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TITLE: Early Identification of Molecular Predictors of Heterotopic Ossification Following Extremity Blast Injury with a Biomarker Assay

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12. DISTRIBUTION / AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of this project is to identify predictive markers of heterotopic ossification in an established animal model that would forecast development of heterotopic ossification (HO) in humans soon after injury. Blast procedures have been completed on all 30 animals (Groups I & II) in the year 1 SOW and 45 animals (Groups III – V) in the year 2 SOW. All animals were biopsied and have been sacrificed according to protocol schedule. Groups I and II animals were also followed with scheduled routine radiographs to monitor progression of HO. Specimen samples are under analysis for gene and protein level expressions with the Nesti partnering molecular biology lab. In year 3, early-appearing gene and protein biomarkers will be identified by analyzing correlations with radiographic HO and will be compared to gene expression signatures in existing human tissue samples known to have gone on to develop HO.

15. SUBJECT TERMS
Heterotopic ossification, blast injury, amputation, bone formation, animal model, rat model, gene expression, protein expression, biomarkers

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Heterotopic ossification (HO), characterized by the pathologic formation of mature bone in the soft tissues, is a frequent complication following high energy orthopaedic trauma. HO is prevalent in patients with severe extremity war-time wounds; specifically, its incidence is reported as high as 57% in patients that sustain a poly-trauma blast injury [1]. Complications related to HO in residual limbs following blast amputation include pain, overlying skin and muscle breakdown, poor fitting and functioning of prosthetic limbs, reoperation for amputation revision, and impaired limb function that delays or limits rehabilitation [2-6]. Current treatments to prevent HO are limited to mitigation rather than prevention. Furthermore, removal of heterotopic bone after it has formed can be difficult; this frequently requires resection of substantial amounts of soft tissue and risks injury to adjacent neurovascular structures that are often intimately associated with the ectopic bone. Hence, it is preferable to address the issue of HO before it begins. Prevention of HO in residual limbs is needed to offer amputation survivors the best possible quality of life and return to function. We have developed a validated blast amputation animal model and confirmed that it replicates the human condition with respect to formation of HO. The current studies are directed at identifying early-appearing biomarkers in the animal model that predict the occurrence of HO in our experimental animals and may well similarly predict the development of HO in the human condition. Patients exhibiting biomarkers predictive of exuberant HO formation can then be identified before the disease process begins and treated prophylactically.

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

Heterotopic ossification, blast injury, amputation, bone formation, animal model, rat model, gene expression, protein expression, biomarkers

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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.

The project is on schedule as proposed and implemented at MUSC, our partnering institution. All hind-limb blast amputation procedures on 75 animals have been completed, as well as related scheduled biopsies as specified under Specific Aims 1 & 2. We have received the harvested specimens from MUSC and the specimens are currently undergoing RNA profiling using an osteogenesis PCR array to examine the correlation of osteogenic marker expression with radiographic HO findings. Human tissue sample collection from wounded service personnel as specified under Specific Aim 3 will start when USUHS IRB approval is obtained. Currently, the USUHS online IRB submission system, IRBnet, is transitioning to a new system and is expected to be online by JUNE 2016 (reported as March by MUSC). This results in a delay in the IRB approval process. However, the continuing review for the IRB has been submitted.
currently under review.

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.

Animal experiments completed on schedule and processing ongoing with data becoming available on a rolling basis as tissue samples are processed and analyzed. Correlation analysis will be performed as data collection from samples is more complete.

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.

Nothing to Report

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

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.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

RNA profiling to continue, human tissue testing (specific aim 3) on hold pending IRB approval

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

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

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

Nothing to Report

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

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

Nothing to Report

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

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

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

- *adoption of new practices.*

Nothing to Report

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

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.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

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.

Awaiting IRBnet replacement

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.

Limited spending due to IRB delay

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

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

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

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

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

Nothing to Report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable;*

bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report

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

Nothing to Report

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

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be 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. State whether an application is provisional or non-provisional and indicate the application number. 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;*
  - *biospecimen 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).*

Name: Leon Nesti
Project Role: PI
Nearest Person month worked: 1
Contribution: Supervision and leadership and coordination with MUSC and support of Senior Scientist

Name: Youngmi Ji
Role: Senior Scientist
Nearest Person month worked: 4
Contribution: working with MUSC on the RNA profiling and biomarker analysis by Western Blot

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.*

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8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site.

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.

IMPORTANT – this page contains unpublished data that should be protected

1. HO radiographic data – Group I & I animals. (Provided by MUSC)

Rat #	Biopsy Time	Post-op Radiographic Measurements (mm)		Postmortem Radiographic Measurements (mm)		%L	%W	HO Grade			HO Severity Score
		Length	Width	Length	Width			L	W	Overall	
1	2 weeks	8.85	10.9	11.3	12.1	27.7	11.0	moderate	mild	moderate	2
2	2 weeks	13.7	8.98	15	12.3	9.5	37.0	mild	moderate	moderate	2
3	2 weeks	11.6	16.8	13.1	17.8	12.9	6.0	mild	mild	mild	1
4	2 weeks	12.4	10.2	14.7	10.5	18.5	2.9	mild	mild	mild	1
5	2 weeks	15	6.66	12.7	7.38	-15.3	10.8	mild	mild	mild	1
6	2 weeks	15	11.9	12.6	7.94	-16.0	-33.3	mild	mild	mild	1
7	2 weeks	12.7	9.36	11.9	10.3	-6.3	10.0	mild	mild	mild	1
8	2 weeks	15.8	7.35	19.3	9.04	22.2	23.0	mild	mild	mild	1
9	2 weeks	8.87	10.8	10.9	12.1	22.9	12.0	mild	mild	mild	1
10	2 weeks	9.83	17.5	15.4	13	56.7	-25.7	severe	mild	severe	3
11	2 weeks	8.01	10.6	5.82	8.31	-27.3	-21.6	mild	mild	mild	1
12	2 weeks	9.89	7.64	8.31	9.88	-16.0	29.3	mild	moderate	moderate	2
13	2 weeks	9.36	10.7	14.8	9.82	58.1	-8.2	severe	mild	severe	3
14	2 weeks	14.9	9.04	15.9	8.63	6.7	-4.5	mild	mild	mild	1
15	2 weeks	10.1	10.7	12.7	10.8	25.7	0.9	moderate	mild	moderate	2
16	4 weeks	8.93	8.13	11.4	8.35	27.7	2.7	moderate	mild	moderate	2
17	4 weeks	15.8	11.1	17	11	7.6	-0.9	mild	mild	mild	1
18	4 weeks	11.5	12.6	8.41	12.7	-26.9	0.8	mild	mild	mild	1
19	4 weeks	7.39	8.29	5.81	11.8	-21.4	42.3	mild	moderate	moderate	2
20	4 weeks	13.5	10.3	15.2	11.6	12.6	12.6	mild	mild	mild	1
21	4 weeks	15.1	8.05	15.3	15.3	1.3	90.1	mild	severe	severe	3
22	4 weeks	16.7	8.29	19.7	16.2	18.0	95.4	mild	severe	severe	3
23	4 weeks	13.5	12.8	14.2	11.1	5.2	-13.3	mild	mild	mild	1
24	4 weeks	9.25	14.8	10.7	18.6	15.7	25.7	mild	moderate	moderate	2
25	4 weeks	19.7	10.6	16.8	16.2	-14.7	52.8	mild	severe	severe	3
26	4 weeks	15	7.81	17	9.68	13.3	23.9	mild	mild	mild	1
27	4 weeks	10.1	20	13.3	19.6	31.7	-2.0	moderate	mild	moderate	2
28	4 weeks	9.41	9.09	9.11	9.25	-3.2	1.8	mild	mild	mild	1
29	4 weeks	20	8.26			0.0	0.0	mild	mild	mild	1
30	4 weeks	15.8	7.19	13.9	15.7	-12.0	118.4	mild	severe	severe	3

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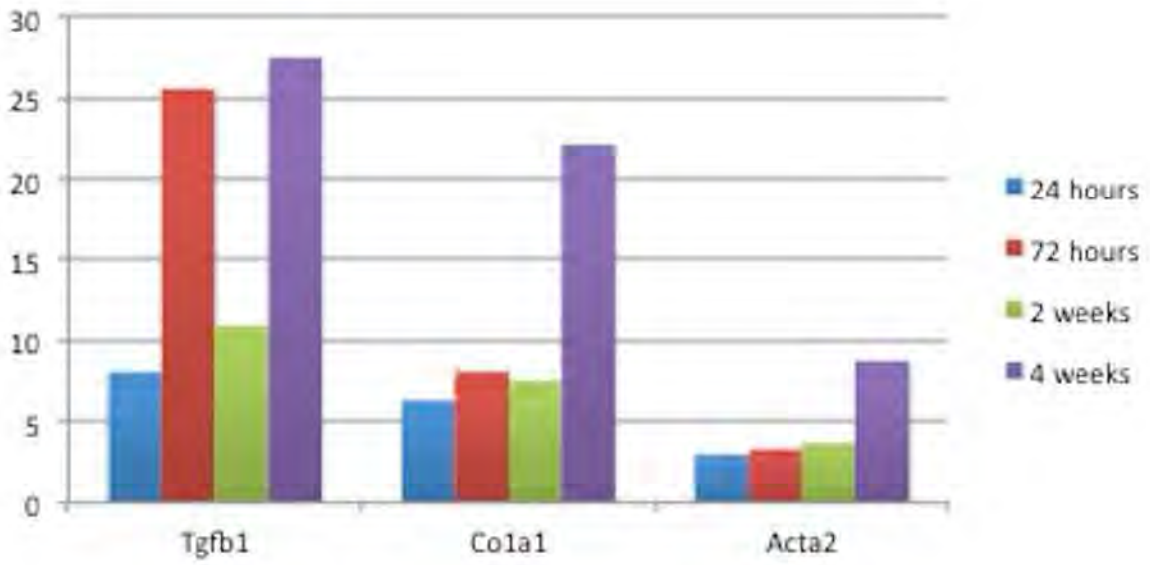
2. Biomarker expression of animal biopsy specimens by qRT-PCR (provided by USUHS)

Biopsy Time points	Fold Changes		
	Tgfb1	Co1a1	Acta2
24 hr BBS	8.005	6.286	2.917
72 hr BBS	25.533	8.034	3.236
2 week BBS	10.9	7.462	3.622
4 week BBS	27.446	22.058	8.704



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3. Biomarker expression of animal biopsy specimens by western blot (provided by USUHS)