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TITLE: A Systems Biology Approach to Radiation Biodosimetry and the Host-Environment Interaction: Applications to Mass Casualty Triage in the Polytrauma Patient

PRINCIPAL INVESTIGATOR: Rasha Hammamieh, PhD

CONTRACTING ORGANIZATION: Geneva Foundation, Tacoma, WA

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Rasha Hammamieh, PhD

E-Mail: rasha.hammamieh1.civ@health.mil

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The Geneva Foundation
917 Pacific Avenue, Suite 600
Tacoma, WA 98402

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Historically, nuclear events, such as the accidental meltdown at Chernobyl, or the bombing of Nagasaki and Hiroshima, are coupled with blasts such that individuals end up with combined injuries of radiation exposure and burn, among other polytraumas. Proper diagnosis of dual injuries (i.e., thermal burn with radiation exposure) is important for determining optimal treatments quickly and correctly to ensure the best positive long-term health outcomes. Moreover, early identification and treatment would be especially valuable for our military and first responders who may suffer the greatest exposures in these scenarios. However, diagnosis in mass casualty situations are characterized by limited resources and availability of healthcare experts. Specifically, first-responders, who have limited experience working with these injury types, will likely be responsible for triaging patients. As such, this proposal aims to address this gap by working towards improving our understanding of these co-injury types and, most importantly, identifying specific biomarkers candidates to call out radiation and burn. In the future, these biomarkers will be integrated into a simple, rapid diagnostic device to enable first-responders and military medics to diagnose these injuries quickly and correctly without any significant expertise. To achieve such a goal, mouse models will be used because these resources are invaluable for unraveling details and demonstrating clear linkages between biological molecules and clinical phenotypes. Additionally, human clinical samples from individuals undergoing radiotherapy will also be collected and analyzed, then integrated with the data mined from the animal models to increase the likelihood of success. Overall, identification of specific biomarkers of radiation and burn injury polytrauma will, in the future, enable responders to make a determination about injury type quickly, which will lead to faster treatments and could save many lives in future incidents.

15. SUBJECT TERMS

Polytrauma, burn wounds, radiation, mouse model, human, biomarker, early signatures

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

In recent years, global events have brought potential mass casualty incidents involving large-scale injury, trauma, and a variety of exposure scenarios to the forefront of our defense and social thinking. We will use both clinical patients and rodent preclinical model under this funding mechanism. This animal model is developed by our collaborator at MedStar Washington Hospital Center (MWHC), to examine existing and novel biomarkers of radiation exposure at varying levels in hair, skin, blood, and major organs. Though the emphasis will be on pan–omic work, we will also implement histopathology and IHC to elucidate relationships between biomarker change and potential clinical impacts. A focus will be on identification of candidate markers (individual markers and/or biological networks) that are obtained least invasively (i.e., blood or hair) and therefore can be applied readily to the field for rapid assessment of military and civilian populations that have potential exposure. This pre-clinical knowledge will inform us to find translational potential markers for this polytrauma model.

Study Design. Mice (C57BL6) will receive a burn injury of either 10% or 20% TBSA, or an exposure to radiation of 0-1 Gy. Two experimental groups will receive both types of injury in combinations of the described exposure/injury severities. Animals will be monitored subsequently, and euthanized at 2 hours, 12 hours, 1, 2, 3, 4, 7, 14, or 21 days post-injury/exposure. Blood, saliva, feces, hair, skin, and other organs will be collected and preserved. DNA and RNA will be isolated from blood, skin, and other samples for use in DNA methylation arrays and mRNA/miRNA microarrays. Hair bulbs will be stained for presence of a commonly studied radiation biomarker, histone H2AX gamma. Data will be analyzed to identify candidate biomarkers molecules of each injury type versus candidates for combination injury. In parallel, the wound samples from patients undergoing radiotherapy will be obtained and multi-omics analysis will be processed. Candidate markers conserved across mouse and human will be of our particular interest.

Relevance. By utilizing a multi-omic approach, we will be able to provide a precision medicine perspective on triaging combined radiation and thermal trauma that has not been previously studied. These markers will be examined in easily obtained specimens for ready application to the field where minimally invasive human sample acquisition will be critical for simple and rapid assessments.

2. KEYWORDS:

Polytrauma, burn wounds, radiation, mouse model, human, biomarker, early signatures

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1: Biomarker Identification in preclinical animal model
1) Identify the signature molecular biomarker modulation that occurs in animals exposed to variable doses of X-ray radiation.
2) Identify the signature molecular biomarker modulation that occurs in animals after 10% or 20% TBSA burn injuries.
3) Identify the signature molecular biomarker modulation that occurs in animals with a combination of both of the above injuries.

Aim 2: Biomarker Detection Timeline Assessment
4) Determine whether there a dose-dependent and time-dependent response that can be seen with this molecule(s).

Aim 3: Biomarker Sample Source Optimization
5) Determine whether this biomarker signal can be detected strongly enough in the least-invasively obtained sample (saliva, then feces/urine, then blood, then skin biopsy) to make it translatable to mass casualty/field triage.

Aim 4: Refine the biomarker panel using human blood samples
6) Validate and/or refine the biomarker panel using the blood samples collected from human patients exposed to this polytrauma paradigm. Human blood samples collected from the patients and the age/race matched controls, respectively will be used for this project.

What was accomplished under these goals?

1) Protocol [ACURO Assigned Number]: BA190153.e001

Title: Refining Early Biomarkers for Detecting Radiation Exposure and Thermal Injury Mass Casualty Triage Applications

Target required for statistical significance: 360

Target approved for statistical significance: Complete

First set of tissues was transferred from MedStar WHC to WRAIR and the inventory was created.

Injury Group	H2	H12	Day 1	Day 3	Day 6
0.5 gray	n=4	n=8	n=8	n=7	n=4
1 gray	n=4		n=4	n=4	n=2
10% burn	n=8		n=4	n=2	
20% burn			n=6		
0.5 gray+ 10% burn	n=8	n=4	n=8	n=7	
0.5 gray+ 20% burn	n=4				
1 gray+ 10% burn					
1 gray+ 20% burn					
Sham	n=8	n=4			

n=8 animals/group
done
group partially done
group not started
Necropsy expected

2) Protocol [HRPO Assigned Number]: Proposal Log Number BA190153; HRPO Log Number: E01724.1a. Title: A Systems Biology Approach to Radiation Biodosimetry
Target required for clinical significance: 20
Target approved for clinical significance: 20
Patient screening began 9/13/22. During the reporting period 38 patients were screened and one patient is enrolled.

3) Teams from WRAIR and MedStar WHC are in regular contact to streamline this effort.

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

By next reporting cycle, we should be able to accomplish the following:
(a) A gene expression analysis of a set of animal cohort to understand the impact of polytrauma
(b) Generate of first set human clinical sample set

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

We anticipate that the present deliverables will include specific and time-independent biomarkers for radiation injury, with a particular focus on those identified from easily obtained biological samples. Additionally, the molecules selected, DNA and proteins, are known to be assayed rapidly and in austere conditions. The long-term objective is to develop a rapid handheld device (such as lateral flow dipstick assays) targeting the molecules (i.e., biomarkers) identified from the present proposal. Such handheld devices will be on high demand during emergencies or on the future battlefield, when victims need to be screened in a timely manner.

What was the impact on other disciplines?

Present work plans to integrate animal and human model to find biomarkers of clinical potential. Thereby, this project will furnish a framework to find biomarker of any polytrauma condition, which are typically difficult to model or finding human samples are often challenging.

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

NA

Changes that had a significant impact on expenditures

NA

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

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**
  Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentation

Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS What individuals have worked on the project?

Name: *Rasha Hammamieh*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): 0000-0001-8643-6233
Nearest person month worked: 1
Contribution to Project: Dr. Hammamieh was responsible for monitoring overall project progression and reporting

Name: *Jeff Shupp*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Dr. Shupp provided direct oversight and collaborating with Sub-Investigators in the MedStar Georgetown Cancer Institute to recruit participants.

Name: *Nabarun Chakraborty*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID): 0000-0002-7883-2013
Nearest person month worked: 1
Contribution to Project: Mr. Chakraborty was engaged in coordinating the work and preparing the reports.

Name: *Aarti Gautam*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID): 0000-0003-3132-5599
Nearest person month worked: 1
Contribution to Project: Dr.. Gautam was working as a liaison between WRAIR and WHC

Name: *Abdulnaser Alkhalil*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: *Dr. Alkhalil was preparing the IACUC protocol*

Name: *Lauren Moffatt*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 0.5
Contribution to Project: *Dr. Moffatt was working with IACUC submission*

Name: *Melissa M McLawhorn*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Ms. McLawhorn worked with Dr. Shupp and Dr. Moffatt to develop the laboratory processing instructions and documentation. She developed the kick off slides and trained the current clinical research staff on the protocol. She has worked with Mr. Dallegge to finalize recruitment materials,

Name: Mason Dallegge
Project Role: Clinical Research Coordinator
Nearest person month worked: 1
Contribution to Project: Mr. Dallegge has worked with Ms. McLawhorn as described above. He conducted in-services with the radiation oncology team and started the day to day screening for the clinical study

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

No

What other organizations were involved as partners?

Organization Name: Walter Reed Army Institute of Research
Location of Organization: (if foreign location list country): Silver Spring MD
Partner's contribution to the project (identify one or more) Facilities (e.g., project staff use the partner's facilities for project activities); Collaboration

Organization Name: MedStar Washington Hospital Center
Location of Organization: (if foreign location list country): Washington DC
Partner's contribution to the project (identify one or more) Facilities (e.g., project staff use the partner's facilities for project activities); Collaboration

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. **APPENDICES:**