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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Depletion of marrow CAR cells, which are early osteoblast progenitors increases bone mass, 10 fold likely by eliminating BMPR inhibitors. Confirming a central role for CAR cells, depleting LepR+ cells in LepR-Cre mice similarly decreases marrow BMPR inhibitors and markedly increases bone mass . Thus, CAR/LepR+ cells are not only osteoblast progenitors, but also the principal source of BMP inhibitors in marrow, negatively regulating bone formation. Using a polymer scaffold to improve toughness and cell infiltration we will assess CAR cell free bone cells as well as viral BMP2 on segmental defect non-union healing in WT mice . Relevant to our prior identification of 3.6Col1a1 cells as essential for periosteal callus formation the critical window for proliferation of these cells is the first 10-14 days after fracture. Single-cell RNAseq of periosteal callus from 3.6Col1-TK mice shows that non-healing bones have no deficit in MSCs but are depleted of osteoblasts as well as chondrocytes. Data using Rosa-TK mice shows that proliferation of both early and mature osteoblasts is essential for fracture callus formation, suggesting that the mature osteoblast is a post-mitotic cell in the setting of fracture healing.

15. SUBJECT TERMS

non-union, BMPR, diphtheria toxin activated adiponectin Cre targeted diphtheria toxin receptor (DT/DTR^{ADQ}), bone formation

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TABLE OF CONTENTS

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" and Major task 2 of Specific Aim 1 states "Explore the role of the BMPR inhibitors CHRDL1 and GREM1 in DT/DTRADQ bone formation". We established the two aims overlap and they were therefore studied in association, Last year we confirmed that elimination of marrow adiponectin expressing cells eventuates in massive bone formation raising the potential of adaptation to non-union healing, a serious event occurring in numerous military personnel. We also demonstrated that the cells eliminated in the marrow are early osteoblast precursors which uniquely express the definitive markers of CAR cells, namely Cxcl12 and the leptin receptor (LepR). CAR cells are early osteoprogenitors and therefore it was particularly surprising that they are eliminated in face of massive bone formation. In fact, the absence of early osteoprogenitor cells is compensated for by induction of those in which the Col3.6 promoter is activated leading to mature osteoblast development. Supporting the concept that Col3.6 promoter positive cells are key to DTR^{ADQ} induced bone formation these moderately mature osteogenic cells express BMPR1a at twice the abundance of MSCs (Fig 1). Suggesting a likely mechanistic relationship between CAR cell depletion and stimulated osteogenesis they are the likely unique producers of the BMPR inhibitors, Grem1 and Chrdl1.

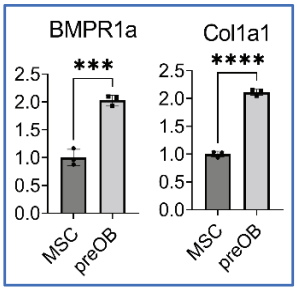


Fig 1. Marrow stromal cells were cultured in the osteoblast differentiation media for 6 days and BMPR1a and col1 gene expression tested by qPCR.

Having established that elimination of marrow ADQ-cre positive cells is profoundly osteogenic we confirmed their identity as CAR cells. To this end, we generated mice expressing Cxcl12-GFP and LepR^{Cre}; tdTomato (*LepR^{Cre}; tdTomato;Cxcl12^{GFP}*). As assessed by confocal microscope (Fig 2) as well as FACS (Fig 3) essentially all GFP+ cells also express tdTomato. Furthermore, Cxcl12 and the BMPR inhibitors are expressed only by LepR cre+ cells (Fig 4) supporting the concept they are CAR cells, elimination of which stimulates bone formation. To further explore this issue, we turned to a strategy to eliminate CAR cells other than DT/DTR^{ADQ}. Specifically, we generated mice expressing DTR and LepR cre (DTR^{LepR}) and administered DT at 2 months of age. Confirming LepR targeting eliminates CAR cells, Cxcl12 is essentially depilated as are BMPR inhibitors (Fig 5). Most importantly, targeting with DT/DTR^{LepR} yields the same osteosclerotic phenotype as DT/DTR^{ADQ} (Fig 6-10).

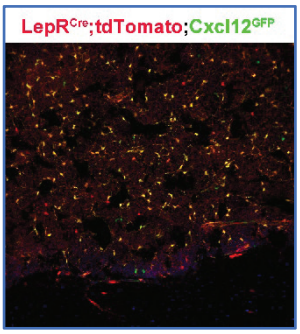


Fig 2. Representative fluorescent microscopic images of femoral diaphysis of *LepR^{Cre};tdTomato;Cxcl12-GFP* mice.

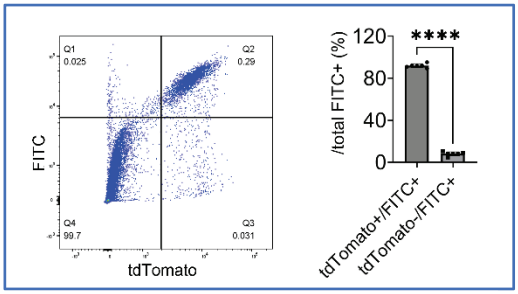


Fig 3. Left: Fluorescence-activated cell sorting (FACS) analysis of Cd45-CD31- stromal cell fractions from enzymatically dissociated bone and bone marrow obtained from 2-month-old *LepR^{Cre};tdTomato;Cxcl12-GFP* mice. Right: Quantification analysis.

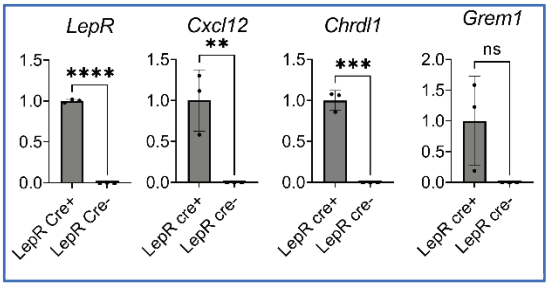


Fig 4. 2 months old *LepR^{Cre}; tdTomato* mice. tdTomato+ and tdTomato- CD45-CD31- stromal cells were sorted and gene expression was analyzed by qPCR.

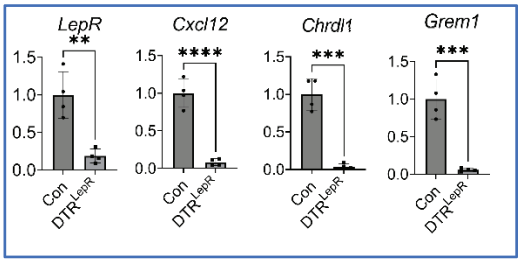


Fig 5. qPCR analysis of LepR, Cxcl12 and Chrdl1, Grem1 mRNA in femoral bone marrow of 2-month-old *DTR^{LepR}* mice treated with DT for 2 days. Con received no DT. n = 4/group.

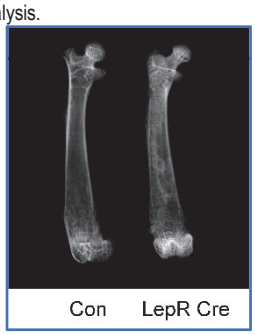


Fig 6. Representative radiographs of *DTR^{LepR}* femurs after 10 days of DT.

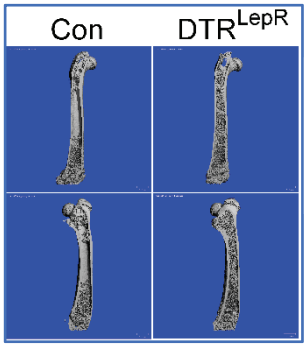


Fig 7. Representative μCT images of Con or *DTR^{LepR}* femurs after 10 days of DT.

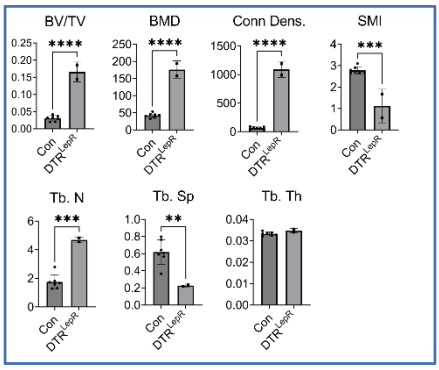


Fig 8. Quantitative µCT analysis of whole femur of Con or *DTR*^{LepR} mice after 10 days of DT injection.

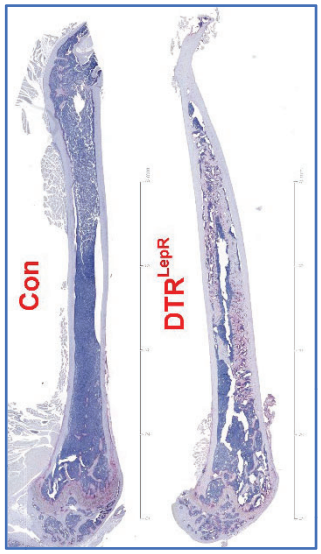


Fig 9. Representative histological section of femur of 2-month-old *DTR*^{LepR} mice treated with DT for 10 days

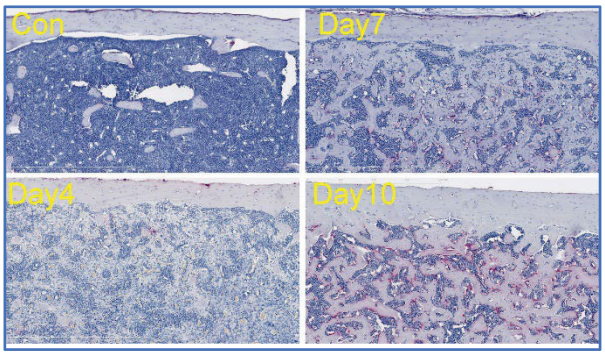


Fig 10. Representative histological section of femur of 2-month-old *DTR*^{LepR} mice treated with DT with time. n = 5/group. Con received PBS for 10 days.

As occurs with DTR^{ADQ}, LepR cre-mediated marrow cell depletion stimulates Smad 1,5 and 8 phosphorylation confirming induction of BMPR signaling (Fig 11). Further confirming its central role, inhibition of BMPR signaling by systemic administration of the anti-fibrous dysplasia ossificans drug, LDN193189, completely prevents DT/DTR^{LepR} induced osteosclerosis (Fig 12-14).

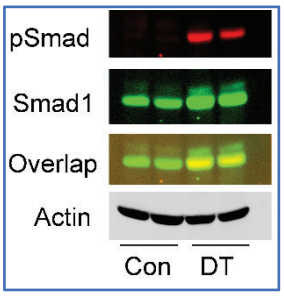


Fig 11. Immunoblot analysis of phospho-Smad1,5,8 and total smad1 in whole-bone marrow lysates of *DTR*^{LepR} mice treated with DT for 3 days. Con received no DT. n = 3

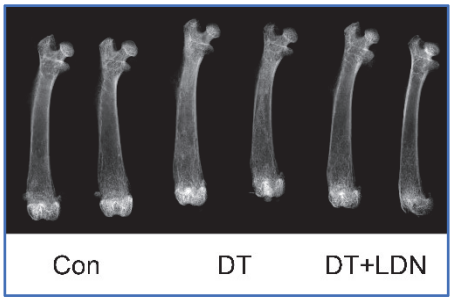


Fig 12. Representative radiograph of femoral diaphysis of *DTR*^{LepR} mice treated with DT and with or without LDN193189 for 10 days. No DT treatment serves as Con.

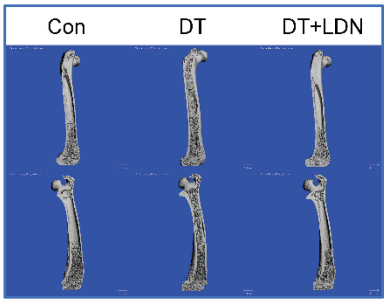


Fig 13. Representative µCT images of femoral diaphysis of *DTR*^{LepR} mice treated with DT and with or without LDN193189 for 10 days. No DT treatment serves as Con.

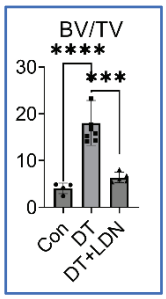


Fig 14. Whole femur quantitative µCT analysis of Fig 13.

Importantly, in contrast to DT/DTR^{ADQ} mice in which mature marrow adipocytes are eliminated, the abundance of these lipid bearing cells does not change in DT/DTR^{LepR} mice despite robust bone formation (Fig 15). This observation challenges the concept that reduction of marrow adiposity accelerates bone formation, an observation of clinical relevance.

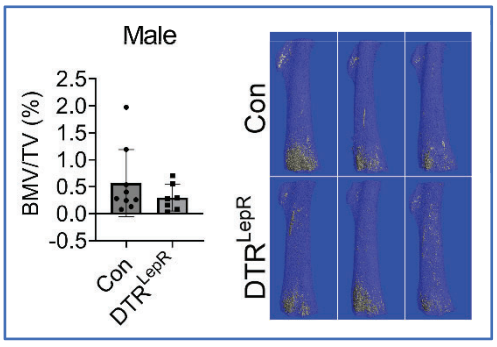


Fig 15. Representative µCT images of osmium-stained femurs of Con and *DTR*^{LepR} mice 10 days after DT injection and µCT analysis. n=5/group.

Marrow fat in dark gray, decalcified bone overlaid in light gray.

Major task 2 of Specific Aim 2 states "Explore cellular induction of non-union defect healing". Our ultimate therapeutic goal is to adapt our observations regarding CAR cell depletion osteosclerosis to healing of non-union fractures. Last year we observed that 3.5 mm segmental defects appear to heal somewhat more rapidly in DT/DTR^{ADQ} than WT mice. We also showed that subcutaneous implanted cryogels containing equal numbers of cells from collagenase digested DTR^{ADQ} bone produced osseous tissue while hydrogels containing the same number of WT cells failed to do so.

This year we extended these subcutaneous observations and asked if segmental defect healing was impacted by implanted DT/DTR^{ADQ} bone cells. To this end we isolated bone cells by collagenase digestion from DTR^{ADQ} and WT mice following 3 days of DT administration. The cells were seeded on cryogel in osteogenic medium for 2 days after which it was implanted in 3.5 mm segmental defects (Fig 16). 3 months later defects containing WT cells exhibited limited healing

which was much more profound it those bearing DT/DTRADQ activated cells (Fig 17). These observations support the concept that CAR cell free bone may provide cells to accelerate non-union repair.

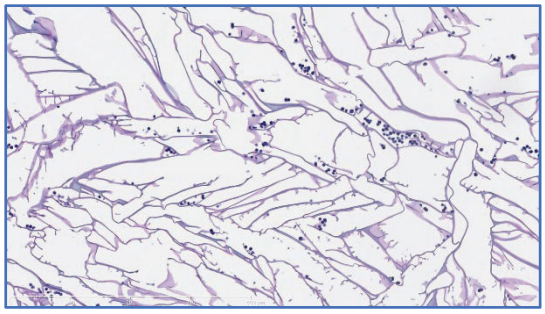


Fig 16. Representative HE microscopic images of cryogel with 2 days whole bone cell seeding. Collagenase digested bone cells were seeded on cryogel for 2 days, and cryogel with cells were fixed with paraformaldehyde and paraffin embedded sections prepared. Black dots represent nuclei.

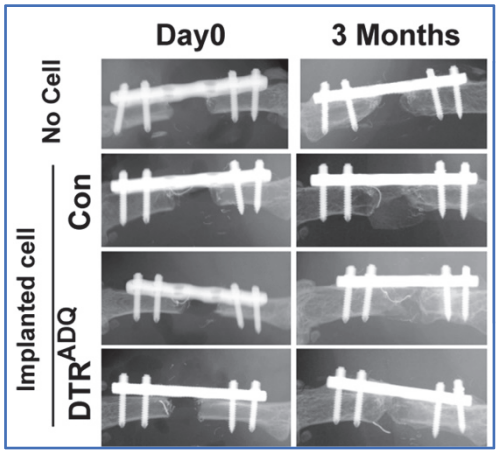


Fig 17. 3.5 mm segmental defects were created in WT femurs and cryogels seeded with WT or *DTR*^{ADQ} cells implanted. X-ray images at after surgery (days 0) and after 3 months.

Major task 1 of Specific Aim 3A states "Perform scRNA-seq on fracture callus from 3.6Col1-TK mice." **Silva lab.**

3A.1 scRNAseq: We recently reported that proliferation of osteoblast lineage cells is essential for periosteal callus formation and healing of long bone fractures (Hixon et al., 2021, https://doi.org/10.1002/jbmr.4424). This was done using 3.6Col1a1-TK mice, which heal fractures normally when dosed with water (Control) but fail to heal fractures when dosed with ganciclovir (GCV). Therefore, we reasoned that by comparing fracture callus from these two conditions (water vs. GCV), we could elucidate differences between healing and non-healing 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 (**CTL**) or ganciclovir (**GCV**) (n=3 mice/group). Callus tissues were collected post mortem and single-cell isolates prepared, followed by RNAseq (NovaSeq S4) using the 10x Chromium 3' single-cell platform. After quality control, cluster analyses were performed, accounting for treatment (CTL, GCV) and time (5, 10 days). *In last year's report, we presented preliminary results of this analysis. Here, we update and extend that report.* Twenty clusters were identified in Control samples, including a closely related set of seven clusters of mesenchymal/skeletal cells (Fig. 18). HSV-TK was expressed in 7% of all cells, primarily in the mesenchymal cluster. Day 5 callus cells comprise many undifferentiated, matrix (ECM)-expressing cells, as well as more differentiated cells (Fig. 19). Pseudotime analysis shows two main branches of cell differentiation, one leading to fibroblasts, and the second leading to osteoblasts and chondrocytes. Of importance, osteoblasts and chondrocytes are markedly depleted in GCV calluses, whereas undifferentiated and fibroblastic cells are not affected (Fig. 20). Similarly, on Day 10, the calluses of GCV-treated mice have 60-80% fewer osteoblasts, chondrocytes and hypertrophic chondrocytes compared to CTL (not shown).

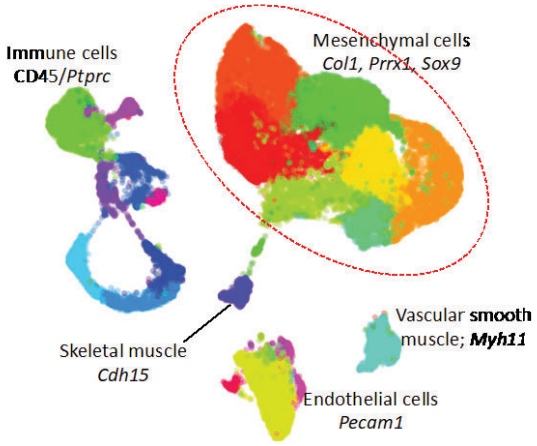


Fig 18. UMAP plot of 32K cells from Control (healing) callus tissue at days 5 and 10 after fracture in Col1-TK mice. Distinct clusters of Immune, Skeletal Muscle, Endothelial, and Vascular Smooth Muscle Cells are evident. We focused analysis on the super-cluster of Mesenchymal cells (----) that highly express *Col1a1, Col1a2*, *Prrx1* and *Sox9* .

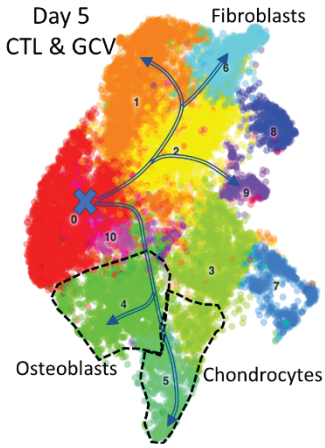


Fig 19. UMAP plot of callus mesenchymal cells at day 5. Pseudotime analysis indicates that cell maturation proceeds from undifferentiated cells (cluster 0; **X**) along one main branch towards fibroblasts and ECM-expressing cells, and another branch toward osteoblasts and chondrocytes.

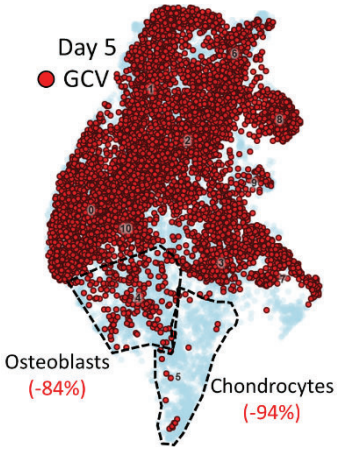


Fig 20. UMAP plot of callus mesenchymal cells at day 5. Cells from the GCV group (red circles) are overlaid on control cells (pale blue). Chondrocytes and osteoblasts are 84 to 94% depleted from the GCV callus, while undifferentiated and fibroblastic cells are relatively unaffected.

3.A.2 Additional histological analysis of Col1-tk mice: To complement the single-cell data, we performed histology at days 5 and 10 after fracture in Col1-tk mice treated with GCV and in control mice (Col1-tk treated with water). Results confirmed normal healing in the Control group but impaired healing in the GCV group, i.e., less bone and cartilage, but more fibrous tissue (Fig. 21). These studies revealed two unexpected findings: i) the depletion of chondrocytes, as well as osteoblasts in GCV-treated 3.6Col1a1-TK mice, which are expected to target osteoblasts but not chondrocytes; and ii) the identification of callus cells expressing chondrocyte markers (eg, *Col2*, Col9) that are TK+ and proliferating (G2/S phase of cell cycle). These unexpected findings have prompted us to further examine which cell populations are critical to fracture callus formation, focusing initially on osteoblasts (next section).

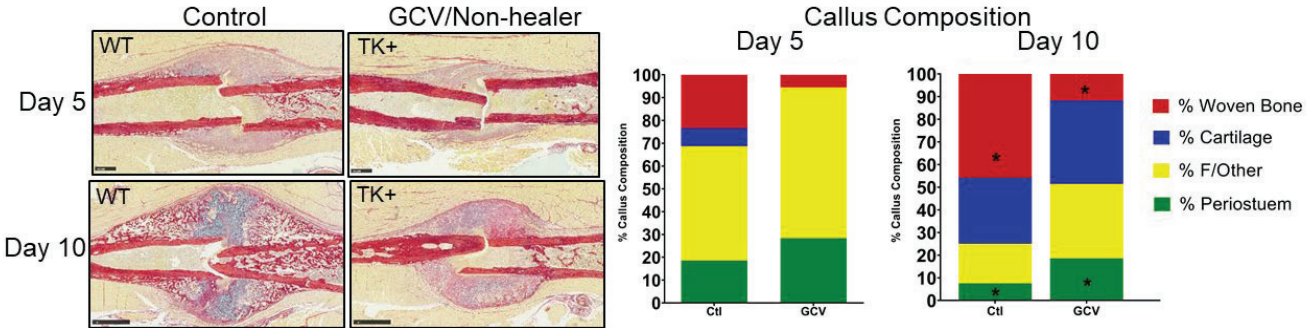


Fig 21. (A) Picrosirius red/Alcian blue-stained fracture callus sections from Control mice show progressive callus maturation from day 5 to 10, with increased woven bone (red) and cartilage (blue). In contrast, samples from GCV/non-healer mice show altered callus composition at each time, and a smaller callus at day 10. (B) Quantification of callus composition indicates less bone (red) and more fibrous and periosteal tissue (yellow, green) in GCV/non-healer mice. (n=5-8/group)

3.A.3 Proliferation of Early and Mature Osteoblasts is Required for Fracture Callus Mineralization. As a next step in identifying which proliferating cell populations are critical to osteochondral callus formation and fracture healing, we have turned to a new mouse, recently generated by our lab. The Rosa-TK mouse was generated by insertion of a deltaTK-mCherry transgene, preceded by a floxed STOP cassette, into the murine Rosa26 (ROSA). By crossing RosaTK with any Cre line, we are able to target specific cell populations (Fig. 22A). Next, RosaTK mice were crossed with Osx-CreERT2 mice to inducibly target early osteoblasts, and Ocn-Cre mice to target mature osteoblasts. After generating femur fractures at 12-wks age, littermate controls (Cre-;TK-, Cre-;TK+, Cre+;TK-) and experimental (Cre+;TK+) mice were dosed with GCV daily. Healing was assessed at 2 weeks using x-ray, microCT, and histology (Fig. 22B). Ablation of replicating pre- and mature osteoblasts in Cre+;TK+ mice with GCV significantly impaired fracture healing. Radiographically, Osx-CreERT2+;TK+ and Ocn-Cre+;TK+ mice treated with GCV have significantly less callus bridging compared to genotype controls. Histological staining to visualize woven bone and cartilage shows that this decrease in bridging is due to significantly less woven bone within the calluses of Cre+;TK+ mice, whereas genotype controls have large, well-formed, mineralized calluses (Figs. 23, 24). Evaluation of fracture calluses by ex vivo microCT shows that both Osx-CreERT2+;TK+ and Ocn-Cre+;TK+ mice treated with GCV have significantly smaller calluses than controls (not shown). Within these calluses, there is significantly less bone volume, which is consistent with the decrease in woven bone seen histologically. We conclude: a) the novel RosaTK mouse can be crossed with different Cre lines and used to target different populations of proliferating cells; and b) blocking proliferation of either early- or late-stage osteoblasts significantly impairs callus mineralization. Ongoing work will validate cell-specific targeting using immunofluorescence for TK and OCN, and future work will use this model to target non-osteoblast lineage cells, such as chondrocytes.

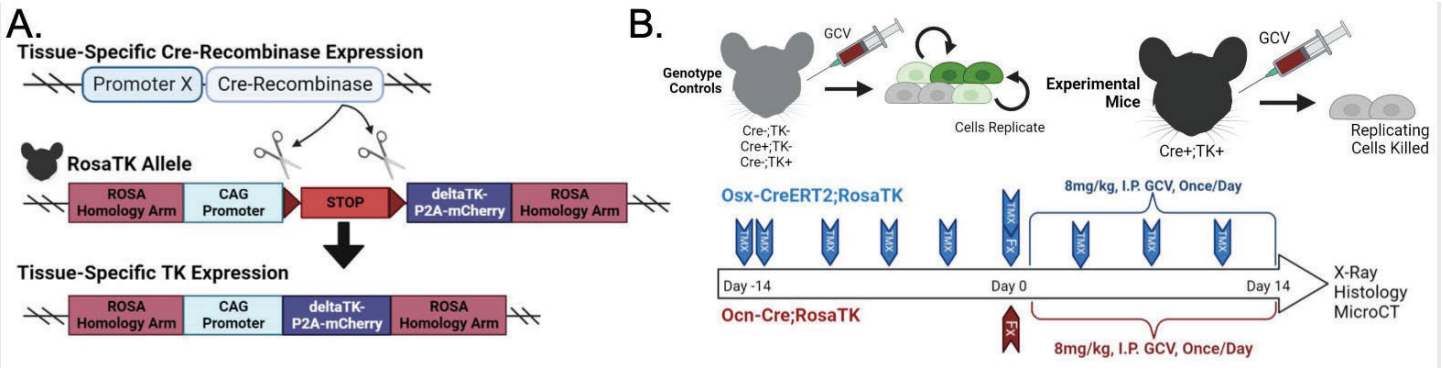


Fig 22. Use of a novel RosaTK mouse line allows for tissue-specific TK expression and ablation of specific populations of replicating cells. (A) A novel mouse line we developed uses the ROSA26 promoter to drive expression of HSV thymidine kinase (TK) downstream of a floxed stop cassette (RosaTK). Breeding to any Cre recombinase mouse line drives tissue-specific expression of TK. (B) Treating Cre+;TK+ mice with ganciclovir (GCV) causes apoptosis in replicating TK-expressing cells. Osx-CreERT2;RosaTK mice were treated with tamoxifen prior to and after fracture. Osx-CreERT2;RosaTK and Ocn-Cre;RosaTK control and experimental mice were fractured at 12 weeks old and treated with GCV once daily. Fracture healing was assessed with x-ray, histology, and microCT 2 weeks post-fracture.

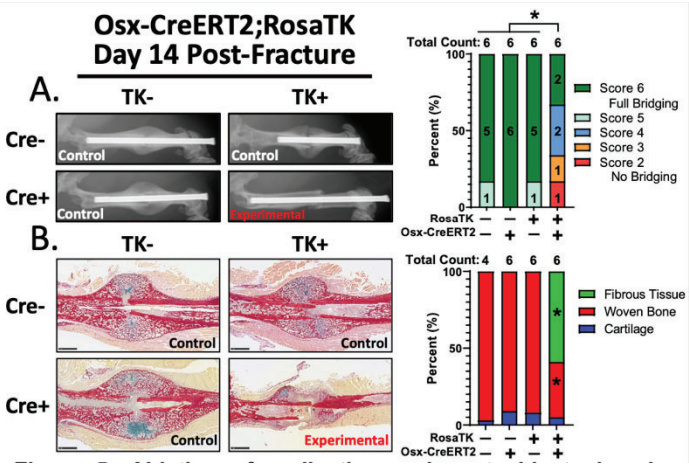


Fig 23. Ablation of replicating early osteoblasts in OsxCreERT2;RosaTK mice impairs fracture healing by X-ray and histological evaluation. A. X-ray scoring using the RUST scale shows experimental (Cre+/TK+) mice have significantly impaired fracture callus bridging. B. Fracture composition evaluated using picrosirius red to visualize collagen- rich bone and alcian blue to visualize proteoglycan-rich cartilage shows significantly less woven bone and significantly more fibrous tissue in experimental mice. Scale bar = 1mm. Significance by Chi- Square (A) and Two-Way ANOVA with Holm-Sidak Post-Hoc (B).

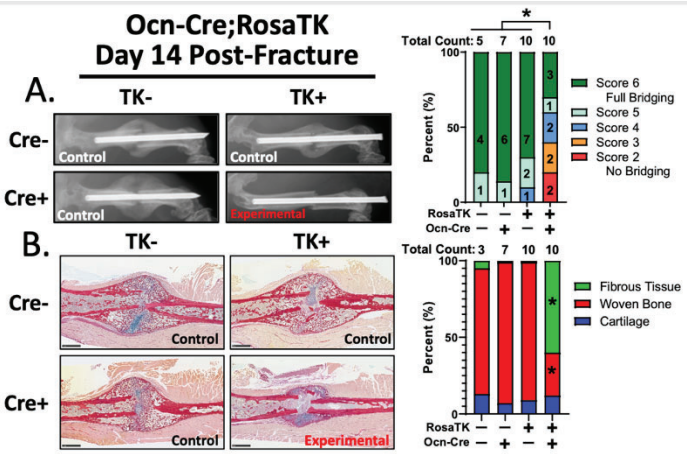


Fig 24. Ablation of replicating mature osteoblasts in OcnCre;RosaTK mice impairs fracture healing by X-ray and histological evaluation. A. X-ray scoring using the RUST scale shows experimental (Cre+/TK+) mice have significantly impaired fracture callus bridging. B. Fracture composition evaluated using picrosirius red to visualize collagen- rich bone and alcian blue to visualize proteoglycan-rich cartilage shows significantly less woven bone and significantly more fibrous tissue in experimental mice. Scale bar = 1mm. Significance by Chi- Square (A) and Two-Way ANOVA with Holm-Sidak Post-Hoc (B).

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.**

DTR^{ADQ} mice demonstrate enhanced BMP-mediated osteogenesis. In last year's report, we described the use of a chitosan-gelatin cryogel scaffold to deliver BMP2-transfected cells at the site of fracture nonunion. This proof-of-concept study was recently published [Hixon et al., 2022; *Cryogel Scaffold-Mediated Delivery of Adipose-Derived Stem Cells Promotes Healing in Murine Model of Atrophic Non-Union*. https://pubmed.ncbi.nlm.nih.gov/35600896/]. This work identified several physical property limitations in the scaffold delivery system: a) too bulky; b) did not stay in position at to fracture site; c) small pore size may impede cell infiltration/seeding. We have worked the past year to address these issues before undertaking additional in vivo experiments. First, we have worked on the physical properties of the scaffold. The original scaffold was fabricated as a 1-mm thick sheet that was cut to size. We created new, 3D-printed molds to make create cylindrical scaffolds with a thickness of 0.5 mm, and dimensions to conform to the shape of the fracture site (Fig. 25). We used different polymer compositions so that we could modulate porosity and pore size of the resulting cryogels. Next, we used microCT imaging to assess the porosity of different chitosan-gelatin (CG, the "original") and chitosan-agarose-gelatin (CAG) compositions fabricated under different conditions (Fig. 26). Pore diameter was larger in CAG scaffolds, and varied as a function of freezing rate which was modulated with different mold dimensions. Next, we tested mechanical properties of CG and CAG scaffolds, and observed that CAG scaffolds have lower elastic modulus and ultimate stress, but have greater post-yield toughness than CG scaffolds (not shown). Taken together, CAG scaffolds have larger pore size, which may enable better cell infiltration, albeit with reduced strength (but similar toughness) compared to CG scaffolds. Next, to assess *in vitro* infiltration we seeded cells onto the four different scaffolds: CG, CAG-small pore, CAG-medium pore, CAG large-pore. Cells readily attached to scaffold surfaces and survived up to 14 days, but did not migrate into the center of the scaffold which we attributed in part to the static, 2D conditions of the culture setup (not shown). Next, we performed *in vivo* subcutaneously implantation of acellular scaffolds. One scaffold of each type was implanted into each of four sites on the backs of control mice; scaffolds were retrieved at 7, 10 and 14 days post-implantation. No evidence of adverse inflammatory reaction was seen; scaffolds were increasingly enveloped by host fibro-adipo-cells and tissue from 7 to 10 to 14 days. Cell infiltration was assessed by confocal microscopy. Cells from the host mice were attached to the scaffold surface and were also seen on the interior of each scaffold type. CAG scaffolds showed better infiltration of cells to the interior. A representative image of a CAG-medium-pore scaffold shows excellent infiltration and cells with strong cytoskeletal staining, indicating active cell attachment (Fig. 27). Taken together, the physical and in vivo implantation results show that each of the four scaffold types are feasible options of additional evaluation. Our next step is to coat the scaffolds with a lentivirus for delivery of BMP2 transgenes. This is the same viral vector used in our proof-of-concept study (Hixon et al, 2022). One difference here is that we will not seed cells on the scaffolds prior to implantation, but will just coat them with virus/BMP2. After lentivirus coating, scaffolds will be implanted subcutaneously and evaluated at 7 days for initial response. We anticipate that cells from the host mice will infiltrate the scaffold and become transfected *in vivo*.

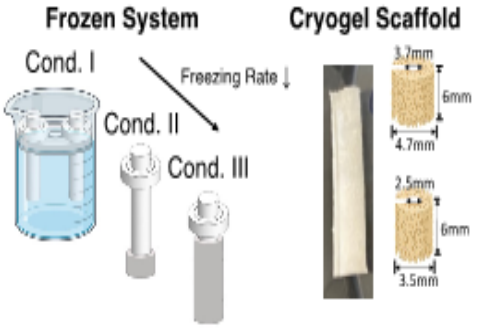
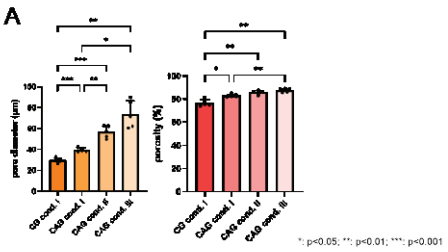


Fig 25. (Left) Molds containing monomer solutions are placed in methanol bath at -4C. By adjusting the wall thickness of the molds, we can alter the freezing rate and subsequently the pore size. (Right) Cryogel scaffold is a hollow cylinder, which is then cut longitudinally to allow it to be wrapped around the fracture site during implantation, while holding its shape.



Scaffolds	Porosity(%)	Pore Diameter(μm)
CG cond. I	76.72±2.980	29.40±2.416
CAG cond. I	82.86±1.436	39.32±2.055
CAG cond. II	85.73±1.566	56.76±5.802
CAG cond. III	87.50±1.520	73.62±13.11

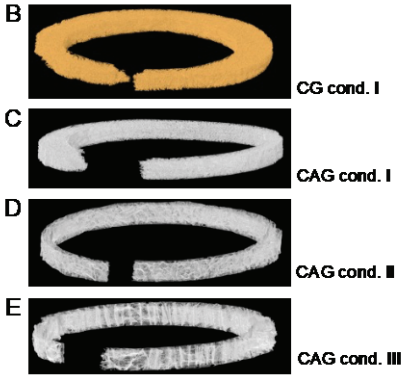
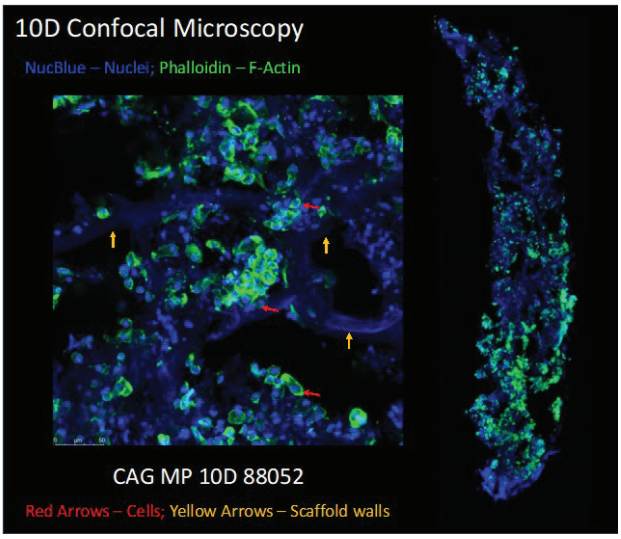


Fig 26. Chitosan-agarose-gelatin (CAG) scaffolds show significantly larger pore size and higher porosity than chitosan-gelatin (CG) scaffolds. Larger pore size was fabricated under slower freezing rate. A) Comparison of pore diameters and porosity between CAG scaffolds and CG scaffolds fabricated with different conditions. B) A 3d-reconstruction of CG scaffolds fabricated with cond. I, C) CAG scaffolds fabricated with condition I, D) CAG scaffolds fabricated with condition II, and E) CAG scaffolds fabricated with condition III.

Fig 27. Representative images of CAG-medium pore scaffold after 10 days in vivo implantation. Nuclei were stained with NucBlue, and F-actin cytoskeleton was stained with Phalloidin (green). Note that gelatin auto-fluoresces blue, so the blue channel shows both nuclei and scaffold. (Right) Section showing the entire rectangular cross-section of the scaffold. (Left) High-power field of central region of scaffold shows abundant cellular staining within the scaffold.



• **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 a colleague Dr. Jie Shen, who provided instruction on generation of segmental non-unions in mice. Combined meetings of the Silva and Teitelbaum labs provided cross-training for their respective lab members. A post-doctoral fellow, Dr. Nicole Gould joined the Silva lab and led the RosaTK work, and gained experience with the fracture nonunion model and with scRNA-seq analysis, as well as advising Mr. Chen on cell culture. Two PhD candidates, Mr. Andre Coello (Biomedical Engineering) and Ms. Leyi Chen (Mechanical Engineering & Materials Science) have led the Aim 3 studies, and Ms. Chen has worked closely with Dr. Zou to produce scaffolds for his use.

• **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 (Spring 2023). Drs. Teitelbaum and Silva were invited speakers at the 2022 Bones and Teeth Gordon Research Conference (Sept. 2022), where they presented talks entitled "Fat Talks to Bone" and "Role of Osteoblast Lineage Cell Proliferation in Fracture Healing", respectively. Dr. Silva presented his talk at the 2022 International Society for Fracture Repair meeting (Sept. 2022). Mr. Coello and Dr. Gould each presented Plenary Posters at the 2022 American Society for Bone and Mineral Research (ASBMR) Meeting (Sept.), and Dr. Gould was awarded the Orthopaedic Research Society (ORS) New Investigator Recognition Award at the ORS Annual Meeting (Feb. 2023) for her abstract “Proliferation of Early and Mature Osteoblasts is Required for Fracture Callus Mineralization“. Mr. Coello and Dr. Gould also presented their relevant work at the Washington University Musculoskeletal Research Center Symposium (May 2023), where Dr. Gould won a Best Abstract award.

• **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 virus or cell seeding for application to segmental defect and atrophic non-union fracture healing models (Tasks 2, 3). 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 as well as the study on Rosa-TK mice. We will evaluate the efficacy of acellular, BMP2-viral coated scaffolds for in vivo osteogenesis.

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 was an invited speaker at the Avioli Bone and Mineral Conference (Spring 2023). Drs. Teitelbaum and Silva were invited speakers at the 2022 Bones and Teeth Gordon Research Conference (Sept. 2022), where they presented talks entitled "Fat Talks to Bone" and "Role of Osteoblast Lineage Cell Proliferation in Fracture Healing", respectively. Dr. Silva presented a paper at the 2022 International Society for Fracture Repair meeting (Sept. 2022), which received a Best Presentation award. Mr. Coello and Dr. Gould each presented Plenary Posters at the 2022 American Society for Bone and Mineral Research (ASBMR)

Meeting (Sept.), and Dr. Gould was awarded the Orthopaedic Research Society (ORS) New Investigator Recognition Award at the ORS Annual Meeting (Feb. 2023) for her abstract "Proliferation of Early and Mature Osteoblasts is Required for Fracture Callus Mineralization". Mr. Coello and Dr. Gould also presented their relevant work at the Washington University Musculoskeletal Research Center Symposium (May 2023), where Dr. Gould won a Best Abstract award. 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?**

  - *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."*

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 Graduate Research Assistant, Ms. Chen, and the Bioinformatics Scientist, Dr Tiandao Li.
Funding Support:	
Name:	Leyi Chen
Project Role:	Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	9
Contribution to Project:	Ms. Chen was recruited to join the project. She produced scaffolds for use by Dr. Zou in Aim 2. She worked on modifying the scaffolds and performing characterization and cell infiltration studies, in preparation for future in vivo studies (Aim 3B). She assisted with the fracture studies using RosaTK mice.
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 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 grant (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: 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

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

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

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

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

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

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): 7/11/2017-6/30/2023 (NCE)
Level of effort (%) in the project: 20% (2.4 person-months)
Total Award Amount:
Point of contact at the funding agency: Todd Le

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

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

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): 7/1/2023-6/30/2024
Level of effort (%) in the project: 0% (0.0 person months)
Total Award Amount:
Point of contact at the funding agency: Donald Buckner

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): 7/1/2023-6/30/2024
Level of effort (%) in the project: 0% (0.0 person months)
Total Award Amount:
Point of contact at the funding agency: Donald Buckner

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): 7/1/2023-6/30/2024
Level of effort (%) in the project: 0% (0.0 person months)
Total Award Amount:
Point of contact at the funding agency: Donald Buckner

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): 7/1/2023-6/30/2024
Level of effort (%) in the project: 0% (0.0 person months)
Total Award Amount:
Point of contact at the funding agency: Donald Buckner

Pending

Overlap
None

Previous/Current/Pending Support for Matthew Silva

PREVIOUS Support

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
Administrative Official: Steve Austin, NIH/NIAMS
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: 0.90 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
Administrative Official: Steve Austin, NIH/NIAMS
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: 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
Administrative Official: Steve Austin, NIH/NIAMS
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
Administrative Official: Karen Brummett, NIH/ORIP
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
Administrative Official: Leslie Littlejohn, NIH/NIAMS
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
Administrative Official: Marisol Espinoza-Pintucci, NIH/NIAMS **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
Administrative Official: Steve Austin, NIH/NIAMS
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 Mechanical Loading
Time commitments: 2.4 cal mos
Supporting agency: NIH
Performance period: 04/10/2023 – 03/31/2028
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: 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
Performance period: 06/01/2021 – 05/31/2025
Level of funding: Total Support

Title: Influence of Genetic Background on Bone Anabolic Response to Mechanical Loading (PI: Silva)

Time commitments: 1.2 cal mos
Supporting agency: NIH/NIAMS
Name and address of the Funding Agency's Procuring Contracting/Grants Officer:
Program Official: Kristy Nicks, NIH/NIAMS
Administrative Official: Steve Austin, NIH/NIAMS
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.

Title: Regulation of Osteocyte Survival by Fibroblast Growth Factor Signaling Pathways (PI: D. Ornitz, Co-Inv: Silva)
Time commitments: 0.84 cal mos
Supporting agency: NIH/NIAMS
Name and address of the Funding Agency's Procuring Contracting/Grants Officer:
Program Official: Kristy Nicks, NIH/NIAMS
Administrative Official: Steve Austin, NIH/NIAMS
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-Inv: 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
Administrative Official: Leslie Littlejohn, NIH/NIAMS
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
Administrative Official: Administrative Official: Leslie Littlejohn, NIH/NIAMS **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.

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
Performance period: 05/01/2011 – 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.

New
Title: The Role of VEGF in the Development of Low Back Pain Following IVD Injury (PI: S. Tang/Gupta, Co-Inv: Silva)
Time commitments: 0.6 cal mos
Supporting agency: NIH
Performance period: 05/01/2023 – 04/30/2025
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.

PENDING Support

New
Title: RAGE-Mediated Osteocyte Regulation of Bone Quality in Diabetes (PI: S. Tang, Co-Inv: Silva)
Project number: R21 AR082597
Level of effort: 0.6 cal mos
Performance period: 10/2023 – 09/2025
Supporting agency: NIH/NIAMS
Supporting agency POC:
Program Official: Kristy Nicks
Administrative Official: Leslie Littlejohn
Level of funding:
Brief description of the project's goals: Intervertebral disc degeneration is strongly associated with low back pain, but the role of the intervertebral disc as the pain generator is not clear. We aim to investigate the role of vascular endothelial growth factor A in regulating the pathological in-growth of blood vessels and nerves in intervertebral discs during low back pain, and the molecular factors that govern the pathoanatomy. The improved understanding of these molecular events will help to develop therapies for treating low back pain.
Specific aims:
1. Ablation of RAGE signaling in promotes survival in mature osteoblasts and osteocytes during diabetes;
2. Promoting osteocyte survival protects against the impairment of bone matrix material properties and consequently maintains bone fracture resistance.

New
Title: Resource Based Center for Musculoskeletal Biology and Medicine (PI: Silva)
Time commitments: 2.52 cal mos
Supporting agency: NIH/NIAMS
Name and address of the Funding Agency's Procuring Contracting/Grants Officer:
Program Official: Anthony Kirilusha, NIH/NIAMS Administrative Official: Steve Austin, NIH/NIAMS **Performance period:** 04/2024 – 03/2029
Level of funding:

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.

OVERLAP

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