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TITLE: Novel Therapeutic Small-Molecule Strategy Targeting Bone Morphogenetic Protein Signaling to Prevent Upper Extremity Heterotopic Ossification

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CONTRACTING ORGANIZATION: Regents of the University of Michigan
Ann Arbor, MI 48109

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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University of Michigan
1150 W Medical Center Dr
MSRB 2 A 570

8. PERFORMING ORGANIZATION REPORT NUMBER

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

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

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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: To validate prophylactic strategies to prevent trauma induced heterotopic ossification using validated small animal models.
Scope: Heterotopic ossification is one of the most challenging problems associated with reconstruction following high-energy trauma wounds due to its insidious development which often leads to chronic pain, open wounds, and loss of range of motion. Ultimately, HO significantly limits soldier function, readiness, and redeployment. Patients who develop these signs or symptoms may undergo radiographic imaging to identify HO, at which point it has already matured and the only option remaining is surgical excision. This results in further morbidity to the patient, and costs for the military healthcare system for the operation, and post-operative management. Furthermore, patients may often develop recurrence which necessitates additional operations, and even successful excision is unable to address the sequelae of HO including chronic pain, open wounds, and loss of function. Our goal is to transform the management strategy for patients with trauma-induced HO, by first targeting two synergistic signaling pathways involved in the development of HO, and then by precisely timing in high-risk patients to minimize duration and maximize efficacy, in order to prevent HO formation.
Major Findings:
TAK1 signaling plays a central role in traumatic heterotopic ossification.
NG25 is effective in preventing traumatic HO
NG25 decreases osteogenic differentiation of HO progenitor cells

15. SUBJECT TERMS

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

TABLE OF CONTENTS

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

Patients with large surface-area burns and musculoskeletal trauma including blast injuries are at risk for development of heterotopic ossification (HO). This condition leads to chronic pain, open wounds, and impaired joint function, all of which impede patient rehabilitation. In our analysis of a large burn database of over 4,000 patients, 95% of the patients with HO developed ectopic bone in the upper extremity. Prevalence of HO (63% of 213 amputees) is higher in wartime injuries compared with those sustained in civilian settings.(*1*) Furthermore, up to 30% of patients who undergo surgical resection of HO in the upper extremity will develop recurrence (*2*). Therefore, prevention of HO includes not only prevention after the inciting trauma, but also prevention of recurrence in the 65% of war-wounded military personnel who already have HO (*3*). Patients with periarticular HO rarely regain complete range of motion and are often left with severely contracted joints (2-5). Additionally, current treatment strategies are inadequate and have severe side effects (6). Due to the tremendous impact of HO, we have a responsibility to develop novel preventative therapeutics that directly target central pathways. In the face of such staggering numbers and suboptimal treatments, a substantial need exists to prevent HO from forming by targeting the pathway of its formation. The central goal of this grant is to demonstrate the efficacy of a new treatment strategy that targets downstream bone morphogenetic protein (BMP) signaling. Specifically, we will analyze novel small molecule inhibitors of both canonical and non-canonical BMP signaling.

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

blast injury, blast overpressure, combat-related heterotopic ossification, amputation, femur fracture

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Goals for Specific Aim 1
1. Receive IACUC approval for Specific Aim 1
   - Due December 2016, completed 100% 12/1/16- UM protocol 5909
   - Due December 2016, completed 100% 12/1/16 NMRC protocol 15-OUMD-19S
   - Renewal of 15-OUMD-19S approval completed 4/1/18, NMRC protocol 18-OUMD-10S
2. ACURO approval for Specific Aim 1
   - Due December 2016, completed 100% 12/1/16
3. Perform longitudinal efficacy studies of NG-25 and LDN 212854 in rodent models of traumatic HO (30 mice, 30 rats)

- Due December 2017, UM burn/tenotomy completed 100% 10/1/16; NMRC blast/amputation completed 50%

4. Perform in vitro treatment of mouse and rat HO and control MSCs with therapetuics
   - Due December 2017, UM burn/tenotomy completed 100% 10/1/16; NMRC blast/amputation completed 25%
5. Bioinformatics and biostatistics processing of data
   - Due December 2017, UM burn/tenotomy completed 100% 10/1/16; NMRC blast/amputation completed 50%.

Goals for Specific Aim 2:

1. Procurement of tissue from blast and burn mediated HO at weekly time points (21 mice, 21 rats)
   - Due 9/1/2017; UM burn/tenotomy completed 80% 10/1/16; NMRC blast/amputation completed 25%
2. Histologic analysis of canonical and non-canonical signaling in traumatic HO: 80 mice, 80 rats.
   - Due 12/1 2017, UM burn/tenotomy completed 85% 10/1/16; NMRC blast/amputation completed 25%.
3. Pulse dose treatments in traumatic HO models; 80 mice, 80 rats (same as previous)
   - Due 5/1/2018. UM burn/tenotomy completed 75% 10/1/16; NMRC blast/amputation completed 0%.
4. Image guided treatment in traumatic HO models; 30 mice, 30 rats
   - Due 8/1/2018. UM burn/tenotomy completed 50% 10/1/16; NMRC blast/amputation completed 0%.
5. Final bioinformatics analysis
   - Due 8/1/2018. UM burn/tenotomy completed 0% 10/1/16; NMRC blast/amputation completed 0%.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Task 1: Performing efficacy studies of NG-25 and LDN-212854 in rodent models of traumatic HO.

a. Perform longitudinal efficacy studies of NG-25 and/or LDN-212854 in the rodent Burn/tenotomy model, assessed by range-of-motion and live animal microCT measurements.

Objective: To prophylax against HO in an animal model of trauma-induced HO with burn/tenotomy injury using small-molecule inhibition of non-canonical (TAK1) or canonical (ALK2) bone morphogenetic protein (BMP) signaling separately or as combination therapy.
Methodology: All animal procedures were carried out in accordance with the guidelines provided in the Guide for the Use and Care of Laboratory Animals from the Institute for Laboratory Animal Research (ILAR, 2011) and were approved by the Institutional Animal Care and Use Committee of the University of Michigan (PRO0007930). All animals were housed in IACUC-supervised facilities, not to exceed five mice housed per cage at 72°F±4°F, receiving 12 hours of light exposure each day, with no diet restrictions. For all *in vitro* and *in vivo* studies requiring wild type mice, young adult (6-8 weeks old) C57BL/6J mice were purchased from The Jackson Laboratory.

Partial-thickness scald burn injury was administered to animals according to a previously described protocol.(*16, 47*) Briefly, mice were anesthetized with inhaled isoflurane. Dorsal hair was closely clipped and an aluminum block heated to 60°C was exposed to the dorsal region over 30% of the total body surface area for 18 seconds to achieve a partial thickness burn injury. Each mouse then received a concurrent sterile dorsal hindlimb tendon transection at the midpoint of the Achilles tendon (Achilles' tenotomy) with placement of a single 5-0 vicryl suture to close the skin. Pain management was achieved with subcutaneous injections of buprenorphine (Buprenex; Reckitt Benckiser Pharmaceuticals Inc.) every 8-12 hours for 48 hours post operatively.

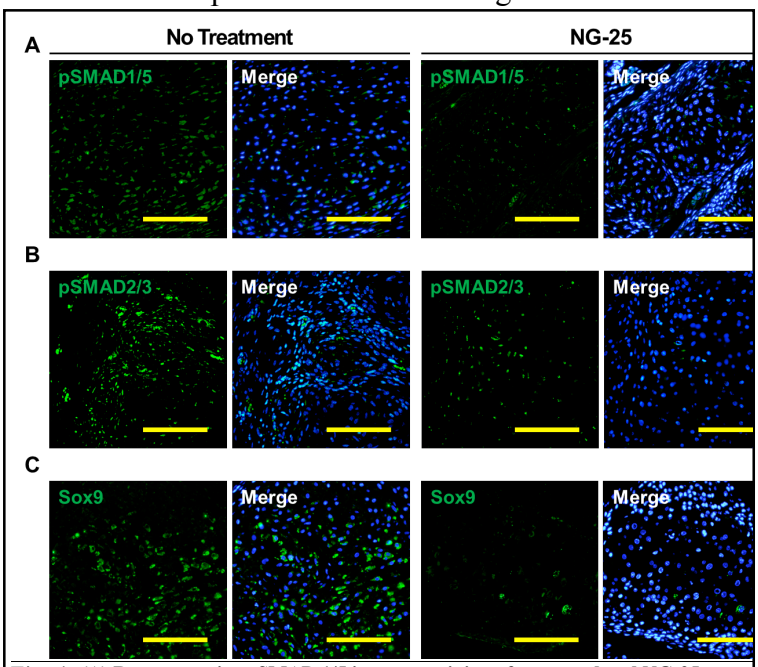


Fig. 1. **(A) Representative pSMAD 1/5 immunostaining of untreated and NG-25 treated mice 3 weeks after injury; (B) Representative pSMAD 2/3 immunostaining of untreated and NG-25 treated mice 3 weeks after injury; (C) Representative SOX9 immunostaining of untreated and NG-25 treated mice 3 weeks after injury. Scale bars = 100 μm**

Results:

Mice treated with NG-25, a TAK1 inhibitor demonstrated less downstream TAK1 signaling and less early pre-HO cartilage signaling (Fig. 1). This is an important validation that our therapeutic is working in the mechanism we hypothesized.

a. Perform longitudinal efficacy studies of NG-25 and/or LDN- 212854 in the rat blast/amputation model, assessed by histology and live animal microCT measurements.

Objective: To prophylax against HO in an animal model of trauma-induced HO with blast/amputation injury using small-molecule inhibition of non-canonical (TAK1) or canonical (ALK2) bone morphogenetic protein (BMP) signaling separately or as combination therapy.

Methodology: All animal procedures were carried out in accordance with the guidelines provided in the Guide for the Use and Care of Laboratory Animals from the Institute for Laboratory Animal Research (ILAR, 2011) and were approved by the Institutional Animal Care and Use Committee of the Naval Medical Research Center (15-OUMD-19S and 18-OUMD-10S). All animals were single housed in VSP-supervised facilities, not to exceed 72°F±4°F, receiving 12 hours of light exposure each day, with no diet restrictions. For adult (8-12 weeks old) rats were purchased from Taconic. Blast and amputation injury was administered to animals according to a previously described protocol (15-OUMD-19S). Briefly, rats were anesthetized with inhaled isoflurane and given Buprenex before receiving a 120 +/- 7 kPa blast. Rats were recovered and transferred to surgery room where they were anesthetized with isoflurane and received a fracture on the hind-limb femur followed by crush injury amputation through the zone of injury before being closed. Pain management was achieved with subcutaneous injections of buprenorphine SR every 48-72 hrs and then BID for 5-9 days or as needed.

Results:

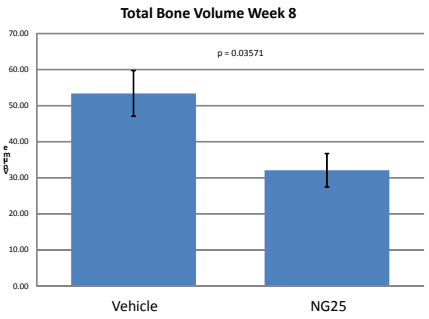


Fig. 2. Total new bone growth at 8 weeks post injury in vehicle or NG-25 treated animals. A reduction of 39.9% new bone volume (mm^3) was observed with NG25 treatment.

A twelve week observational study has been completed where rats treated with NG-25, a TAK1 inhibitor. At 8 weeks post injury vehicle controls showed 53.4 mm3 total new bone growth compared to a 39.9% reduction (32.1 mm3) in NG25 treated animals. (**Fig. 2**). Additional data at 8 and 12 weeks are still being analyzed.

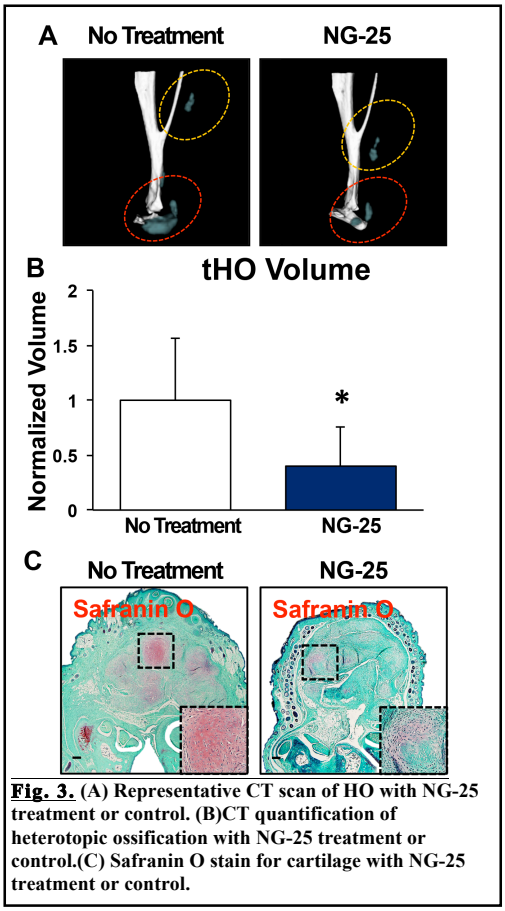


Fig. 3. (A) Representative CT scan of HO with NG-25 treatment or control. (B)CT quantification of heterotopic ossification with NG-25 treatment or control.(C) Safranin O stain for cartilage with NG-25 treatment or control.

Mice treated with NG-25, a TAK1 inhibitor demonstrated less end time point HO than control (**Fig. 3**). This is an important validation that our therapeutic is working in the mechanism we hypothesized.

Next we set out to validate that NG-25 treatment could be guided by BoneTag NIR imaging. We found that the ideal timepoint where we first detected HO by NIR imaging was at days 18 and 25 (**Fig. 4,5**). We thus set out the following experimental plan as described in Figure 6.

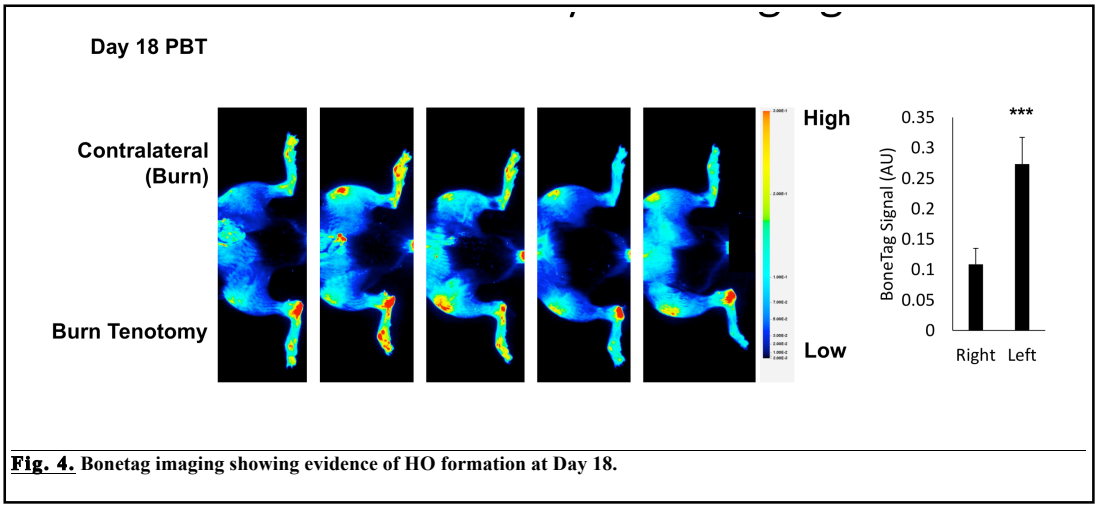


Fig. 4. Bonetag imaging showing evidence of HO formation at Day 18.

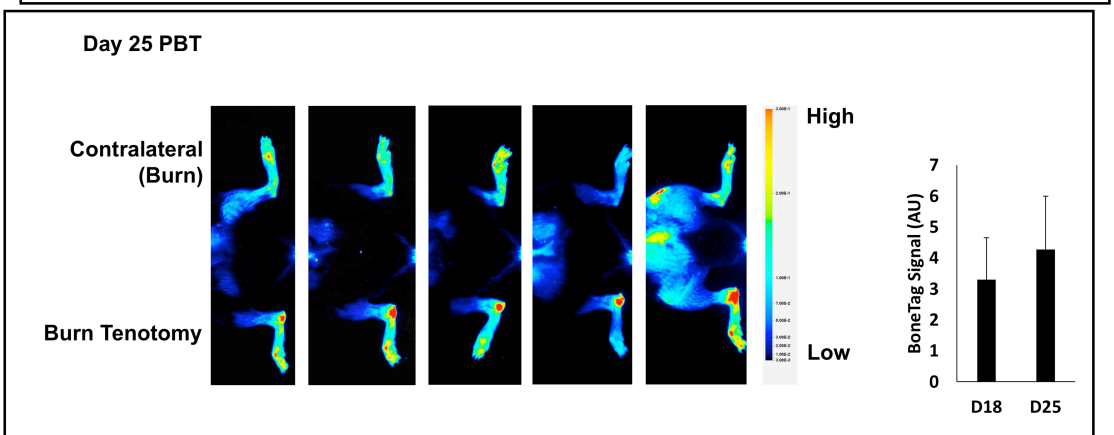


Fig. 5. Bonetag imaging showing evidence of HO formation at Day 25

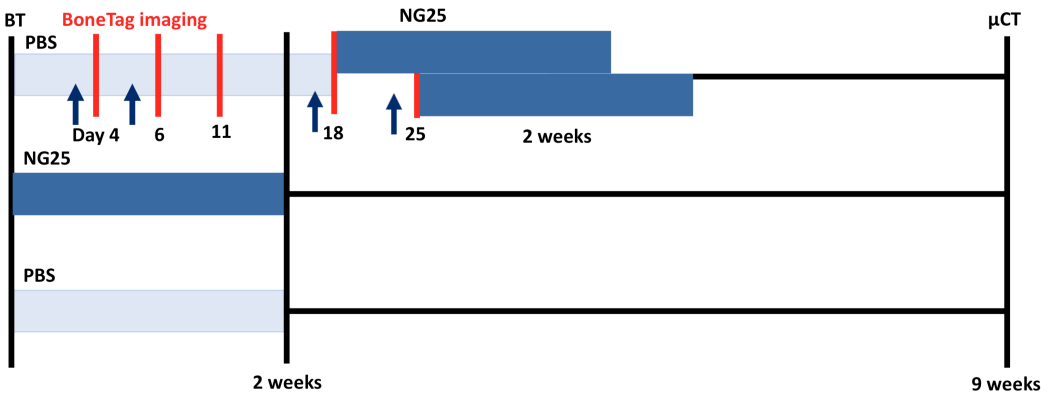


Fig. 6. Experimental plan

Major Task 2: Performing *in vitro* treatment of mouse and rat HO and control MSCs with NG-25 and/or LDN-212854 for chondrogenic and osteogenic differentiation studies.

Objective:

Methodology: MSCs were harvested from the soft tissue of the injured extremity. All tissue was mechanically minced, digested with collagenase A and dispase, and subsequently plated. Cells used were all passage 2 through 6. We used WT MSCs treated with NG-25 or vehicle control. For proliferation assays, cells were seeded in 12-well plates at a density of $5x10^3$ cells per well (n = 3). Cells were grown in standard growth medium. At days 1, 2, 4, and 6 the numbers of viable cells harvested cells following trypsin-EDTA treatment were manually enumerated using Trypan blue stain and a hemocytometer. Additionally, cell proliferation was assessed by bromodeoxyuridine (BrdU) incorporation. Cells obtained from wild type mice were treated with either DMSO or 5Z-7-Oxozeaenol during either proliferation or differentiation assays.

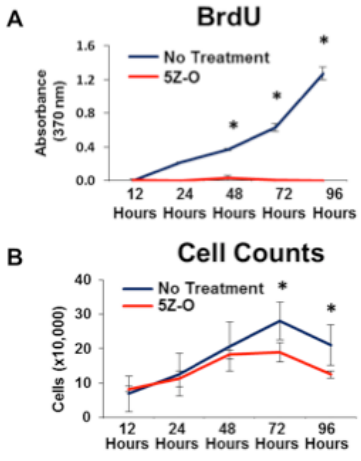


Fig. 7. ***In vitro*** **proliferation with pharmacologic inhibition of TAK1 using 5Z-7-Oxozeaenol (5Z-O). (A) BrdU assay of MSCs treated with TAK1 inhibitor 5Z-O or vehicle control. (B) Cell counting assay of MSCs treated with TAK1 inhibitor 5Z-O or vehicle control.**

Results: *In vitro,* mesenchymal stem cells (MSCs) isolated and treated with TAK1 inibitor exhibited markedly reduced proliferation (**Fig. 7**)

In vitro, mesenchymal stem cells (MSCs) isolated and treated with TAK1 inhibitor exhibited markedly reduced osteogenic differentiation and gene expression (**Fig. 8**) Both alkaline phosphatase stain and alizarin stain were decreased in NG-25

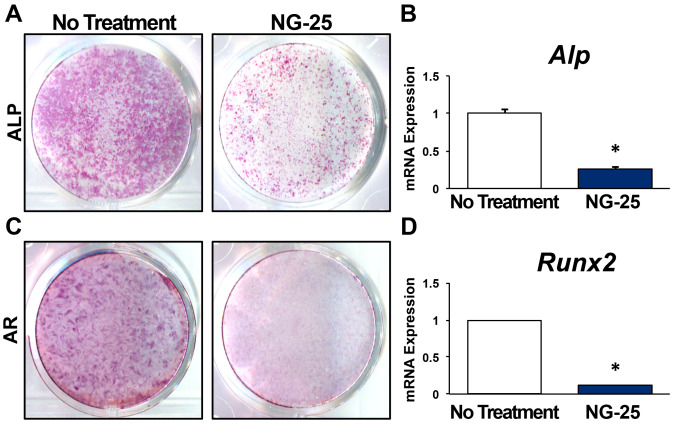


Fig. 8. **(A) Alkaline phosphatase stain with NG-25 or control. (B) Alp gene expression by qRT PCR in NG-25 treated and control. (C) Alizarin red stain with NG-25 treated or control. (D) Runx2 gene expression with NG-25 treatment or control.**

treated cells. To validate that this was due to TAK1 inhibition, we next used siRNA to knockout TAK1. Similar to our NG-25 treatment, TAK1 knockout by siRNA decreased *in vitro* osteogenic differentiation (**Fig. 9**).

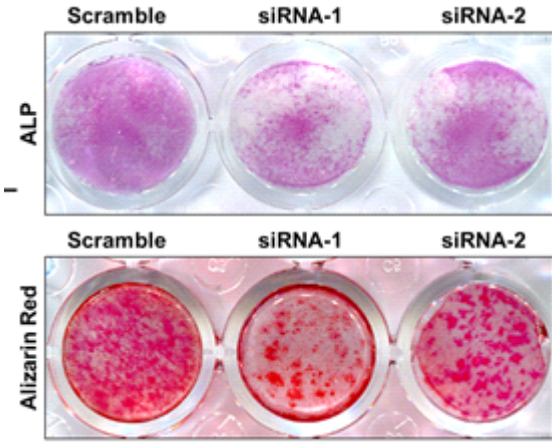


Fig.9. **(Top) Alkaline phosphatase stain of MSCs treated with TAK1 siRNA or control. (Bottom) Alizarin red stain (mineral deposition) of MSCs treated with TAK1 siRNA or control. Findings: TAK1 siRNA, like NG-25 decreases MSC osteogenic differentiation.**

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Post-doctoral fellows, plastic surgery residents and medical students who worked on this project were trained in animal surgery, trauma models, histology, CT imaging and bioinformatics. Additionally, they gained professional development by having the opportunity to attend research meetings, symposia, seminars, journals clubs and animal care training.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The findings in Aims 1 and 2 were presented at podium presentation at:
Plastic Surgery Research Council, Raleigh, NC, May, 2017
American Burn Association, Boston, MA, May 2017
American Association of Plastic Surgery, Austin, TX, April 2017
University of Michigan Moses Gunn Symposium, Ann Arbor, MI, May 2017

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

1. Continue studies to measure the impact of TAK1 and BMP inhibitor therapies (NG-25 and LDN-212854) in the tenotomy/burn induced heterotopic ossification model.
2. Perform analyses of osteogenic and chondrogenic signaling in both rodent HO models, using histology and imaging.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

TAK1 inhibitors are currently under investigation for their potential therapeutic role for other disease states including malignancy. In this study, we investigate TAK1 inhibitors to prevent heterotopic ossification. Our study also investigates combined inhibition with a highly specific BMP receptor inhibitor in order to provide a dual approach to the pSmad 1/5 signaling cascade – canonical (BMP receptor) and non-canonical (TAK1). Finally, our study investigates three different durations of treatment. We recognize the importance of minimizing treatment times to ensure adherence and minimize adverse effects.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

These pathways we are defining in heterotopic ossification can be translated to other traumatic bone injuries including fractures, osteoporosis and non-unions. Additionally, these findings can be translated to tissue engineering to improve bone formation which is of significant interest to plastic surgery, orthopedic surgery, neurosurgery, oral surgery and otolaryngology.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Upon completion of this project, we believe it is likely that BMP targeted therapies can be used in military and civilian settings to prevent heterotopic ossification. Additionally, these models and the idea of image guided therapy can be used as other therapies are developed.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

The effects of successfully preventing and treating heterotopic ossification are substantial. Preventing heterotopic ossification at the time of injury will radically decrease the need for revision surgeries in this population as well as decreasing pain making it easier for reintegration and redeoployment. In non-combat injuries such as hip replacements, traumatic brain injury, spinal cord injury, a strategy to prevent HO would also greatly improve patient quality of life.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

None

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

NMRC site has experienced delays during 2018 due to extensive construction work at the site which overran by 5 months resulted in the blast tube apparatus being unavailable for experiments and therefore the traumatic HO models were delayed. This was compounded by an additional several month delay in supply of osteogenic media due to manufacturer issues resulted in an additional delay of the rat trauma-HO model kinetics being completed. Supplies now available as of late October 2018 and kinetics groups are scheduled for completion prior to end of 2018 calendar year at NMRC.

Changes that had a significant impact on expenditures

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

Project is financially on track. No cost extension approved.

Expenditures to date:
$219,294 UM
$172,757 NMRC

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable

Significant changes in use of biohazards and/or select agents

None

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

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

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

Strategic Targeting of Multiple BMP Receptors Prevents Trauma-Induced Heterotopic Ossification.
Agarwal S, Loder SJ, Breuler C, Li J, Cholok D, Brownley C, Peterson J, Hsieh HH, Drake J, Ranganathan K, Niknafs YS, Xiao W, Li S, Kumar R, Tompkins R, Longaker MT, Davis TA, Yu PB, Mishina Y, **Levi B**.
Mol Ther. 2017 Aug 2;25(8):1974-1987. doi: 10.1016/j.ymthe.2017.01.008. Epub 2017 Jul 15.

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

Nothing to report

Other publications, conference papers and presentations.

1. Loder S, Agarwal S, Cholok D, Breuler C, Priest C, Hsieh S, Chung M, Li J, Brownley C, Peterson J, Habbouche J, Kaura A, Butts J, Reimer J, Drake J, Ucer S, Li S, Longaker M, Mishina Y, **Levi B**. *Simultaneous Targeting of Multiple BMP Receptors Prevents Trauma-Induced Heterotopic Ossification.* Moses Gunn 29th Annual Research Conference, Ann Arbor, MI. May 2017.
2. Agarwal S, Loder S, Cholok D, Breuler C, Chung M, Brownley C, Peterson J, Li J, Hsung H, Ranganathan K, Priest C, Li S, Mishina Y, **Levi B**. *A Translational Strategy Targeting Type I BMP Receptors to Prevent Heterotopic Ossification.* PSRC Annual Meeting 2017, Durham, North Carolina.
3. Loder S, Agarwal S, Hsung H, Cholok D, Chung M, Li J, Breuler C, Priest C, Ranganathan K, Habbouche J, Kaura A, Butts J, Li S, Mishina Y, **Levi B**. *Loss Of TGF-beta Activated Kinase (TAK1) Activity Induces Cellular Proliferation And Diminishes Differentiation During Bone Healing.* PSRC Annual Meeting 2017, Durham, North Carolina.

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

None

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

Nothing to report

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

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Personnel	Role	Months	Contribution
Benjamin Levi	PI	1.2	Guided experiments, analyzed data, helped write abstracts and manuscripts
Thomas A, Davis, PhD	Associate Investigator	0.6	Guided experiments, analyzed data, helped write abstracts and manuscripts
Devaveena Dey, PhD	Stem Cell Biologist	1.2	Performed experiments and analyzed data
Matthew Bradley, MD	Associate Investigator	0.6	Guided experiments, analyzed data, helped write abstracts and manuscripts
Allison Tomasino, BS	Research Associate/Surgical Tech	1.8	Performed experiments and analyzed data
Benjamin Wheatley	4th yr Orthopaedic Resident/Fellow	1.8	Performed experiments and analyzed data
Shuli Li	Research Scientist	6	Performed experiments and analyzed data
Amanda Huber	Research Investigator	6	Performed experiments and analyzed data
Kaetlin Vasquez	Lab manager	6	Performed experiments and analyzed data

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organization Name: Naval Medical Research Center – Regenerative Medicine Department
Location of Organization: 503 Robert Grant Ave. Silver Spring Maryland 20910
Partner's contribution to project: Collaboration

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

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

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Novel therapeutic small molecule strategy targeting bone morphogenetic protein signaling to prevent upper extremity heterotopic ossification.

PI: Benjamin Levi — **Org:** University of Michigan, Ann Arbor, MI
Co-PI: CDR Matthew Bradley — **Org:** Naval Medical Research Center, Silver Spring, MD

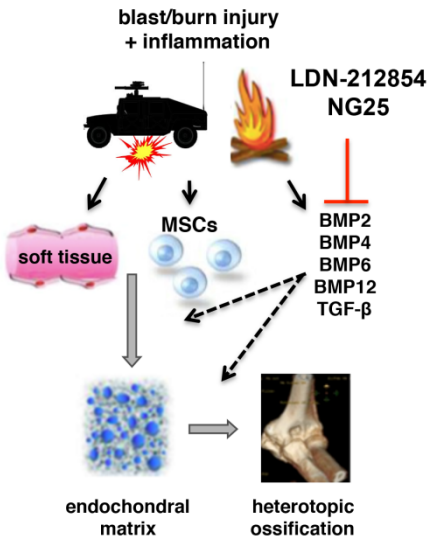
Award Amount: $500,000

Study/Product Aim(s)

The goals of this grant are to establish the efficacy of non-canonical and canonical BMP inhibition for HO prevention, and to optimize these therapies with respect to timing and duration using non-invasive, clinically translatable diagnostic methods to precisely identify the optimal timing of treatment. Specifically, we will inhibit TAK1 (non-canonical) and ALK2 (canonical) using the novel small molecule inhibitors NG-25 and LDN-212854 to prevent trauma induce-HO in animal models of military and civilian populations.

Approach

Specific aim 1: To prophylax against HO in two validated animal models of trauma-induced HO using small-molecule inhibition of non-canonical (TAK1) or canonical (ALK2) bone morphogenetic protein (BMP) signaling separately or as combination therapy.
Specific aim 2: To define and characterize the time course and stages of HO and to use novel imaging strategies to optimize treatment timing and duration.



Clinical use of recombinant BMP-2 is associated with heterotopic ossification (HO). Our data confirm a link between blast/burn injury and BMP signaling. We have found elevated levels of both TAK1 and ALK2 at sites destined for HO. We hypothesize that by targeting the specific BMP pathways involved, we can effectively prevent HO in models of trauma, and in a model of recurrence applicable to patients with excised HO.

Timeline and Cost

Activities / CY	16	17	18
Test inhibitory efficacy of small novel molecules (NG-25 and LDN-212854)			
Defining the timing of canonical and non-canonical BMP signaling during HO development			
Pulsed-dose and NIR-guided treatment with NG-25 and LDN-212854			
Requested Budget (500$K)	**$100K**	**$200K**	**$200K**

Goals/Milestones

CY16-17 Goals

- ❑ We will use the Achilles tenotomy-burn, recurrence, and blast-fracture rodent models to assess the effect of non-canonical and canonical BMP inhibition on HO formation *in vivo* using novel small molecules NG-25 and LDN-212854. (NMRC, UM)
- ❑ We will validate the ability of NG-25 and LDN-212854 to mitigate chondrogenic and osteogenic differentiation of mouse derived MSCs.

CY17-18 Goals

- ❑ We will define the stages of HO development with respect to histology and presence of non-canonical and canonical BMP signaling.
- ❑ We will test treatment with NG-25 and/or LDN-212854 during abbreviated periods of time, or guided by near-infrared (NIR) diagnosis of HO to minimize treatment duration. (NMRC,UM)

Comments/Challenges/Issues/Concerns

- N/A

Budget Expenditure to Date

Projected Expenditure: $0.5M
Actual Expenditure: $219,294 UM, $172,757 NMRC