**AWARD NUMBER: W81XWH-16-1-0760**

**TITLE: The role of tissue-resident donor T cells in rejection of clinical face transplants**

**PRINCIPAL INVESTIGATOR: Bohdan Pomahac, MD**

**RECIPIENT: Brigham and Women's Hospital**
**Boston MA 02115**

**REPORT DATE: Jan 2019**

**TYPE OF REPORT: Final Report**

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

# REPORT DOCUMENTATION PAGE

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*OMB No. 0704-0188*



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| Jan 2019 | Final | 30 Sep 2016 - 29 Sep 2018 |

**4. TITLE AND SUBTITLE**
The role of tissue-resident donor T cells in rejection of clinical face transplants

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**5b. GRANT NUMBER**
W81XWH-16-1-0760

**5c. PROGRAM ELEMENT NUMBER**

**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)**
Brigham and Women's Hospital

75 Francis Street
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**
Unlike solid organ transplants, face transplants have a unique immunological characteristic – the presence of skin, which contains approximately 1 million T cells/$cm^2$. A full face transplant is 600-700 $cm^2$ in size and therefore, contains approximately 600-700 million donor T cells. We proposed to study 1) whether donor T cells persist long-term within facial allografts following transplantation, 2) the relative contribution of donor vs. recipient T cells in face transplant rejection, and 3) whether pathogenic T cell clones are measurable in blood during episodes of rejection and could therefore serve as an early and personalized rejection biomarker.

Using high throughput T-cell receptor sequencing of donor and recipient tissues, we found that donor T cells persisted within facial allograft after transplant and may contribute to early rejection episodes. Recipient T cells migrated into the allograft during rejection and over time became predominant. Clonally expanded T cells infiltrating the rejecting allograft were detected in peripheral blood, raising the possibility that the frequency of patient-specific T cell clones in blood may serve as a non-invasive and personalized rejection biomarker for rejection.

**15. SUBJECT TERMS**
T cells, Face transplants, Rejection

| 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)* |

**TABLE OF CONTENTS**

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

Unlike solid organ transplants, face transplants have a unique immunologic characteristic – the presence of skin, which contains approximately 1 million T cells/$cm^2$. A full face transplant is 600-700 $cm^2$ in size and therefore, contains approximately 600-700 million donor T cells. Although the role of T cells in rejection of face transplants is well established, the relative contribution of donor versus recipient T cells in the rejection process is unexamined. The aims of this research are to test the central hypothesis that donor T cells contribute to face transplant rejection, and that pathogenic T cells (both donor and recipient-derived) are detectable in blood during rejection to serve as personalized rejection biomarkers.

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

T cells, Face transplants, Rejection

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.*

Task 1. Obtaining HRPO approval – estimated at month 3, actual percentage of completion 100%. Completed on May 24, 2017.

Task 2. Determining turnover of donor and recipient T cells within facial allografts following transplant. Estimated to start at months 3-5, current percentage of completion is 100%.

Task 3: Confirmation of the role of pathogenic T cell clones in graft damage. Estimated completion: months 6-7. Current percentage of completion: 0%.

Task 4: Detection of pathogenic T cell clones in blood. Estimated completion months 8-10. Current percentage of completion: 100%.

**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.*

High throughput T-cell receptor sequencing (HTS) was used to track donor and recipient T cells following face transplantation using archived specimens from 3 pairs of donors and recipients. For each donor-recipient pair, we first established the donor and recipient T cell repertoires, and tracked those T cell clones in facial allograft and in the circulation after transplant during rejection and non-rejection.

**Findings**

***Donor T cells persisted within allograft for up to 1 year post-transplant and recipient T cell clones dominated over time***

Donor T cell clones predominated early within facial allograft, persisted for up to 1 year post-transplant and disappeared over time, whereas recipient T cell clones migrated into the allograft and over time became predominant (Figure 1).

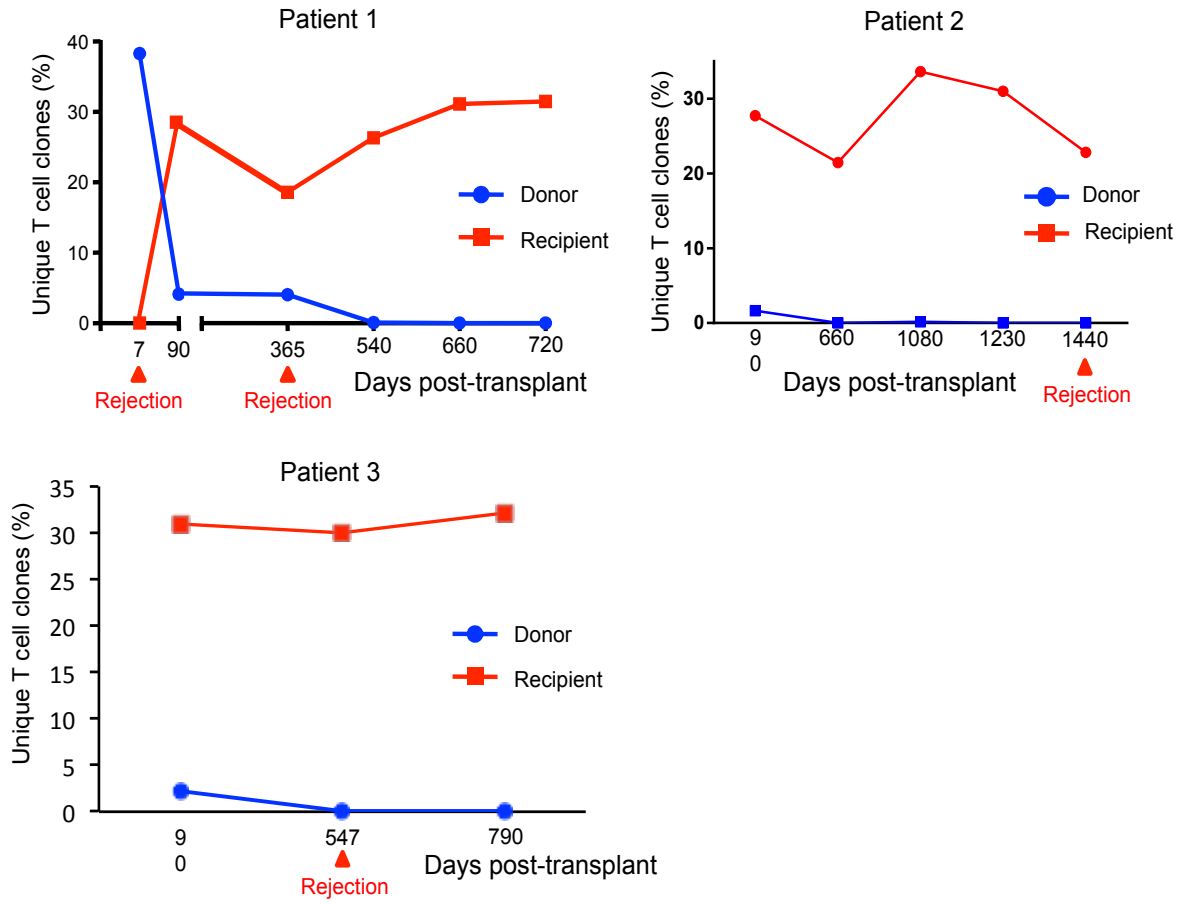



**Figure 1. Donor and recipient T cell clones within facial allograft following transplantation.**

***Donor T cells may contribute to early rejection episodes and recipient T cell clones dominated at later rejection episodes***

Donor T cells showed clonal expansion during early rejection episodes (Figure 2), suggesting that they may participate in rejection. In contrast, during late rejection episodes, recipient T cell clones dominated (Figure 3 and 4).

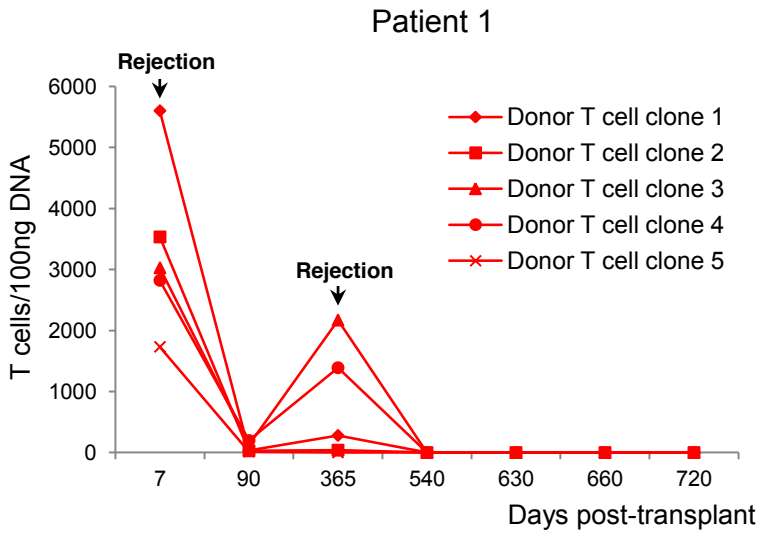



**Figure 2. The total number of top 5 most abundant T cell clones in the face transplant skin showed that 5/5 clones that underwent clonal expansion during early rejection episodes in Patient 1 were donor-derived.**

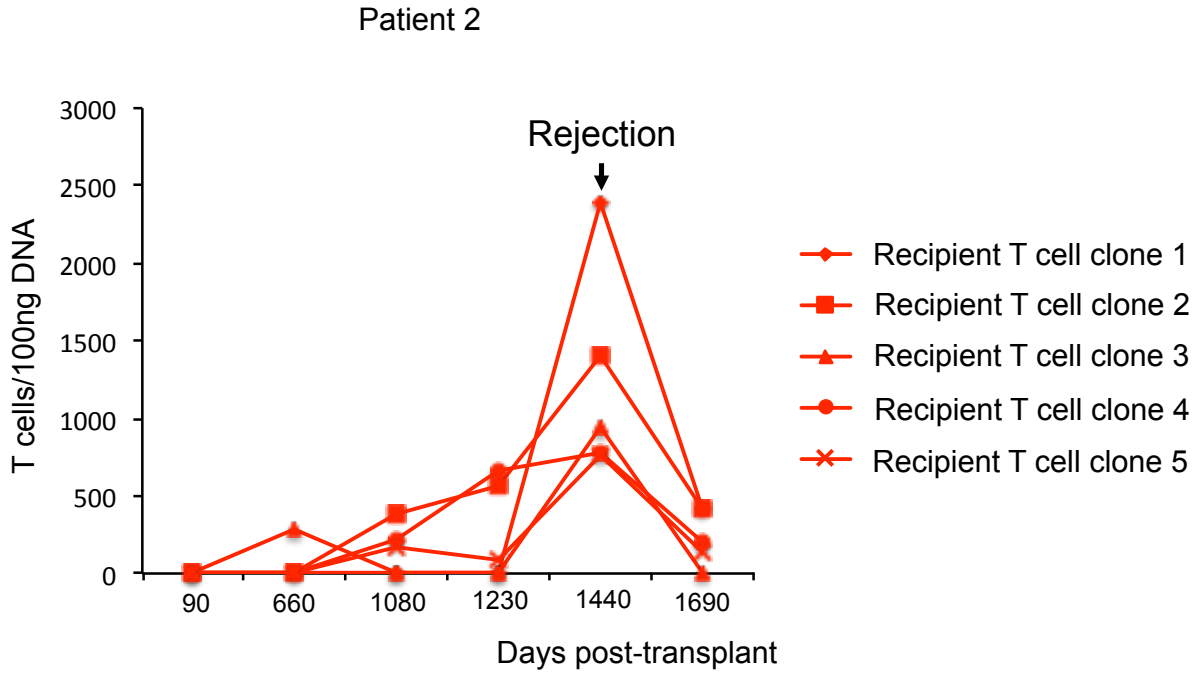



**Figure 3. The total number of top 5 most abundant T cell clones in the face transplant skin showed that 5/5 clones that underwent clonal expansion during late rejection episode in Patient 2 were recipient-derived.**

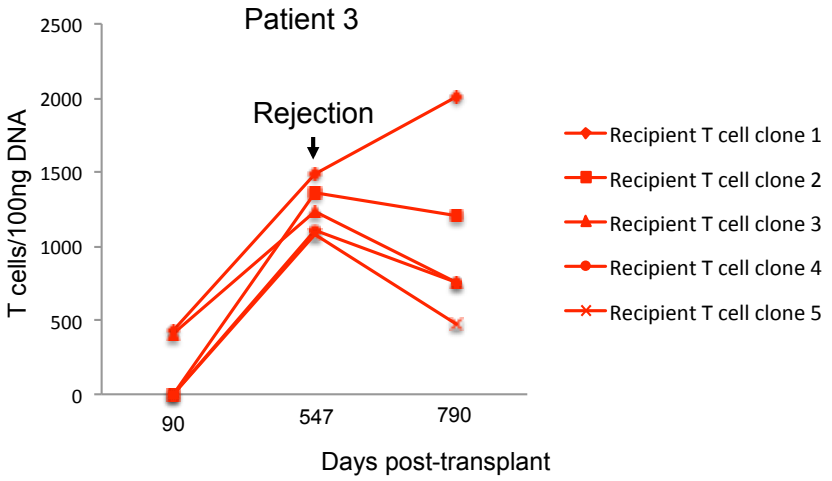



**Figure 4. The total number of top 5 most abundant T cell clones in the face transplant skin showed that 5/5 clones that underwent clonal expansion during late rejection episode in Patient 3 were recipient-derived.**

***T cells infiltration is associated with rejection***

To investigate the pathogenicity of T cells in rejection, we analysed the association between rejection episodes and the number of T cells infiltrating the face transplant skin. The number of T cells infiltrating the allograft increased during rejection episodes in all 3 patients. Importantly, the number of T cells decreased once the rejection episodes resolved (Figure 5), suggesting that T cell infiltration is associated with rejection.

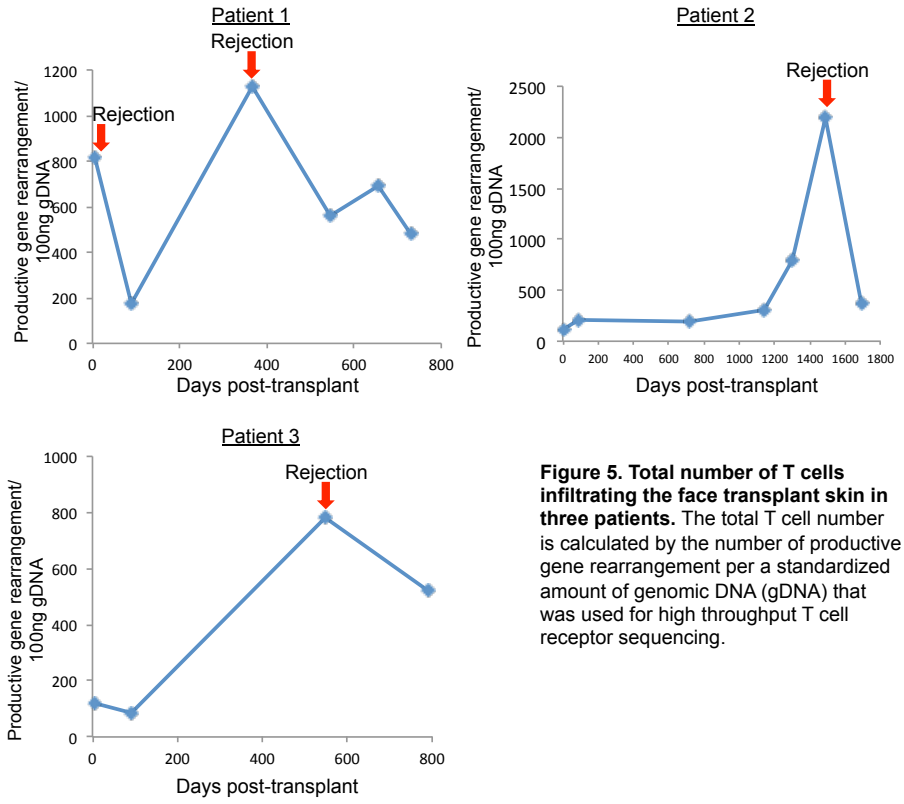
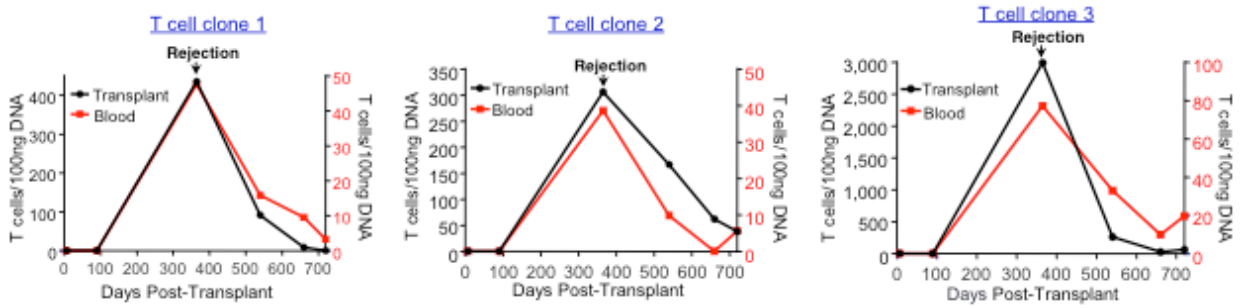



**Figure 5. Total number of T cells infiltrating the face transplant skin in three patients.** The total T cell number is calculated by the number of productive gene rearrangement per a standardized amount of genomic DNA (gDNA) that was used for high throughput T cell receptor sequencing.

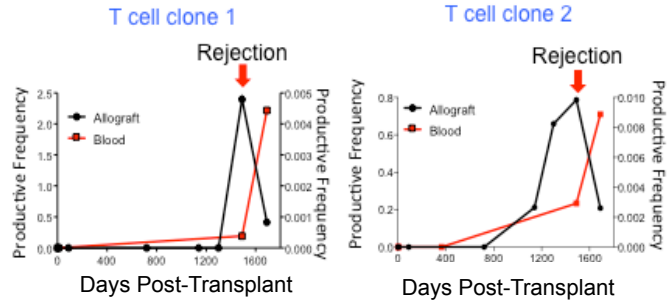
***Identical T cell clones that expanded within the face transplant skin during rejection also showed simultaneous clonal expansion in peripheral circulation***

We analyzed paired face transplant skin biopsies and blood collected at the same time using HTS. Remarkably, in all 3 patients, identical T cell clones (identified by TCR CDR3 V-beta nucleotide sequences) that expanded within the transplant during rejection also showed clonal expansion in blood (Figure 6). Therefore, our data raises the possibility that testing blood samples to monitor the frequencies of pathogenic T cell clones may serve as non-invasive rejection biomarkers. Because the T cell clones that cause rejection in each transplant is unique, these findings raise the potential for personalized management of immune suppression for each patient.

Patient 1




Patient 2




Patient 3

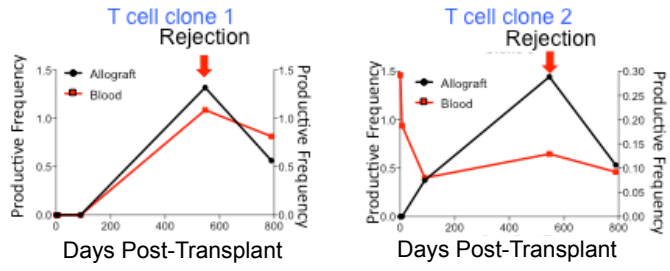



**Figure 6. Clonally expanded T cells infiltrating the transplant during rejection were also detectable and expanded in blood.** The frequencies of T cells within the transplant skin biopsies (left Y axis) were higher than in blood (right Y axis).

**Other Achievements**
Using the findings generated from this award as preliminary data, we submitted a proposal for an investigator-initiated award, which was successfully funded (Award number: W81XWH-18-1-0784). For the current award, we initially proposed to carry out immunostaining to co-localize inflammatory cytokine production within pathogenic T cell clones to confirm the role of pathogenic T cell clones in graft damage (Subtask 1 of Major Task 3). However, we were unable to obtain specific staining. Given the limited amount of archived specimens from face transplants, we decided not to pursue this method. Instead, we plan to utilize single nucleus RNA sequencing (sNucSeq) to determine the pathogenicity of T cell clones as part of the new award (W81XWH-18-1-0784).

**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.*

Nothing to Report.

*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).*

Our findings suggest the potential role of donor T cells in the rejection process and challenge the current paradigm that recipient T cells are exclusively responsible for rejection. Our data also suggest that pathogenic T cells, which are unique in each recipient, are detectable in the circulation and may serve as personalized rejection biomarkers. We are currently preparing a manuscript to report these findings in a peer-reviewed journal.

**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.*

HRPO approval took longer than we anticipated. It was approved on Month 7.

**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.*

Nothing to Report.

**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**

- HRPO (log #A-19710), approved on 05/24/2017
- IRB (Protocol #2016P002185), approved on 12/01/2016
- This study utilized archived specimens and therefore, target and enrollment numbers are not applicable.

**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. |

  *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: Bohdan Pomahac
Project Role: Principal Investigator
Nearest person month worked: 1
Contribution to project: Dr. Pomahac has provided scientific oversight as the principal investigator for this project.

Name: Thet Su Win
Project Role: Research Fellow
Nearest person month worked: 4
Contribution to project: Dr. Win has performed all experiments and data analyses. Also, she has drafted the progress reports.

**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.*

Nothing to Report

**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.*

| |
|---|
| Nothing to Report. |

## 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.*

# The role of tissue-resident donor T cells in rejection of clinical face transplants

W81XWH-16-1-0760

RT150073

**PI:** Bohdan Pomahac, MD **Org:** Brigham and Women's Hospital **Award Amount:** $150,000

## Study/Product Aim(s)

- Quantify the number and diversity of donor vs. recipient T cells within face transplants over time using cutting edge high throughput TCR sequencing (HTS)
- Identify pathogenic cell clones implicated in rejection using a combination of HTS and multicolor immunostaining/spectral imaging
- Identify if these pathogenic T cell clones are of donor vs. recipient origin
- Determine if these pathogenic cell clones are measurable in blood during episodes of rejection and could therefore serve as an early and specific rejection biomarker

## Approach

- Use banked skin biopsies and blood samples from 6 recipients of face transplants at different time points.
- Correlate results with clinical findings.

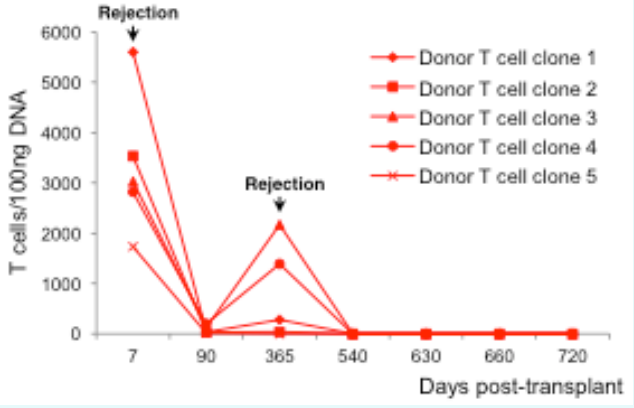



Figure. The total number of top 5 most abundant T cell clones in the face transplant skin showed that 5/5 clones that underwent clonal expansion during acute rejection were donor-derived.

Accomplishment: Generated evidence that donor T cells may contribute to early rejection episodes following face transplantation.

## Timeline and Cost

| Activities CY | 15 | 16 | 17 | 18 |
|---|---|---|---|---|
| Task 1. IRB and HRPO approval | | | | |
| Task 2. Quantify donor & recipient T cells | | | | |
| Task 3. Identify pathogenic T cell clones in grafts | | | | |
| Task 4. Identify pathogenic T cell clones in blood | | | | |
| **Estimated Budget ($K)** | **$0** | **$37.5K** | **$112.5K** | **$0** |

**Updated: 19 April, 2019**

**Goals/Milestones**

**CY16 Goal –** IRB/HRPO approval

☑ Both IRB and HRPO approved

**CY17 and CY18 Goals – Perform experiments, analyze and disseminate results**

☑ Quantify donor and recipient T cells in grafts

☐ Identify pathogenic T cell clones in grafts

☑ Identify pathogenic T cell clones in blood

☐ Disseminate results

**Comments/Challenges/Issues/Concerns**

- Timelines have changed with respect to the original proposal because of delay in obtaining IRB/HRPO approval.
- In original proposal, we proposed to carry out immunostaining to co-localize inflammatory cytokines within pathogenic T cell clones (Subtask 1 of Major Task 3). However, we were unable to obtain specific staining.

**Budget Expenditure to Date**

Projected Expenditure: **$150,000**

Actual Expenditure: **$149,980.24**