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TITLE: T Cells and Rejection in Vascularized Composite Allotransplants

PRINCIPAL INVESTIGATOR: Rachael Clark MD PhD

CONTRACTING ORGANIZATION: Brigham and Women's Hospital
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T cells and Rejection in Vascularized Composite Allotransplants

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Brigham and Women's Hospital

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Boston, MA 02115

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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
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

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 reiection.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Vascular composite allograft, rejection, biomarker, T cell

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

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 are awaiting HRPO approval before starting work on Major Task 2.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We plan first to obtain HRPO approval for the project, then proceed with Major Tasks 2 and 3. 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:** D*escribe 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).

Nothing to Report

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 PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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

Nothing to Report

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

IRB and HRPO approvals took longer than expected. We have replied to the HRPO's request for further information and are awaiting their approval.

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.

Less funds were spent in Year 1 due to human subjects approval delays, but, we intend to spend the funds appropriately once project is approved.

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

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

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).*

Nothing to Report

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. Describe the technologies or techniques were 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. 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;*
- *physical 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: 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: Thet Su Win, MD PhD
Project Role: Research Fellow
Nearest person month worked: 7.2
Contribution to Project: Dr. Win has worked on regulatory submissions as well as preparing the samples and procedures for Major Task 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?
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.

Rachael Clark, MD, PhD
NIH CTCL Immunobiology: Lessons from Alemtuzumab – closed 11/30/2018

NIH Generation of Robust Resident Memory T cell in Barrier Tissues through Skin Vaccination – increased effort to 1.80 CM from 1.44CM

New support: American Skin Association, Characterizing the Inflammatory Synapse between Mast Cells, Benign T cells, and Malignant T Cells in CTCL – 0.12 CM

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*

Organization Name: Broad Institute
Location of Organization: Cambridge, MA
Partner's contribution to the project: Discussed plans with a post-doc and supervisor for future work once approvals are in place. For future, partner's staff will facilitate our performance of sequencing.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

T cells and Rejection in Vascularized Composite Allotransplants

W81XWH-18-1-0784
RT170025

PI: Rachael Clark, MD PhD **Org:** Brigham and Women's Hospital **Award Amount:** $1,236,771

Study/Product Aim(s)

- Determine the role of donor vs. recipient T cells in VCA rejection.
- Determine if monitoring pathogenic T cell clones in the circulation can be used as a rejection biomarker.
- Establish the correlation of rejection in sentinel flaps and VCA allografts.

Approach

- Analyze banked blood and tissue samples from face and upper extremity transplants using a combination of techniques, including high throughput T-cell receptor sequencing (HTS), NanoString nCounter gene expression profiling, single nucleus-RNA sequencing (sNuc-seq) and immunofluorecence staining.
- Correlate results with clinical findings.

Figure. Post-operative frontal view of three full-facial recipients at Brigham and Women's Hospital, 17 months (left), 18 months (center), and 12 months (right) after the transplantation.

Accomplishment: Submitted HRPO application.

Timeline and Cost

Activities CY	18	19	20	21
Task 1. IRB and HRPO approval				
Task 2. Determine role of donor vs. recipient T cells in rejection				
Task 3. Determine if monitoring T cells in circulation can be used as rejection biomarker				
Task 4. Establish correlation of rejection in sentinel flaps and VCA allografts				
Estimated Budget ($K)	**$166.4K**	**$500.3K**	**$413.6K**	**$156.4K**

Updated: October 1, 2019

Goals/Milestones

CY18 Goal – IRB/HRPO approval

☑ IRB approval

CY19, CY20 and CY21 Goals

☐ HRPO approval

☐Determine the role of donor vs. recipient T cells in VCA rejection

☐Determine if monitoring pathogenic T cell clones in the circulation can be used as a rejection biomarker

☐ Establish the correlation of rejection in sentinel flaps and VCA allografts

Comments/Challenges/Issues/Concerns

- Timelines have changed with respect to the original proposal because of delay in obtaining IRB and HRPO approval.

Budget Expenditure to Date

Projected Expenditure: $1,236,771

Actual Expenditure: $ **75, 521.01**