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TITLE: Adjuvant Immunotherapy to Reverse Immunosuppression in Burn-Injured Patients with Antibiotic-Resistant Infections

PRINCIPAL INVESTIGATOR: Dr. Isaiah Turnbull, MD, PhD

CONTRACTING ORGANIZATION: Washington University in St. Louis - School of Medicine

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Fort Detrick, Maryland 21702-5012

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Adjuvant Immunotherapy to Reverse Immunosuppression in Burn-Injured Patients with Antibiotic-Resistant Infections

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6. AUTHOR(S)

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

Washington University in St. Louis - School of Medicine

8. PERFORMING ORGANIZATION REPORT NUMBER

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12. DISTRIBUTION / AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: 10% of combat casualties from recent military conflicts suffered burn injuries. Infection is the most common complication of burns, and more than 70% of burn patients suffer an infectious complication. Severe burns with associated traumatic injuries dramatically disrupt the function of the immune system resulting in immunosuppression. This immunosuppression both increases the risk of infection and also makes antibiotic treatment for infection less effective. Simply put: antibiotic therapy is inadequate to the task of treating infections in burn-injured soldiers. Because of this infection is the leading cause of death for warfighters who succumb to their burns. Advancing the treatment of infections in burn patients will require identifying burn patients with severe immunosuppression and developing treatments to restore immune function. The goal of this project is to use immunophenotyping approaches to identify immunosuppressed burn-injured patients and to define the molecular pathways underlying this immunosuppression. Burn injured subjects in the USAISR Burn Center and healthy controls will be enrolled and blood samples obtained for immune function and molecular immunophenotyping studies. We are identifying the molecular pathways that are disrupted in the immunosuppressed patients. Identifying these pathways will allow clinicians to deploy existing immunomodulatory and immunoadjuvent therapies to restore immune function to immunosuppressed burn injured patients

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
U	U	U	UU	11	**19b. TELEPHONE NUMBER** *(include area code)*

1. Introduction:

10% of combat casualties from recent military conflicts suffered burn injuries. Infection is the most common complication of burns, and more than 70% of burn patients suffer an infectious complication. Severe burns with associated traumatic injuries dramatically disrupt the function of the immune system resulting in immunosuppression. This immunosuppression both increases the risk of infection and also makes antibiotic treatment for infection less effective. Simply put: antibiotic therapy is inadequate to the task of treating infections in burn-injured soldiers. Because of this infection is the leading cause of death for warfighters who succumb to their burns. Advancing the treatment of infections in burn patients will require identifying burn patients with severe immunosuppression and developing treatments to restore immune function. The goal of this project is to use immunophenotyping approaches to identify immunosuppressed burn-injured patients and to define the molecular pathways underlying this immunosuppression. Burn injured subjects in the USAISR Burn Center and healthy controls will be enrolled and blood samples obtained for immune function and molecular immunophenotyping studies. We are identifying the molecular pathways that are disrupted in the immunosuppressed patients. Identifying these pathways will allow clinicians to deploy existing immunomodulatory and immunoadjuvent therapies to restore immune function to immunosuppressed burn injured patients,

2. Keywords

burn, sepsis, immune dysfunction, immunophenotyping, immunosuppression, cytokine, phosphoproteins

3. Accomplishments

3.1 What were the major goals of the project?

Specific Aim 1: Validate the ELISpot assay at identifying functional immunoppression and infection in critically ill burn patients. Determine whether ELISpot can predict sepsis I nearly serious burn injury.	**Timeline**	**Completion Date**	**% Complete**
Major Task 1.1: Recruit Burn Injured Patients and Healthy Controls for observational Trial.	Months		
Submit IRB protocol for prospective observational trial and peripheral blood sample collection to the US Medical Research and Development Command Institutional Review Board	1-3	12/1/2023	95%
Develop REDCap database, subject recruitment and bio-sample collection protocols	1-3	12/1/2023	80%
Recruit critically ill burn injured subjects Clinical Sepsis Screen N=750 potential subjects for suspected infection in the USAISR Burn ICU Recruit ad consent N=175 subjects with suspected infection Collect peripheral blood samples at time of consent, 24 hours, and 72 hours after consent Process and biobank blood samples	3-40		
Recruit critically ill burn injured subjects without suspected infection Screen N=750 potential subjects for suspected infection in the USAISR Burn ICU Recruit and consent N=75 subjects without "suspected infection" Collect peripheral blood samples at time of consent, 24 hours and 72 hours after consent Process and biobank blood sample	6-36		
Recruit healthy control subjects Recruit and consent n=50 healthy donors from the general population in St. Louis. Collect peripheral blood samples at time of consent, 24 hours and 72 hours after consent Process and biobank blood samples for analysis including banking PMBC, fixed whole blood and plasma	12-36		
Milestone Achieved: Burn injured and healthy control subjects recruited, blood samples obtained, processed and biobanked and transferred to Washington University	40		
Milestone Achieved: Clinical data aggregated in REDCap	40		
Major Task 1.2: Define the functional immunologic defects associated with severe burns using the ELISpot assay			
Measure IFN-gamma and TNF-alpha production by PBMC using the ELISpot Assay using peripheral blood samples.	6-42		

Analyze data to compare immune function burn-injured subjects (n=250) and health controls (n=50). Interim analysis at 18 months (after 15 months of enrollment) to ensure appropriate subject accrual rate and to evaluate for initial effect size.	18-42		
Milestone Achieved: Define the utility of the ELISpot assay to measure immune dysfunction in burn injured patients vs. healthy controls.	18-42		
Major Task 1.3: Determine if the ELISpot immune function assay can identify patients with clinically relevant infection.			
Measure IFN-gamma and TNF-alpha production by PBMC using the ELISpot Assay using samples collected/banked.	24-42		
Analyze data to compare immune function burn-injured subjects and healthy controls. Interim analysis at 18 months to ensure appropriate subject accrual rate and to evaluate for initial effect size.	18-42		
Milestone Achieved: Determine if the ELISpot assay can discriminate burn patients with sepsis vs. those with sterile SIRS	18-42		
Specific Aim 2: Use high-resolution immunophenotyping to identify the mechanisms of immune dysfunction cause by severe burns and to identify candidate molecular pathways for immunomodulation.			
Major Task 2.1: Define the effect of burn injury on the cellular proteomics of circulating leukocytes			
Measure cellular phosphoproteome by mass cytometry from burn injured subjects and controls (n=300). Samples will be fixed whole blood biobanked.	9-44		
Upload/analyze mass cytometry data on cytobank platform	42-46		
Milestone Achieved: Define the effect of burn injury on the intracellular phosphoproteome of circulating leukocytes	42-48		
Major Task 2.2: identify changes in soluble biomarkers induced by burn injury.			
Run Luminex soluble marker assay on banked plasma samples.	9-44		
Analyze cytokine data on SPSS platform	42-46		
Milestone Achieved: Define the effect of burn injury on circulating plasma cytokine levels measured longitudinally in both burn patients with sepsis and uninfected burn patients.			
Major Task 2.3: Determine effect of burn injury on the cellular transcriptome of circulating leukocytes			
Sort PBMC boibanked specimens using FACS to isolate RNA from CD4+T Cells, DC8+T cells and monocyte populations. Isolate cells from n=25 subjects with microbial proved sepsis and n=25 subjects with suspected infection subsequently deemed not infected.	9-44		

Measure transcriptome of isolated RNA samples from above using affymetric gene arrays	42-46		
Analyze data to identify transcriptional markers to true sepsis vs. sterile SIRS.	42-46		
Milestone Achieved: identify the transcriptional signature of burn sepsis at the time of initial clinical suspicion of infection.	42-48		

3.2 What was accomplished under these goals?

- Procedure manual for laboratory specimen collection, processing, and storing created
- Negotiate cooperative research and development agreement (CRADA) between WU and USAISR (near completion)
- Sample collection and processing supplies stocked at ISR
- USAISR site specific documents near completion
- REDCap data dictionary completed
- REDCap database in progress
- Healthy donor enrollment initiated
- Improved mass cytometry assay throughput

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

NOTHING TO REPORT

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

- Submit USAISR site specific protocol documents to WCG-IRB
- Submit USAISR site specific documents to OHRO
- Finalize REDCap database
- Submit DSAA to DHA
- Recruit and enroll healthy volunteers
- Initiate enrollment of burn injured subjects
- Initiate immunophentyping assays of samples from healthy controls and burn injured subjects as samples accrue.

4. Impact:

4.1 What was the impact on the development of the principal discipline(s) of the project? NOTHING TO REPORT

4.2 What was the impact on other disciplines? NOTHING TO REPORT

4.3 What was the impact on technology transfer? NOTHING TO REPORT

4.4 What was the impact on society beyond science and technology? NOTHING TO REPORT

5. Changes/Problems

5.1 Changes in approach and reasons for change: NOTHING TO REPORT

5.2. Actual or anticipated problems or delays and actions or plans to resolve them?

1. We have been delayed in initiating sample collection while working through the HRPO process. We have navigated the sIRB process by using the private WCG IRB. We were delayed finalizing the CRADA due to complex negotiations surrounding sample and data security protocols as well as IP considerations. **These issues are resolved and the protocol is submitted for final approval.**

2. Completing site-specific documents for the USAISR has been complicated by turnover in Clinical Research Nurse and short staffing in USAISR Research Regulatory – this has severely affected the review of the site specific documents. **Personnel have been recruited and documents have been submitted.**

3. Washington University changed the protocols for compensating volunteers for their time and travel. The University has developed a specific program to obtain and track volunteer compensation which we are required to use. Deploying this program required retraining study personnel and developing a new procurement protocol. **This process is complete and gift cards (compensation for healthy donors) are in-hand.**

4. Enrollment of healthy donors at Washington University site has been complicated by changes in Washington University environmental health and safety policies which required locating a new location for healthy donor procedures.
    a. **We have identified appropriate clinical space to perform phlebotomy procedures and have recruited a nurse coordinator to perform the phlebotomy.**
    b. **We have successfully negotiated appropriate clinical space that meets EH&S and IRB requirements for study procedures.**

    These new procedures are a significant improvement over our previous approaches and anticipate that these new procedures will dramatically increase the pace of healthy donor recruitment. **We are actively recruiting healthy subjects.** We have a log of interested healthy volunteers and anticipate enrollment in the next 1-2 weeks.

5. Delayed enrollment has also led to a concomitant delay in initiating immunophenotyping assays. We anticipate that we will need to increase the rate at which we perform these assays. The mass cytometry assay is the primary rate limiting step. We plan to enroll 300 total subjects with 3 samples per subject, for a total of 900 samples to be assayed by mass cytometry. Initially we planned to a 10-sample assay approximately each week over years 2-3.5 of the study. **We have used the delay in our enrollment to improve our assay throughput and now can comfortably execute two 10-sample assays each week. This will allow us to complete the 900 sample assays in <50 weeks. We will run the assays as samples become available, but this increased throughput offsets the delay in enrollment we have experienced due to regulatory delays.**

5.3. Changes that had a significant impact on expenditures: NOTHING TO REPORT

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

5.4.1 Human Use Regulatory Protocols

PROTOCOL (1 of 1 total):

Protocol: *E03570.1a*

Title: *Adjuvant immunotherapy to Reverse Immunosuppression in burn-injured patients with antibiotic-resistant infections*

Target required for clinical significance: 300

Target approved for clinical significance: 300

SUBMITTED TO AND APPROVED BY:

- OHRO initial approvals:
  E03570.1a Study Level 2/2/2023
  E03570.1b Washington University 4/4/2023

STATUS:

(i) Number of subjects recruited/original planned target: 0
Number of subjects screened/original planned target: 0
Number of patients enrolled/original planned target: 0
Number of patients completed/original planned target: 0

(ii) Report amendments submitted to the IRB and USAMRMC HRPO for review:

Nothing to report.

(iii) Adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation:

Nothing to report

5.4.2 Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training: *No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW).*

5.4.3. Animal Use Regulatory Protocols: *No animal use research will be performed to complete the SOW*

6. Products

6.1 Publications, conference papers, and presentations: NOTHING TO REPORT

6.2 Website(s) or other Internet site(s): NOTHING TO REPORT

6.3 Technologies or techniques: NOTHING TO REPORT

6.4 Inventions, patent applications, and/or licenses: NOTHING TO REPORT

6.5 Other Products: NOTHING TO REPORT

7. Participants & Other Collaborating Organizations

7.1 What individuals have worked on the project?

Name	Project Role	Nearest person month worked	% Effort	Contribution to the project
Isaiah Turnbull, MD, PhD	Principal Investigator	May	20%	Oversight and leads all aspects of the study.
Richard Hotchkiss	Co-Investigator	May	5%	Developing Bio-collection Protocols.
Matthew Montoya-Rush, PhD	Postdoctoral Scientist	May	50%	Developing REDCap Database and Bio-collection Protocols

7.2 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

7.3 What other organizations were involved as partners?

Collaborating Organizations

Organization	Location	Contribution to Project
Institute of Surgical Research and US Army Burn Center	3698 Chambers Pass, JBSA Fort Sam Houston, TX 78234-6315	Dr. Leopoldo Cancio (Study PI) Dr. James Bynum (AI)
Coalition for National Trauma Research	7970 Fredericksburg Road, San Antonio, TX 78230	Monica Phillips (Clinical Coordinator) Joel Baker (Clinical Research Nurse at USAISR)

8. Special Reporting Requirements

8.1 Quad Chart: See attached.