to determine the capacity of gain of function RANKL to reverse Bis-P-suppressed bone remodeling.
**Specific aims/tasks:**
1. assess the efficacy of a single chain TNF trimer which selectively inhibits TNFR1 receptor1 while sparing TFNR2 in preventing inflammatory osteolysis and
2. determine the capacity of gain of function RANKL to reverse Bis-P-suppressed bone remodeling.
**Estimated start and end dates (month/day/year - month/day/year):** 1/1/15-12/31/19 **Level of effort (%) in the project:** 10% (1.2 person months)
**Total Award Amount:**
**Point of contact at the funding agency:** Amy Reeves; areeves@shrinenet.org; (314) 872-8342 ext1022

**Title of the project:** Fatty Liver Promotes Hepatic Breast Cancer Metastasis
**Funding agency:** Siteman Cancer Center
**Goals of the project:** Hepatic metastasis is a major contributor to breast cancer-associated death and compromises chemotherapeutic efficacy. Thus, understanding the factors which promote liver metastasis would profoundly impact the prognosis of this common malignancy.
**Specific aims/tasks:**

1.Hepatic metastasis is a major contributor to breast cancer-associated death and compromises chemotherapeutic efficacy. Thus, understanding the factors which promote liver metastasis would profoundly impact the prognosis of this common malignancy.
**Estimated start and end dates (month/day/year - month/day/year):** 1/1/17-12/31/18
**Level of effort (%) in the project: 5% (0.60 person months)**
**Total Award Amount:**
**Point of contact at the funding agency:** Jaclyn McGuire; mcguirej@wustl.edu; Donald Buckner; Donald.buckner@bjc.org

**Current**

**Title of the project:** Fat Talks To Bone
**Funding agency:** National Institutes of Health
**Goals of the project:** This project will study a mouse that completely lacks fat yet has a 4-5 fold increase in bone proving that fat decreases skeletal mass. The results will determine how fat regulates bone and as such provide potential therapeutic strategies to diminish osteoporosis and fractures in obese patients.
**Specific aims/tasks:**
1. Determine the fat-derived signals which decrease bone mass.
2. Determine the mechanisms whereby fat suppresses osteoblast activation.
3. Determine the role of osteoclasts in FF mice.
**Start and end dates (month/day/year - month/day/year):** 7/11/2017-6/30/2022
**Level of effort (%) in the project: 20% (2.4 person-months)**
**Total Award Amount:**
**Point of contact at the funding agency:** Todd Le; 301-594-7794; let@extra.niddk.nih.gov

**Title of the project:** Accelerated healing of traumatic fractures and nonunion
**Funding agency:** Department of Defense
**Goals of the project:** The overarching goal of this proposal is therefore to extend the mechanistic observations of the two partnering laboratories and assess their potential to promote defect and atrophic nonunion repair.
**Specific aims/tasks:**
1. Determine the mechanism of DT/DTRADQ-induced bone formation.
2. Effect of DT/DTRADQ activation on defect nonunion healing.
3. Aim 3A. Characterize the periosteal osteoprogenitor cells in normal fracture callus and their deficit in atrophic nonunion.
Aim 3B: Test the potential of 1) implanted DTRADQ MSCs and 2) local delivery of antibodies that block BMPR inhibitors to rescue atrophic nonunion.
**Start and end dates (month/day/year - month/day/year):** 6/1/2021-5/31/2025
**Level of effort (%) in the project:** 15% (1.8 person months)
**Total Award Amount:**
**Point of contact at the funding agency:** Zabarsky, Zachary K; zachary.k.zabarsky.civ@mail.mil

**NEW**
**Title of the project:** Hepatic steatosis promotes liver metastasis

**Funding agency:** National Institutes of Health
**Goals of the project:** Our goal is to determine if prevention of NAFLD retards liver metastasis and the mechanisms by which hepatocyte-derived lipids are transferred to tumor cells and promote their growth.
**Specific aims/tasks:**
1. Explore the effect of suppressing steatosis and related events on liver metastasis.
2. Explore the role of hepatocyte lipids in promoting liver metastasis.
3. Explore the role of macrophages in steatosis-stimulated metastasis.
**Start and end dates (month/day/year - month/day/year):** 1/1/2022-11/30/2026
**Level of effort (%) in the project: 15% (1.8 person months)**
**Total Award Amount:**
**Point of contact at the funding agency:** Monica Benjamin; 240-276-5253; monica.benjamin@nih.gov

**NEW**
**Title of the project:** Avioli Lecture Award
**Funding agency:** Foundation for Barnes-Jewish Hospital (BJHF)
**Goals of the project:** The fund sponsors weekly Avioli Seminar Series, among the most attended lecture series as WUSM. It has also provided funds for junior faculty to present at scientific meetings.
**Specific aims/tasks:**
1. n/a
**Start and end dates (month/day/year - month/day/year):** 7/1/2021-6/30/2022
**Level of effort (%) in the project: 0% (0.0 person months)**
**Total Award Amount:**
**Point of contact at the funding agency:** Donald Buckner; Donald.buckner@bjc.org

**NEW**
**Title of the project:** Messing Chair Fund
**Funding agency:** Foundation for Barnes-Jewish Hospital (BJHF)
**Goals of the project:** Our goal is now to extend these observations to developing a drug to treat diseases such as osteoporosis and stimulate fracture healing when it is impaired.
**Specific aims/tasks:**
1. n/a.
**Start and end dates (month/day/year - month/day/year):** 10/1/2020-7/30/2023
**Level of effort (%) in the project: 0% (0.0 person months)**
**Total Award Amount:**
**Point of contact at the funding agency:** Donald Buckner; Donald.buckner@bjc.org

**NEW**
**Title of the project:** Breast Cancer Research Award
**Funding agency:** Foundation for Barnes-Jewish Hospital (BJHF)
**Goals of the project:** The goal is to study the osteoclast as this cell mediates the bone destruction that occurs in breast cancer patients with metastasis to the skeleton
**Specific aims/tasks:**
1. n/a

**Start and end dates (month/day/year - month/day/year):** 7/1/2021-6/30/2022
**Level of effort (%) in the project: 0% (0.0 person months)**
**Total Award Amount:**
**Point of contact at the funding agency:** Donald Buckner; Donald.buckner@bjc.org

**Pending**

**NEW**
**Title of the project:** Avioli Lecture Award
**Funding agency:** Foundation for Barnes-Jewish Hospital (BJHF)
**Goals of the project:** The fund sponsors weekly Avioli Seminar Series, among the most attended lecture series as WUSM. It has also provided funds for junior faculty to present at scientific meetings.
**Specific aims/tasks:**
1. n/a
**Start and end dates (month/day/year - month/day/year):** 7/1/2022-6/30/2023
**Level of effort (%) in the project: 0% (0.0 person months)**
**Total Award Amount:**
**Point of contact at the funding agency:** Donald Buckner; Donald.buckner@bjc.org

**NEW**
**Title of the project:** Breast Cancer Research Award
**Funding agency:** Foundation for Barnes-Jewish Hospital (BJHF)
**Goals of the project:** The goal is to study the osteoclast as this cell mediates the bone destruction that occurs in breast cancer patients with metastasis to the skeleton
**Specific aims/tasks:**
1. n/a
**Start and end dates (month/day/year - month/day/year):** 7/1/2022-6/30/2023
**Level of effort (%) in the project: 0% (0.0 person months)**
**Total Award Amount:**
**Point of contact at the funding agency:** Donald Buckner; Donald.buckner@bjc.org

**NEW**
**Title of the project:** Sonnenwirth Lecture Award
**Funding agency:** Foundation for Barnes-Jewish Hospital (BJHF)
**Goals of the project:** Lectureship that will fund ongoing lectures.
**Specific aims/tasks:**
1. n/a
**Start and end dates (month/day/year - month/day/year):** 7/1/2022-6/30/2023
**Level of effort (%) in the project: 0% (0.0 person months)**
**Total Award Amount:**
**Point of contact at the funding agency:** Donald Buckner; Donald.buckner@bjc.org

**Overlap**
None

**Previous/Current/Pending Support for Matthew Silva**

**PREVIOUS Support**

**Title:** Osteogenic and Angiogenic Response to Skeletal Loading
**Time commitments:** 2.4 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Kristy Nicks, NIH/NIAMS, 301-594-5055, Kristy.nicks@nih.gov
Administrative Official: Steve Austin, NIH/NIAMS, Steve.Austin@nih.gov
**Performance period:** 07/25/2003 – 02/28/2022 NCE
**Level of funding:** Annual Direct Support
**Brief description of the project's goals:** Our goal is to determine the mechanobiological pathways that lead to bone formation after mechanical loading.

**Title:** Cabinet microCT System for Musculoskeletal Specimen Imaging
**Time commitments:** 0.0 cal mos
**Supporting agency:** NIH/ORIP
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Alena Horska, NIH/ORIP, alena.horska@nih.gov
Administrative Official: Karen Brummett, NIH/ORIP, brummettk@mail.nih.gov
**Performance period:** 07/15/2020 – 07/14/2021
**Level of funding:** Total Support
**Brief description of the project's goals:** This proposal requests funds to purchase a research CT scanner which will be used for imaging musculoskeletal tissue samples, such as bone, cartilage, tendon and intervertebral disk.

**Title:** Rotator Cuff Degeneration and Repair
**Time commitments:** 0.6 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Anthony Kirilusha, NIH/NIAMS, anthony.kirilusha@nih.gov
Administrative Official: Leslie Littlejohn, NIH/NIAMS, littlele@mail.nih.gov
**Performance period:** 09/15/2010 – 08/31/2021
**Level of funding:** Annual Direct Support
**Brief description of the project's goals:** The goal of this project is to determine the mechanisms of rotator cuff degeneration and the effects of degeneration on tendon-to-bone healing.

**Title:** Skeletal Disorders Training Program
**Time commitments:** 0.0 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Faye H. Chen, NIH/NIAMS, chenf1@mail.nih.gov
Administrative Official: Marisol Espinoza-Pintucci, NIH/NIAMS, marisol.espinoza-pintucci@nih.gov
**Performance period:** 05/01/2011 – 04/30/2021
**Level of funding:** Annual Direct Support
**Brief description of the project's goals:** The goal of this project is to determine the mechanisms of rotator cuff degeneration and the effects of degeneration on tendon-to-bone healing.

**CURRENT Support**

**Title:** Resource Based Center for Musculoskeletal Biology and Medicine (PI: Silva
**Time commitments:** 2.4 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Anthony Kirilusha, NIH/NIAMS, anthony.kirilusha@nih.gov
Administrative Official: Steve Austin, NIH/NIAMS, Steve.Austin@nih.gov
**Performance period:** 04/01/2019 – 03/31/2024
**Level of funding:** Total Support (includes Core A: ; Core B: )
**Brief description of the project's goals:** Director of the Center (Core A and directs Core B, which supports evaluation of musculoskeletal structure and strength in mouse models.

**Title:** Response of the Osteoporotic Skeleton to in Vivo Loading (PI: Silva
**Time commitments:** 2.28 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Kristy Nicks, NIH/NIAMS, 301-594-5055, Kristy.nicks@nih.gov
Administrative Official: Steve Austin, NIH/NIAMS, Steve.Austin@nih.gov
**Performance period:** 04/01/2018 – 03/31/2023 NCE
**Level of funding:** Total Support
**Brief description of the project's goals:** Assess the responsiveness of the osteoporotic skeleton to increased mechanical loading using mouse models of aging, with focus on the roles of osteoblast recruitment and Wnt signaling.

**Title:** New Murine Models for Study of Atrophic Fracture Nonunion (PI: Silva
**Time commitments:** .90 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Faye H. Chen, NIH/NIAMS, chenf1@mail.nih.gov
Administrative Official: Steve Austin, NIH/NIAMS, Steve.Austin@nih.gov
**Performance period:** 03/01/2020 – 02/28/2023 NCE
**Level of funding:**
**Brief description of the project's goals:** This project will develop new nonunion models using mice, with the future goal of using the models to test new therapies for treatment of fracture nonunion.

**Title:** Accelerated Healing of Traumatic Fractures and Nonunion (PI: S.Teitelbaum, Partnering PI:Silva
**Time commitments:** 2.4 cal mos
**Supporting agency:** Department of Defense (DoD
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Zachary K. Zabarsky, zachary.k.zabarsky.civ@mail.mil
**Performance period:** 06/01/2021 – 05/31/2025
**Level of funding:** Total Support

***NEW***
**Title:** Influence of Genetic Background on Bone Anabolic Response to Mechanical Loading (PI: Silva
**Time commitments:** 1.5 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Kristy Nicks, NIH/NIAMS, Kristy.nicks@nih.gov
Administrative Official: Steve Austin, NIH/NIAMS, Steve.Austin@nih.gov
**Performance period:** 02/01/2022 – 01/31/2024

**Level of funding:** Total Support
**Brief description of the project's goals:** We aim to study a large set of genetically diverse mice to test whether the ability of physical loading to increase their bone mass is under genetic control.

***NEW***
**Title:** Regulation of Osteocyte Survival by Fibroblast Growth Factor Signaling Pathways (PI: D. Ornitz, Co-PI: Silva)
**Time commitments:** 1.0 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Kristy Nicks, NIH/NIAMS, , Kristy.nicks@nih.gov
Administrative Official: Steve Austin, NIH/NIAMS, Steve.Austin@nih.gov
**Performance period:** 02/01/2022 – 01/31/2027
**Level of funding:** Total Support
**Brief description of the project's goals:** This project will establish a role and identify mechanisms for FGFR signaling in the maintenance of osteocyte viability and bone homeostasis in adults, it will evaluate potential adverse effects of FGFR inhibition on bone, and identify new genes that could be targeted to promote skeletal homeostasis.

**Title:** The Role Of Physiologic And Pathologic Ages On Rage Signaling In IVD Degeneration (PI: S. Tang, Co-PI: Silva)
**Time commitments:** 0.36 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Anthony Kirilusha, NIH/NIAMS, anthony.kirilusha@nih.gov
Administrative Official: Leslie Littlejohn, NIH/NIAMS, littlele@mail.nih.gov
**Performance period:** 07/01/2019 – 06/30/2024
**Level of funding:** Total Support
**Brief description of the project's goals:** Investigate the role of signaling of the AGE-RAGE axis in intervertebral disc homeostasis, injury, and disease.

**Title:** MicroRNA Regulation of Bone Formation and Repair
**Time commitments:** 0.48 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Program Official: Faye H. Chen, NIH/NIAMS, chenf1@mail.nih.gov
Administrative Official: Administrative Official: Leslie Littlejohn, NIH/NIAMS, littlele@mail.nih.gov
**Performance period:** 05/01/2020 – 04/01/2025
**Level of funding:** Total Support
**Brief description of the project's goals:** The goals of this project are to elucidate the function and mechanism of specific microRNAs on: i) in vitro osteogenesis; ii) in vivo bone repair processes in established mouse models of bone fracture and iii) heterotopic ossification formation in a clinically relevant mouse model.

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**PENDING Support**

**Title:** Skeletal Disorders Training Program (PI: R. Civitelli, Associate Director: Silva)
**Time commitments:** 0.0 cal mos
**Supporting agency:** NIH/NIAMS
**Name and address of the Funding Agency's Procuring Contracting/Grants Officer:**
Aron Marquitz, Program Official, aron.marquitz@nih.gov
**Performance period:** 05/01/2022 – 04/30/2027

**Level of funding:** Total Support
**Brief description of the project's goals:** This training program educates and forms the next generation of scientists and physicians committed to skeletal disorders, so that research in this area can be perpetuated, a better understanding of the causes of these diseases can be achieved, and the search for new treatment modalities can progress.

**Title:** The Role of VEGF in the Development of Low Back Pain Following IVD Injury (PI: S. Tang/Gupta, Co-PI: Silva)
**Time commitments:** 0.6 cal mos
**Supporting agency:** NIH
**Performance period:** 07/01/2022 – 06/30/2024
**Level of funding:** Total Support
**Brief description of the project's goals:** This work will elucidate the role of vascular endothelian growth factor (VEGF) in angiogenesis and neurogenesis in low back pain behavior in a mouse model of intervertebral disc injury.

**Title:** Dietary lipids and sugars in age-related bone loss and treatment response
**Time commitments:** 2.0 cal mos
**Supporting agency:** NIH/DHHS
**Performance period:** 09/01/2022 – 08/31/2027
**Level of funding:** Total Support
**Brief description of the project's goals:** Our bone health is critical as we age. It impacts our physical function which is key to our quality of life. This project explores the relationship between our genes, diet, and therapies that prevent bone loss.

**Title:** Response of the Osteoporotic Skeleton to Mechanical Loading
**Time commitments:** 3.0 cal mos
**Supporting agency:** NIH
**Performance period:** 10/01/2022 – 09/30/2027
**Level of funding:** Total Support
**Brief description of the project's goals:** The major goal of this project is to enhance the response to mechanical loading in osteoporotic mice using WNT anabolics.

**Title:** RE-JOIN at WU - multiscale nerve mapping in healthy and diseased joints
**Time commitments:** 0.6 cal mos
**Supporting agency:** NIH
**Performance period:** 09/01/2022 – 08/31/2027
**Level of funding:** Total Support
**Brief description of the project's goals:** RE-JOIN at Washington University will lead efforts to better understand the structural, functional, and molecular complexities of the sensory innervation of the joints through technology adaptation and collaborative data sharing. The efforts will result in a new multidimensional understanding of the neuroanatomy and pathophysiology of painful joint conditions to inform the development of novel non-opioid management.

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## OVERLAP

There is no scientific overlap between any other funded or pending projects.