AWARD NUMBER: W81XWH-18-1-0798

TITLE: Harnessing Single-Cell Technologies to Understand and Diagnose Rejection in Clinical Face and Upper Extremity Transplantations

PRINCIPAL INVESTIGATOR: Rachael Clark MD PhD

CONTRACTING ORGANIZATION: Brigham and Women's Hospital
Boston, MA 02115

REPORT DATE: Oct 2019

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Oct 2019	Annual Report	09/30/2018-09/29/2019

4. TITLE AND SUBTITLE
Harnessing Single-Cell Technologies to Understand and Diagnose Rejection in Clinical Face and Upper Extremity Transplantations

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0798

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Rachael Clark MD PhD

E-Mail: rclark@bwh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Brigham and Women's Hospital

75 Francis St
Boston, MA 02115

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Monitoring and immunosuppression of patients following vascular composite allograft (VCA) transplantation is currently based on protocols used in solid organ transplantation (SOT). However, VCAs pose unique immunological challenges because they contain a significant load of passenger donor immune cells. A deeper understanding of mechanisms underlying VCA rejection and identification of biomarkers that can reliably distinguish rejection from other skin inflammation are crucial for the development of more selective immunosuppression targeted specifically for VCA recipients. We will retrospectively study cryopreserved allograft skin biopsies from 7 face and 3 upper extremity transplant patients by single nucleus RNA sequencing (sNucSeq), to (1) identify the cell type, frequency and gene expression profiles (hence, their functional states and phenotypes) of graft-derived and infiltrating cells within VCAs during ACR and non-rejection, and (2) compare single nucleus gene expression profiles of ACR with that of non-transplant related skin inflammation to discover unique molecular changes occur within VCA transplants during ACR, which are distinct from non-rejection related skin inflammation. IRB approval for this project has been obtained and HRPO approval is pending.

15. SUBJECT TERMS

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	15	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.*

Monitoring and immunosuppression of patients following vascular composite allograft (VCA) transplantation is based on protocols used in solid organ transplantation (SOT). However, VCAs pose unique immunological challenges because they contain many passenger donor immune cells. A deeper understanding of mechanisms underlying VCA rejection and identification of biomarkers that can distinguish rejection from other skin inflammation are crucial for the development of more selective immunosuppression targeted specifically for VCA recipients. We will study cryopreserved allograft skin biopsies from 7 face and 3 upper extremity transplant patients by single nucleus RNA sequencing (sNucSeq), to (1) identify the cell type, frequency and gene expression profiles (hence, their functional states and phenotypes) of graft-derived and infiltrating cells within VCAs during ACR and non-rejection, and (2) compare single nucleus gene expression profiles of ACR with that of non-transplant related skin inflammation to discover unique molecular changes occur within VCA transplants during ACR, which are distinct from non-rejection related skin inflammation.

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

Vascular composite allograft, rejection, single nucleus RNA sequencing

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 as happening in Month 1; IRB approved on 18 April, 2019 (Month 6). HRPO approval estimated to occur in Month 2 is currently pending. Current percentage of completion is 75%.

Major Task 2: Identify graft-derived and infiltrating immune cells within VCA allografts during acute cellular rejection versus non-rejection. Estimated to start at Month 3, complete at Month 12; 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 April 2019. We submitted the HRPO forms on 21 May 2019. HRPO asked for further information 2 September 2019. We responded to request from HRPO 30 September 2019 and are awaiting approval.

Major Task 2 has not yet been started as we are awaiting HRPO approval.

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

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "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 single nucleus RNA sequencing (sNuc-seq) on immune cells from VCA allografts during rejection and non-rejection, correlate sNuc-seq data with clinical outcomes, and validate selected gene expression findings with multiplex immunostaining. For Major Task 3, we will perform the same tasks as Major Task 2, except on non-transplanted skin from patients with rosacea, delayed-type hypersensitivity reaction, or normal skin.

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
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

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 # 2018P001496): Approved 4/17/2019

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: *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: 0.60
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: 4.80
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*
- *Other.*

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: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Harnessing Single-Cell Technologies to Understand and Diagnose Rejection in Clinical Face and Upper Extremity Transplantations

W81XWH-18-1-0798

RT170144

PI: Rachael Clark, MD PhD **Org:** Brigham and Women's Hospital **Award Amount:** $199,999

Study/Product Aim(s)

- Identify graft-derived and infiltrating cells within VCAs during acute cellular rejection and non-rejection.
- Compare transcriptional profiles of acute cellular rejection with that of non-transplant related skin inflammation.

Approach

- Analyze archived skin biopsies from clinical face and upper extremity transplants.
- Examine transcriptional profiles of single cells extracted from skin biopsies collected during rejection and non-rejection.
- Compare with biopsies from non-transplanted patients with skin inflammation.

Figure. Clinical photographs of a full face transplant recipient during non-rejection (BANFF Grade 0) and severe rejection (BANFF Grade 3).

Accomplishment: Submitted HRPO application and replied to their request for more information..

Timeline and Cost

Activities CY	18	19	20
Task 1. IRB and HRPO approval			
Task 2. Identify graft-derived & infiltrating cells within VCAs during rejection & non-rejection			
Task 3. Compare rejection with non-transplant related skin inflammation			
Task 4. Dissemination of findings			
Estimated Budget ($K)	**$37.5K**	**$104.2K**	**$58.2K**

Updated: October 1, 2019

Goals/Milestones

CY18 Goal – IRB/HRPO approval

☑ IRB approval

CY19 and CY20 Goals – Perform experiments, analyze data and disseminate results

☐ HRPO approval

☐Identify graft-derived and infiltrating cells within VCAs during rejection and non-rejection

☐Compare transplant rejection with non-transplant related skin inflammation

Comments/Challenges/Issues/Concerns

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

Budget Expenditure to Date

Projected Expenditure: **$199,999**

Actual Expenditure: **$49,075.78**