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CDMRPL-16-0-DM160525

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

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

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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USACEHR
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MedStar Health Research Institute, Washington DC

8. PERFORMING ORGANIZATION REPORT NUMBER

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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We are using a rodent model, of radiation and thermal injury to examine existing and novel biomarkers of radiation exposure alone and combination injuries (radiation plus thermal) at varying levels in hair, skin, blood, and major organs. The emphasis is on pan–omic work, be we also are implementing histopathology and IHC to elucidate relationships between biomarker change and potential clinical impacts in samples collected. A focus is on identification of candidate markers 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. Many of the single injury animal exposures have been completed for both burn and radiation only exposures, with the majority of animals exposed completing a 14 day time course. The highest radiation only exposure group however only survives until day 7, therefore this group does not complete the full proposed time course. Many of the combination injury animal exposures have also been completed. We have observed a clear combinatorial effect on mortality however, and have adjusted our experimental groups accordingly to end the time courses following 20% burn injury and radiation exposure by Day 2 in these combination groups in order to ensure viable sample collection. *All samples generated to date and preserved for subsequent molecular analysis have been transferred to USACEHR.*

15. SUBJECT TERMS
radiation, burn injury, mass casualty, biomarkers, systems biology

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)*

1. **INTRODUCTION:**

We are using rodent models of radiation and thermal injury to examine existing and novel biomarkers for radiation exposure alone, thermal injury alone, or their combination. We're monitoring markers at varying levels in hair, skin, blood, and major organs. The emphasis is on pan–omic work, but we also are implementing histopathology and immunohistochemistry (IHC) to elucidate relationships between biomarker change and potential clinical impacts in samples collected. A focus will be on identification of candidate markers 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 had potential radiation exposure and/or thermal injury.

2. **KEYWORDS:** radiation, burn injury, mass casualty, biomarker, systems biology
3. **ACCOMPLISHMENTS:**
    - **What were the major goals of the project?**

Aim 1: Biomarker Identification - *55% complete*

Major Task 1: Secure IACUC and ACURO Approval and Plan for Exposures with Consultation from AFRRI - *100% complete*

Major Task 2: Perform Single Injury Animal Exposures (n = 432 animals, C57BL6 mouse) - *100% complete*

Major Task 3: Perform Combined Injury/Exposure groups (n= 486 animals, C57BL6 mouse) - *100% complete*

Major Task 4: -omics Assays for molecular biomarker identification - *60% complete*

Major Task 5: H2AX/Histologic Assays for biomarker identification and confirmation. - *25% complete*

1) Identify the signature molecular biomarker modulation that occurs in animals exposed to variable doses of X-ray radiation. - *25% complete*
2) Identify the signature molecular biomarker modulation that occurs in animals after 10% or 20% TBSA burn injuries. - *25% complete*
3) Identify the signature molecular biomarker modulation that occurs in animals with a combination of both of the above injuries. - *25% complete*

Aim 2: Biomarker Detection and Timeline Assessment - *20% complete*

Major Task 1: Temporal/Chronologic Analyses of Data to assess for biomarker dynamics over time and optimal window for detectable signals. - *25% complete*

4) Determine whether there a dose-dependent and time-dependent response that can be seen with an individual molecule(s). - *25% complete*

Aim 3: Biomarker Sample Source Optimization - *10% complete*

Major Task 1: Comparison of sample types for identification of best sample source - *10% complete*

5) Determine whether a given 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. - *10% complete*

Aim 4: Analysis, Reporting, and Communications of Findings - *30% complete*

Major Task 1: Final Data Analysis - *0% complete*

- **What was accomplished under these goals?**

Major Task 1: Secure IACUC and ACURO Approval and Plan for Exposures with Consultation from AFRRI

All appropriate regulatory approvals are being maintained for animal use in this study. Protocols have been renewed at the MedStar Health Research Institute IACUC, and subsequently will be submitted with the ACURO office when that approval is set to expire.

Major Task 2: Perform Single Injury Animal Exposures: n = 432 animals

All of the single injury animal exposures have been completed for both burn and radiation only exposures, with the majority of animals exposed completing a 14 day time course. The highest radiation only exposure group however only survives until day 6, therefore this group did not complete the full proposed time course.

Major Task 3: Perform Combined Injury/Exposure groups n= 486 animals

All of the combination injury animal exposures have also been completed. We have observed a clear combinatorial effect on mortality however, and have had to adjust our experimental groups accordingly to end the time courses following the combination 20% burn injury and radiation exposure to 2 days, in order to ensure viable sample collection.

Major Task 4: -omics Assays for molecular biomarker identification

All samples generated to date and preserved for subsequent molecular analysis have been transferred to USACEHR for processing and assay. Additionally, samples have been included as "extra" in order to optimize processing from skin samples. Optimization is complete and all blood, skin, and liver RNA samples have been purified. Blood and skin samples sets have been applied to DNA microarray hybridization and scanning. Initial quality control (QC) analysis of the blood and samples is complete and is underway for the liver samples. Downstream analysis on blood and skin microarray results is underway.

Major Task 5: H2AX/Histologic Assays for histologic biomarker identification and confirmation.

Initial H&E stains have been completed on most of the skin samples.

- **What opportunities for training and professional development has the project provided?**
  - *Two medical students have had the opportunity to learn skill sets in sample preservation and processing for histology with samples generated in this project. This learning occurred with the students under the mentorship of more senior lab staff. In addition, One summer student that was part of the Army Educational Outreach Program (AEOP) has gained*

hands on experience purifying RNA from collected skin and blood samples from the experiment.

- **How were the results disseminated to communities of interest?**
  - Results were presented at the 2018 MHSRS conference and at the 2019 Wound Healing Society conference.
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - *We plan to complete the animal exposures and begin transcriptomic analysis of samples generated to date, beginning with the blood and skin samples. Genes of interest demonstrating differential regulation will be interrogated further using confirmatory real time RT-PCR and histologic methods to assess protein levels.*

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - *Nothing to Report Thus Far.*
   - **What was the impact on other disciplines?**
     - *Nothing to Report.*
   - **What was the impact on technology transfer?**
     - *Nothing to Report*
   - **What was the impact on society beyond science and technology?**
     - *Nothing to Report.*
5. **CHANGES/PROBLEMS:** *Nothing to Report*
6. **PRODUCTS:** *Nothing to Report*
   - **Other Products**
     - *Biospecimen collections; We have collected all organs from experimental animals and therefore will have accumulated samples that will be kept in a repository if full samples are not used in presently described assays.*
7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   - **What individuals have worked on the project?**

Name: John Clifford, PhD
Project Role: Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.0
Contribution to Project: Planning, coordination, re-analysis of preliminary data, experimental design development and initial execution

Name: Lauren Moffatt, PhD
Project Role: Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.0
Contribution to Project: Regulatory approvals, planning, coordination, experimental design development and initial execution

Name: Abdulnaser Alkhalil, PhD
Project Role: Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.0

Contribution to Project: Planning, coordination, re-analysis of preliminary data, experimental design development and execution and coordination of animal exposures

Name: Campbell, Ross, PhD
Project Role: Statistician, Data Manager
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.5
Contribution to Project: Re-analysis of preliminary data, experimental design development

Name: Duncan Donohue, PhD
Project Role: Statistician, Data Manager
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.0
Contribution to Project: Re-analysis of preliminary data, experimental design development

Name: Robert Christy, PhD
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.5
Contribution to Project: Oversight of planning, coordination, experimental design development

Name: Sanchita Ghosh, PhD
Project Role: Consultant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.0
Contribution to Project: Radiation-related regulatory approvals, and associated planning, coordination, experimental design development

Name: Anna Day, BS
Project Role: Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3.0
Contribution to Project: Execution of animal exposures and materials acquisition, sample tracking

Name: Kyle Monger, BS
Project Role: Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3.0
Contribution to Project: Execution of animal exposures, sample tracking

Name: Karina Charipova, BS
Project Role: Technician/Student
Researcher Identifier: N/A

Nearest person month worked: 1.0
Contribution to project: Sample processing, initial histology

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - *Nothing to Report*
- **What other organizations were involved as partners?**
  - **No additional organizations were involved, other than those originally listed in the SOW and funded as subawardees.**

8. **SPECIAL REPORTING REQUIREMENTS**
   - **QUAD CHARTS:** *See attached*

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

DM160525

Clinical Research Intramural Initiative Program, Precision Medicine Research Award FOA# DHA-16-CRII-PMRA

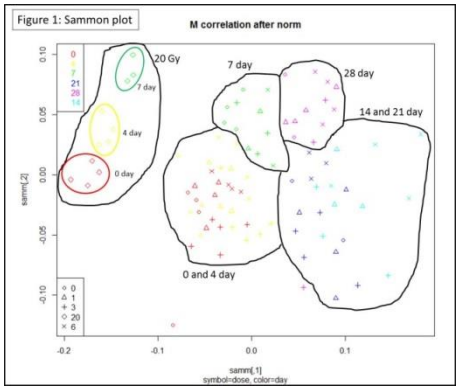
PI: Robert J. Christy, PhD **Org:** Institute Of Surgical Research **Award Amount:** 750,000

Study/Product Aim(s)

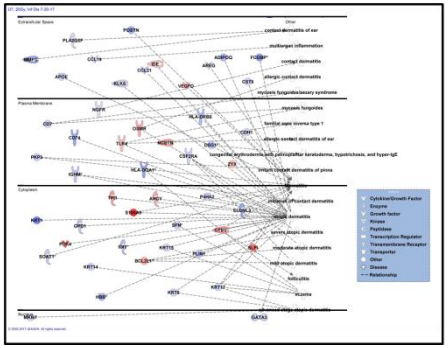
1)Identify the signature molecular biomarker modulation that occurs in animals exposed to variable doses of X-ray radiation, or variable severity burn injury.
2) Identify the signature molecular biomarker modulation that occurs in animals with a combination of both of the above injuries.
3) Determine whether there a dose-dependent and time-dependent response that can be seen with this molecule(s).
4)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.

Approach

We will use a rodent model to examine existing and novel biomarkers of combination 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 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.



B. Processes related to inflammatory disease (Day 7, 20Gy)



Accomplishment: Preliminary data indicate differential gene expression that is time and dose dependent. The highest dose previously tested (20Gy) is lethal by >7days and exerts effects related to inflammatory disease states in skin. We have verified this in the present animal work and have completed animal model work according to modifications related to observations of mortality.

Timeline and Cost

Activities / CY	17	18	19
Animal Work			
-Omics and Histologic Assays			
Data analysis and harmonization			
Translatability Assessment			
Estimated Budget ($K)	**$000**	**$000**	**$000**

Goals/Milestones

CY17 Goal – Animal Model
☑Secure ACURO approval and begin animal model
☑Sample collection and processing
☑Assay initiation
CY18 Goals – Continue Animal Model and Assays
✓ Animal model completion
✓Continue assays
CY19 Goal – Analysis and biomarker suitability assessment
☐Validate markers