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TITLE: T cells and Rejection in Vascularized Composite Allotransplants

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CONTRACTING ORGANIZATION: Brigham and Women's Hospital**,** Boston, MA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Immune rejection is a major barrier to wider implementation of vascular composite allografts (VCAs) that hold great promise for restoring function in American service members, who have suffered devastating traumatic injuries. Despite systemic immunosuppression, T cell mediated rejection (TCMR) occurs much more frequently in VCA than in solid organ transplants, likely due to the significant number of T donor T cells that survive in the allografts. This study will used banked tissues from VCA patients to comprehensively analyze the contributions of donor versus recipient T cells in VCA rejection. Another question that will be addressed is whether sentinel flaps, transplanted concomitantly with the allograft from the same donor to a distant anatomical site, or circulating levels of clonally expanded T cells, are useful as reliable markers for VCA rejection. IRB approval for this project has been obtained and HRPO approval is pending.

15. SUBJECT TERMS
None listed.

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

Immune rejection is a major barrier to wider implementation of vascular composite allografts (VCAs) that hold great promise for restoring function in American service members, who have suffered devastating traumatic injuries. Despite systemic immunosuppression, T cell mediated rejection (TCMR) occurs much more frequently in VCA than in solid organ transplants, likely due to the significant number of donor T cells that survive in the allografts. This study will used banked tissues from VCA patients to comprehensively analyze the contributions of donor versus recipient T cells in VCA rejection. Another question that will be addressed is whether sentinel flaps, transplanted concomitantly with the allograft from the same donor to a distant anatomical site, or circulating levels of clonally expanded T cells, are useful as reliable markers for VCA rejection.

2. KEYWORDS:

Vascular composite allograft, rejection, biomarker, T cell

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Regulatory approval by sponsor and institution. IRB approval estimated at Month 1; Actual approval achieved at Month 3. HRPO approval estimated to occur in Month 3 is currently pending. Current percentage of completion 75%.

Major Task 2: Activities for Specific Aim 1.
Subtask 1: High throughput TCR sequencing (HTS) of donor and recipient tissues from 6 face transplant patients during rejection and non-rejection. Estimated to start at Month 4, complete at Month 7; current percentage of completion is 0%.
Subtask 2: Determine functional phenotypes of clonally expanded donor and recipient T cells using single nucleus RNA sequencing (sNucSeq), using samples from 7 face transplant patients. Estimated to start at Month 8, complete at Month 11; current percentage of completion is 0%.
Subtask 3: Validation of findings (HTS and sNucSeq) from face transplant cohort in additional VCA type (using tissue samples from 3 upper extremity transplant patients). Estimated to start at Month 12, complete at Month 15; current percentage of completion is 0%.

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.

Under Major Task 1, we obtained IRB approval 18 January 2019. We submitted HRPO application 10 March 2019 and responded to HRPO request for further information 5 June 2019. We received HRPO approval in late 2019. Current percentage of completion: 100%

Under Major Task 2, we have addressed the questions posed in Subtask 1 by studying high throughput sequencing of samples from three human face transplant recipients at all four stages of rejection (BANFF Grades 0,1,2,3). We have tracked donor sequences in recipient biopsies over time. Completion of Subtask 1 is 85%.
For Subtask 2, personnel is being trained to optimize the novel protocol for sNucSeq using human skin samples. One optimized, the VCA patient samples will be studied using sNucSeq. Current Completion: 15%

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

This project has provided training for the postdoctoral fellow William J. Crisler to learn new lab skills. Specifically, he has been trained on analyzing high throughput sequencing data using the software of Adaptive Biotechnologies as well as the NanoString nSolver software. He is currently being trained to optimize single nucleus sequencing in skin, a novel human tissue for this protocol.

How were the results disseminated to communities of interest?

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Nothing to Report

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

For Major Task 2, we will perform high throughput TCR sequencing (HTS) and single nucleus RNA sequencing (sNucSeq) on face transplant donor and recipient tissues, then validate findings by HTS and sNucSeq in upper extremity transplant VCA patient samples. For Major Task 3, we will perform HTS on face transplant patient blood cells to investigate if monitoring circulating T cell clones can be used as rejection biomarkers. This will be validated in blood samples from upper extremity patient blood samples.

4. **IMPACT:**

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

Nothing to Report

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:

Changes in approach and reasons for change

Nothing to Report

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

IRB and HRPO approvals took longer than expected. Shortly after receiving the HRPO approval, Brigham & Women's shut down in-person research activity as a result of the coronavirus pandemic. Personnel continued working on this project remotely, and key personnel are now able to be back in the lab.

Changes that had a significant impact on expenditures

Less funds have been spend due to approval delays and the coronavirus pandemic, but we are now planning major experiments to address Major Tasks 2 and 3.

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

IRB (Protocol # 2018P003007): Approved 18 January 2019

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:

- **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 presentations.

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: Rachael Clark, MD PhD
Project Role: Principal Investigator
Nearest person month worked: 1.2
Contribution to Project: Dr. Clark provided scientific oversight and provided feedback and support on regulatory and protocol submissions.

Name: William J. Crisler, PhD
Project Role: Research Fellow
Nearest person month worked: 10.8
Contribution to Project: Dr. Crisler has worked on regulatory submissions as well as preparing the samples and procedures for Major Task 2. He has also performed the analysis for Major Task 2 subtask 1.

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

Rachael Clark, MD, PhD
New support: NIH-NCI Evaluation of an implantable microdevice for rapid cancer drug screening directly in T cell lymphoma patients – 0.60 CM

New support: NIH Optimizing pre-analytic sample handling for high throughput TCR sequencing in cutaneous T cell lymphoma – 2.18 CM

Recently closed support: NIH Skin Inflammation in Human Health and Disease: 2018 International Conference 4/30/2019 0.24 CM

What other organizations were involved as partners?

Organization Name: Broad Institute
Location of Organization: Cambridge, MA
Partner's contribution to the project: Discussed plans with a postdoc and supervisor for future work once approvals are in place. Partner's staff will facilitate our performance of sequencing (sNucSeq) on human skin samples. Once optimized, we will perform sNucSeq on VCA samples.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS: /

9. APPENDICES: