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

PRINCIPAL INVESTIGATOR: Dr. Leon Nesti

CONTRACTING ORGANIZATION: Henry M. Jackson Foundation for the Advancement of Military Medicine
Bethesda, MD 20817

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Dr. Leon Nesti

E-Mail: leon.nesti@usuhs.edu

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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 were analyzed for gene and protein level expression with the Nesti partnering molecular biology lab. Early-appearing gene and protein biomarkers were identified by correlation between animals exhibiting radiographic evidence of 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
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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 between 57-63% in patients that sustain a poly-trauma blast injury [1,2]. 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 [3-7]. 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 determine if a correlation exists to 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.

All 75 hind-limb blast amputation procedures under Specific Aims 1 & 2 in year 1 & 2 SOW (Groups I – V) have been completed, and all 150 specimens from both amputated and contralateral control limbs have been collected. Group I and II animals (15 each) were followed with serial radiographs to monitor progression of HO and sacrificed at 24 weeks post-blast. Group I animals underwent bilateral muscle biopsy procedure at two weeks, while Group II animals underwent biopsy procedure at four weeks. Group III – V animals (15 each) were biopsied at 24 hours, 24 hours, and 72 hours, respectively, and sacrificed at the same time as biopsy procedures, as per protocol. All animals, except those in Group V, underwent standard wound care with bulb syringe irrigation prior to wound closure following blast amputation. Prior to wound closure, group V animals underwent pulsed lavage irrigation. The biopsy specimens were processed to collect total RNAs and protein lysates for both gene- and protein-level biomarkers.

Results: HO progression has been assessed and graded between immediate post-blast and post-mortem radiographs on Group I & II animals. **Radiographic HO data acquired from Group I & II animals and biomarker expression data are included in appendix a. Supporting Data.**

- HO progression has been assessed and graded between immediate post-blast and post-mortem radiographs on Group I & II animals. Radiographic HO data acquired from Group I & II animals are included in **Figure 1**.
- Rat biopsy samples at 2 weeks, 4 weeks, 24h, and 72h post-injury were used for RNA biomarker screening.
    - The Wound healing and Osteogenesis pathway specific PCR Array, which contains 84 genes, was performed.
    - Data analysis was performed using the RT^2 Profiler PCR Array Data Analysis software (SABiosciences) and Venn Diagram analysis.
    - From these analyses, we found that many of genes in Wound healing pathway were related to fibrosis and inflammation (**Figure 2**) and as a result, we extended our analysis using the Fibrosis pathway specific PCR array.
    - We generated a list of genes with significantly altered expression (fold change ≥2) from each of these stages and applied it to a Venn diagram analysis.
    - We found that 47 genes overlapped among these four stages (**Figure 3A**). **Figure 3B** shows the list of 47 genes.
    - From these 47 genes, we categorized 3 patterns:
        - First, common genes (7: Bcl2, Cxcr4, Grem1, Itgav, Mmp14, Mmp2, and Tgfbr2) showed the increased gene expression pattern through the stages (**Figure 4**).
        - Second, Common genes (13: Ccl12, Ccl3, Hgf, Lox, Mmp3, Nfkb1, Plat, Serpinh1, Snai1, Stat6, Tgfb1, Thbs2, and Tnf) showed the same gene expression pattern between the early stages (24h and 72h) and late stages (2 weeks and 4 weeks) (**Figure 5**).
        - Third, Common genes (13: Akt1, Ccr2, Eng, Il10, Ilk, Itga2, Itgb3, Itgb6, Plau, Serpine1, Smad2, Thbs1, and Timp1) showed the different gene expression pattern between the early stages (24h and 72h) and late stages (2 weeks and 4 weeks) (**Figure 6**).
    - The first pattern may be used as prognostic markers of HO development.
    - The second and third pattern may be used as a marker for early detection of HO.

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).

Up-regulation of genes in the Sprague-Dawley rat contributing to fibrosis and inflammation have been correlated with the development of heterotopic ossification after traumatic blast amputation in an animal model. Correlation of similar gene expression in human specimens from the partnering PI lab may provide insights into mechanisms of HO that are operative following blast injury in humans. These observations may identify mechanisms that are potentially modifiable by therapeutic interventions designed to mitigate heterotopic ossification after blast injury.

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

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

Nothing to Report

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.

Nothing to Report

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).*

Manuscript in Preparation

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
Contribution: Supervision and leadership and coordination with MUSC and support of Senior Scientist

Name: Youngmi Ji
Role: Senior Scientist
Contribution: working with MUSC on the RNA profiling and biomarker analysis

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.*

Medical University of South Carolina Department of Orthopaedics 96 Jonathan Lucas Street Suite 708 MSC 622 Charleston SC 29425-8908

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.

MUSC has independently submitted a duplicative report, tasks have been clearly marked with the responsible PI.

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.

References:

1. Potter, B.K., T.C. Burns, A.P. Lacap, R.R. Granville, and D.A. Gajewski, Heterotopic ossification following traumatic and combat-related amputations. Prevalence, risk factors, and preliminary results of excision. *J Bone Joint Surg Am*, 2007. 89(3): p. 476-86. [PMID: 17332095]
2. Alfieri KA, Forsberg JA, Potter BK. Blast injuries and heterotopic ossification. *Bone & Joint Research*. 2012;1(8):174-179. doi:10.1302/2046-3758.18.2000102.
3. Andersen, R.C., H.M. Frisch, G.L. Farber, and R.A. Hayda, Definitive treatment of combat casualties at military medical centers. *J Am Acad Orthop Surg*, 2006. 14(10 Spec No.): p. S24-31. [PMID: 17003202]
4. Covey, D.C., Combat orthopaedics: a view from the trenches. *The Journal of the American Academy of Orthopaedic Surgeons*, 2006. 14(10 Spec No.): p. S10-7. [PMID: 17003178]
5. Dudek, N.L., M.N. DeHaan, and M.B. Marks, Bone overgrowth in the adult traumatic amputee. *American journal of physical medicine & rehabilitation / Assoc of Academic Physiatrists*, 2003. 82(11): p. 897-900. [PMID: 14566159]
6. Owens, B.D., J.C. Wenke, S.J. Svoboda, and D.W. White, Extremity trauma research in the United States Army. *The Journal of the American Academy of Orthopaedic Surgeons*, 2006. 14(10 Spec No.): p. S37-40. [PMID: 17003204]
7. Potter, B.K., T.C. Burns, A.P. Lacap, R.R. Granville, and D. Gajewski, Heterotopic ossification in the residual limbs of traumatic and combat-related amputees. *J Am Acad Orthop Surg*, 2006. 14(10 Spec No.): p. S191-7. [PMID: 17003198]

Supporting Data:

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

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

(A)

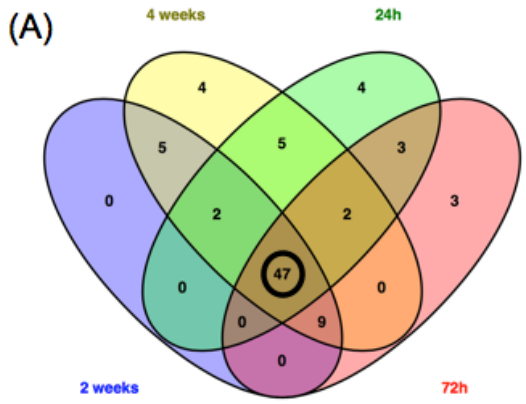
Venn Diagram

(B)

Common elements (32) gene list

Ccl12	Ctgf	Mmp2	Itga5
Ccl7	Egfr	Mmp9	Itgb3
Col4a1	F3	**Timp1**	Plat
Col5a1	Hgf	Ptgs2	Plau
Col5a3	Il10	Serpine1	Actb
Cxcl1	Il1b	**Tgfb1**	Fgf2
Cxcl3	**Tnf**	Tgfbr3	Hbegf
Cxcl5	Plaur	**Wisp1**	Itgb6

Figure 2. Wound Healing pathway specific PCR array. (A) Venn Diagram analysis. The gene lists were generated from different stages (FC≥2, P≤0.05) and applied to the Venny website (*Oliveros, J.C. (2007) VENNY. An interactive tool for comparing lists with Venn Diagrams.* http://bioinfogp.cnb.csic.es/tools/venny/index.html). 32 genes commonly appeared in 4 different stages. (B) A list of the common genes (32). The genes that were related to fibrosis and inflammation are in bold. (USU)



(B)

Gene Symbol (47)			
Akt1	Hgf	Mmp13	Smad2
Bcl2	Il10	Mmp14	Snai1
Ccl12	Il1a	Mmp2	Stat6
Ccl3	Il1b	Mmp3	Tgfb1
Ccr2	Ilk	Mmp8	Tgfbr2
Ctgf	Itga2	Mmp9	Thbs1
Cxcr4	Itga3	Myc	Thbs2
Dcn	Itgav	Nfkb1	Timp1
Edn1	Itgb1	Plat	Tnf
Eng	Itgb3	Plau	Actb
Faslg	Itgb8	Serpine1	Itgb6
Grem1	Lox	Serpinh1	

Figure 3. Fibrosis pathway specific PCR array Analysis. (A) Venn Diagram analysis. The gene lists were generated from different stages (FC≥2) and applied to the Venny website (*Oliveros, J.C. (2007) VENNY. An interactive tool for comparing lists with Venn Diagrams.* http://bioinfogp.cnb.csic.es/tools/venny/index.html.). 47 genes commonly appeared in four different stages. (B) List for common elements (47) genes. (USU)

↗	24h		72h		2 weeks		4 weeks	
Gene Symbol	Fold Regulation	p-value	Fold Regulation	p-value	Fold Regulation	p-value	Fold Regulation	p-value
Bcl2	2.014	6.50E-02	3.314	3.12E-04	6.886	3.00E-05	7.872	4.00E-06
Cxcr4	4.133	3.50E-02	8.504	2.12E-02	12.808	8.00E-05	13.793	1.53E-03
Grem1	15.263	4.92E-02	28.753	1.54E-02	89.793	4.09E-02	126.962	2.72E-02
Itgav	3.134	1.28E-02	3.429	2.42E-04	5.457	6.99E-04	7.008	4.30E-05
Mmp14	6.296	1.03E-02	19.062	4.00E-06	23.138	1.11E-02	97.951	8.00E-05
Mmp2	5.527	7.29E-03	7.354	1.11E-04	15.286	9.75E-02	27.820	1.14E-03
Tgfbr2	2.919	2.71E-02	6.183	8.70E-05	6.207	4.27E-03	10.259	3.40E-05

Figure 4. Common genes (7) showed the increased gene expression pattern through the stages with significant p-value (at least from three stages, P≤0.05) and fold change (FC≥2). (USU)

↑↑ ↓↓	24h		72h		2 weeks		4 weeks	
Gene Symbol	Fold Regulation	p-value	Fold Regulation	p-value	Fold Regulation	p-value	Fold Regulation	p-value
Ccl12	51.173	2.78E-02	91.460	5.50E-05	29.370	7.79E-02	67.236	7.06E-03
Ccl3	195.431	2.14E-01	60.676	9.29E-04	397.456	3.21E-03	282.620	4.42E-04
Hgf	3.279	1.51E-02	14.182	4.38E-04	14.037	1.61E-04	20.143	9.22E-04
Lox	21.549	2.89E-04	27.376	2.94E-04	11.133	6.33E-03	23.355	2.04E-04
Mmp3	146.574	2.34E-01	5.860	4.98E-02	1194.924	5.03E-03	455.079	5.02E-04
Nfkb1	3.093	4.88E-02	4.695	1.20E-03	3.050	6.60E-02	5.162	6.80E-04
Plat	6.281	9.11E-03	6.545	1.26E-02	5.254	1.63E-02	10.693	1.98E-03
Serpinh1	3.582	1.70E-02	11.073	1.07E-02	4.270	5.89E-02	10.077	6.04E-03
Snai1	17.689	2.57E-03	19.809	1.10E-05	6.131	5.32E-02	17.265	5.20E-03
Stat6	8.140	1.68E-03	9.408	1.00E-06	6.575	8.34E-03	10.234	1.38E-04
Tgfb1	10.354	3.08E-04	38.460	2.84E-04	31.176	5.52E-04	43.768	1.48E-04
Thbs2	12.423	4.56E-02	43.203	0.00E+00	30.660	1.68E-01	70.204	3.13E-04
Tnf	21.861	7.06E-03	21.732	7.00E-06	22.749	1.00E-06	19.584	1.89E-02

Figure 5. Common genes (13) showed the same gene expression pattern between the early stages (24h and 72h) and late stages (2 weeks and 4 weeks) with significant p-value (at least from three stages, P≤0.05) and fold change (FC≥2). (USU)

↑↓ ↓↑	24h		72h		2 weeks		4 weeks	
Gene Symbol	Fold Regulation	p-value	Fold Regulation	p-value	Fold Regulation	p-value	Fold Regulation	p-value
Akt1	4.313	1.20E-01	4.107	2.54E-02	2.946	3.76E-02	4.649	6.39E-04
Ccr2	7.214	4.92E-02	18.349	5.25E-03	19.935	2.07E-04	17.751	1.23E-03
Eng	10.150	1.39E-02	6.689	7.45E-03	3.342	3.04E-02	8.054	2.33E-04
Il10	56.495	3.26E-03	50.854	3.61E-04	31.282	6.80E-03	46.560	1.78E-04
Ilk	2.297	4.55E-03	2.201	9.42E-03	2.402	1.16E-02	3.173	9.45E-03
Itga2	4.387	6.72E-02	3.615	5.09E-03	2.923	1.44E-02	4.752	1.97E-03
Itgb3	15.987	3.02E-02	12.569	2.52E-02	20.768	1.37E-02	45.752	2.55E-04
Itgb6	-7.294	1.11E-02	-4.657	1.36E-02	-2.189	5.61E-02	-2.949	2.57E-02
Plau	5.082	2.63E-02	4.831	1.30E-05	3.399	2.34E-03	6.018	1.63E-04
Serpine1	236.609	2.54E-02	40.772	1.17E-02	22.938	5.47E-02	28.870	2.32E-02
Smad2	2.542	2.31E-03	2.265	6.42E-04	2.287	1.61E-02	3.456	9.30E-05
Thbs1	212.522	1.52E-01	50.852	7.99E-03	10.892	3.50E-05	32.504	4.38E-03
Timp1	71.977	6.69E-03	26.177	4.40E-05	18.370	6.00E-06	20.014	1.68E-04

Figure 6. Common genes (13) showed the different gene expression pattern between the early stages (24h and 72h) and late stages (2 weeks and 4 weeks) with significant p-value (at least from three stages, $P \leq 0.05$) and fold change ($FC \geq 2$). (USU)

A Final Quad Chart is also provided (USU)

Early Identification of Molecular Predictors of Heterotopic Ossification following Extremity Blast Injury with a Biomarker Assay

OR120071PI Translational Research Partnership **Award: W81XWH-13-2-0083**

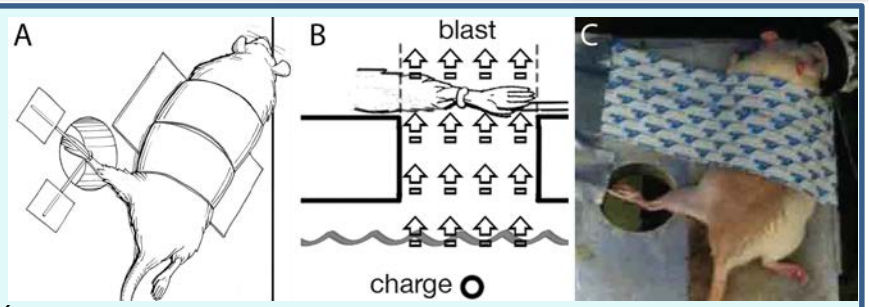
PI:Leon Nesti MD, PhD **Org: Henry M. Jackson for the Advancement of Military Medicine** **Award Amount: $434,497.00**

Study/Product Aim(s)

1) *To correlate gene- and protein- level expression related to osteogenesis in the animal model and human tissue.*
2) *To identify early-appearing gene- and protein-level expression in the animal model that predicts eventual development of human HO.*
3) *To validate early-appearing biomarkers to predict development of HO.*

Approach

Our **hypothesis** is that the biologic processes that characterize heterotopic ossification in a blast amputation model in the Sprague-Dawley rat will closely resemble those observed in battle-injured soldiers. Correlation of animal and human HO findings will allow identification of common biomarkers that are present early in the process and are predictive of HO formation in wounded soldiers at greatest risk. These high-risk individuals would ultimately be enrolled in a clinical trial of therapeutic interventions known to effectively prevent HO in the civilian setting.



(A) Schematic demonstrating the position of the rat during blast treatment, and (B) cross-sectional schematic of pressure wave that generates the traumatic amputation. (C) A representative image of a rat prior to blast treatment.

Status Update: We received the approval of IRB and HRPO on this study and waiting for CRADA placing in among HJF, WRNMMC, USUHS, and MUSC. All initial blast specimens have been received. Prepared and set up the conditions for RNA and protein isolation and analysis to use biopsy samples from the rats. RNA samples from different stages were applied to the pathway specific array (e.g. Wound healing and Osteogenesis) and analyzed the data. Currently, microRNA profiling is progressing.

Timeline and Cost

Activities CY	13-14	15	16	17
Task 1) Correlate HO biomarkers in animal model and human tissue				
Task 2) Identify early animal HO biomarkers that predict human HO				
Milestone 1; Mx comparing molecular HO mechanisms in animal & humans.				
Task 3) Validate HO predictive value of early post-blast tissue biomarkers				
Estimated Budget ($434K)	**$0**	**$98**	**$140**	**$196**

Updated: 03/31/2018

Goals/Milestones

CY13/14 Goal – Receive and process MUSC blast specimens.

- ☐ Blast specimens have been received
- ☐ Blast specimens have been initially processed.

CY15 Goal – Animal / human biomarker correlation

- ☐ Correlation of animal HO biomarkers in existing late human tissue
- ☐ Identify early animal biomarkers that might predict human HO

CY16 Goal – Predictive early biomarker animal/human correlation

- ☐ Identify early predictive animal biomarkers in early human tissues
- ☐ Validate predictive value of early HO biomarkers in humans

CY17 Goal – Observational human clinical biomarker validation

- ☐ Enrollment completion and human data analysis for HO biomarkers

Comments/Challenges/Issues/Concerns

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Budget Expenditure to Date

Projected Expenditure: $434,497 Actual Expenditure: $416,484