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AWARD NUMBER: W81XWH-17-1-0278

TITLE: Molecular Signature of Acute Rejection in Clinical Face Transplants

PRINCIPAL INVESTIGATOR: Bohdan Pomahac, MD

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

REPORT DATE: August 2018

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Fort Detrick, Maryland §21702-5012

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

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

Brigham and Women's Hospital
75 Francis St
Boston MA 02115

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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Vascularized composite allotransplantation (VCA), such as face and limb transplantation, is life transforming. Immunosuppression following VCA transplantation is based on protocols used in solid organ transplantation, but it is unclear if this is appropriate. The aims of this project are to i) determine the molecular phenotype of human face transplant rejection, ii) compare gene expression profiles of facial transplant rejection with that of inflammatory dermatoses, iii) compare gene expression profiles of facial transplant rejection with publically available gene expression datasets in solid organ transplants. We hypothesize that there are unique as well as shared molecular features in face transplant rejection compared to solid organ transplant rejection. Determination of unique features of face transplant rejection will highlight the need for and facilitate the development of targeted therapies for VCA recipients.

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.

Although post-transplant monitoring and immunosuppression protocols for vascularized composite allotransplants (VCA) are based on solid organ transplantation, VCAs have unique immunological characteristics. The purpose of the study is to firstly, determine the molecular landscape of rejecting and stable facial allografts, secondly, to compare gene expression profiles of facial allograft with that of inflammatory dermatoses, and lastly, to compare gene expression profiles of facial allograft rejection with publically available gene expression database in solid organ transplants.

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

Face transplants, gene expression, rejection

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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 2, actual percentage of completion 100%. Completed on 10th April 2018.
Task 2. To determine the molecular phenotype of rejection in facial allografts using 60 biopsies from 7 face transplant patients. Estimated completion at month 9. Current percentage of completion 35%.
Task 3: To compare molecular phenotype of rejecting facial allografts with that of biopsies taken from non-transplanted patients with rosacea and delayed-hypersensitivity reaction. Estimated completion at month 10. Current percentage of completion 0%.
Task 4: To compare molecular signature of acute rejection in facial allografts with kidney allografts using publically available gene expression datasets. Estimated completion at month 12. Current percentage of completion 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 Task 2, we extracted RNA from facial transplant skin biopsies collected during non-rejection (Grade 0, n=10) and severe rejection (Grade 3, n=11), from 7 face transplant patients. We then used the extracted RNA to quantify the expression of 730 genes using the NanoString nCounter technology. To identify the molecular changes associated with severe rejection, we compared the gene expression profiles of Grade 0 biopsies with those obtained at the time of severe Grade 3 rejection.

Findings

Unsupervised principal component analysis clustered Grade 3 biopsies separately from Grade 0 along the first principal component (Figure 1).

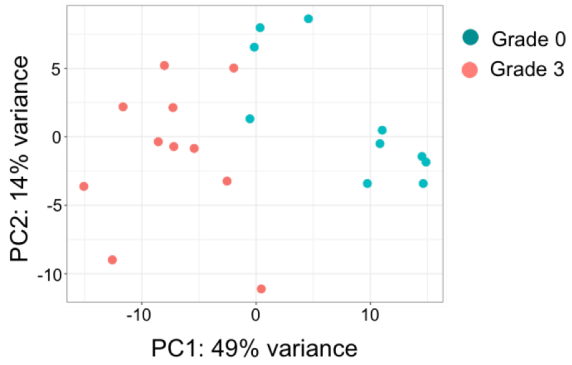

Figure 1. Unsupervised principal component analysis clustered Grade 3 rejection biopsies separately from Grade 0 samples

Subsequent differential expression analysis revealed that a total of 202 genes were differentially expressed in Grade 3 biopsies (all up-regulated compared to Grade 0; log2 fold change > 1; adjusted p value < 0.05). The top 50 differentially expressed genes are shown in Figure 2. The single most up-regulated gene was *GZMB* (granzyme B) (log2 fold change = 3.41 in comparison to Grade 0 biopsies). Many of the top up-regulated genes encode for proteins associated with T cell infiltration (*CD3D*, *CD3G*, *CD8A*), interferon-gamma signaling and effects (*STAT1*, *HLA-DRA*, *HLA-DOB*, *HLA-DPB1*, *HLA-DOB*) and effector molecules (*GZMB*, *GZMK*, *GZMA*, *PRF1*, *GNLY*). Interestingly, Grade 3 biopsies had increased expression of genes associated with T cell inhibition (*IDO*, *LAG3*, *CTLA4*), suggesting that regulatory processes are induced within face transplants during rejection.

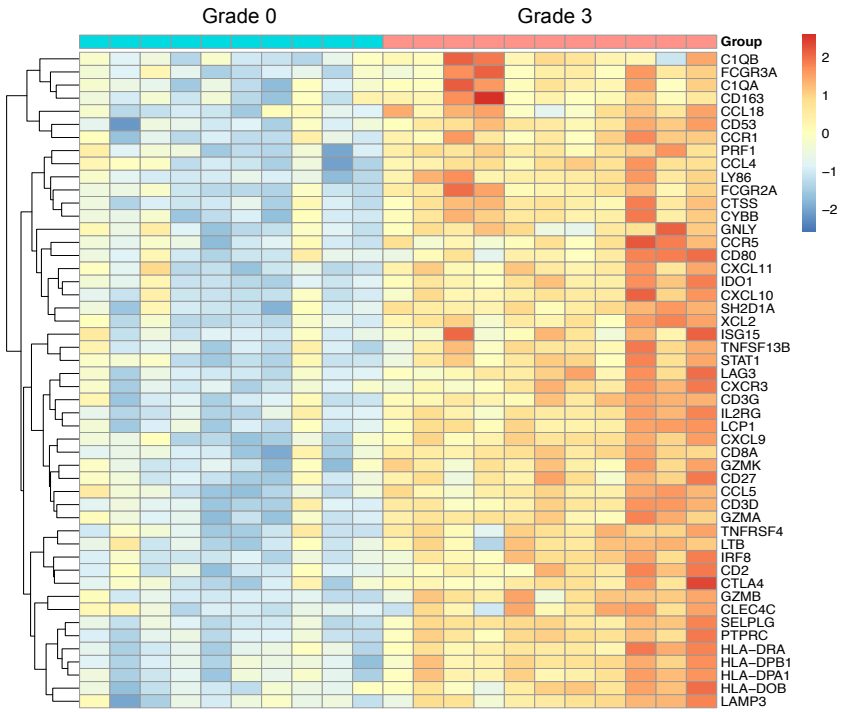

Figure 2. Heatmap of the top 50 genes differentially expressed in Grade 3 compared to Grade 0 biopsies (log2 fold change>1; adjusted p value<0.05). Each column represents a facial allograft biopsy. Gene expressions row scaled.

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.

During the next reporting period, we plan to continue with Task 2: To determine the molecular phenotype of rejection in facial allografts. Towards this task, we are performing gene expression profiles on biopsies collected during mild (Grade 1) and moderate (Grade 2) rejection episodes. Next, we will compare these gene expression profiles with those of Grade 0 and Grade 3 biopsies. Subsequently, we will validate the expression of genes of interest at the protein level using immunofluorescence staining. Over the next year, we plan to make progress towards Task 3: Compare molecular phenotype of rejecting facial allograft with that of inflammatory dermatoses. Towards this task, we will undertake gene expression profiling of facial skin biopsies from non-transplanted patients with rosacea and delayed-type hypersensitivity reaction, and compared them with the gene expression profiles of face transplant skin biopsies collected during rejection. In addition, we plan to make progress towards Task 4: Compare molecular signature of facial allograft rejection with kidney transplant rejection using publically available gene expression datasets.

4. **IMPACT:** Describe 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 Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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. However, we are now proceeding at full speed with the planned experiments.

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

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. In addition to a description of the technologies or techniques, describe how they will be 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. State whether an application is provisional or non-provisional and indicate the application number. 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;*
  - *biospecimen 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 MD
Project Role: PI
Nearest person month worked: 1 CM
Contribution to Project: Dr. Pomahac is a renowned surgeon-scientist. He provided scientific oversight and provided research samples utilized in the project. He also provided feedback and support on regulatory and protocol submissions.

Name: Thet Su Win MD, PhD
Project Role: Research Fellow
Nearest person month worked: 1 CM
Contribution to Project: Dr. Win has worked on regulatory submissions as well as the experimental procedures and data analysis.

Name: Sotirios Tasigiorgos, MD
Project Role: Research Fellow
Nearest person month worked: 3 CM
Contribution to Project: Dr. Tasigiorgos has assisted with the regulatory submissions as well as experimental procedures.

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 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.

Molecular signature of acute rejection in face transplants
W81XWH-17-1-0278
RT160119

PI: Bohdan Pomahac, MD **Org:** Brigham and Women's Hospital **Award Amount:** $200,000

Study/Product Aim(s)

- Determine the molecular signature of acute rejection in face transplants
- Compare the molecular phenotype of facial allograft rejection with that of inflammatory dermatoses
- Compare the molecular signature of facial allograft rejection with kidney transplant rejection

Approach

- Archived facial allograft biopsies from 7 face transplant patients
- Extract RNA from 60 biopsies
- Examine expression of 730 candidate genes and correlate with rejection grades
- Validate findings using independent set of 40 archived biopsies
- Compare with biopsies from non-transplanted patients with dermatoses and to kidney transplants

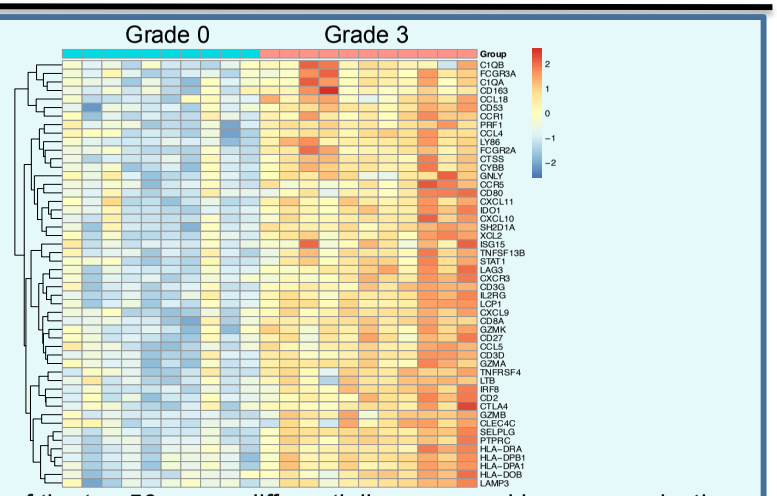

Figure. Heatmap of the top 50 genes differentially expressed in severe rejection (Grade 3) compared to Grade 0 biopsies.

Accomplishment: HRPO approval, determined molecular signature of severe rejection using biopsies from 7 clinical face transplants.

Timeline and Cost

Activities CY	17	18	19	20
Task 1. IRB and HRPO approval				
Task 2. Molecular phenotype of rejection				
Task 3. Compare molecular phenotype of rejection with dermatoses				
Task 4. Compare face transplant rejection with kidney transplant rejection				
Estimated Budget ($K)	**$60,853**	**$139,147**	**$0**	**$0**

Updated: August 12, 2018

Goals/Milestones

CY17 Goals – Months 1 - 6

- ☑ IRB approved
- ☑ HRPO approved
- ☑ RNA extraction of face transplant biopsies
- ☑ Gene expression profiling
- ☑ Bioinformatics analysesd
- ☐ Validation of findings

CY18 Goals – Months 7 – 12

- ☐ RNA extraction from biopsies of patients with dermatoses
- ☐ Gene expression profiling
- ☐ Bioinformatics analyses and comparison of molecular signature of acute rejection in face vs. kidney transplants

Comments/Challenges/Issues/Concerns

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

Budget Expenditure to Date

Projected Expenditure: $200,000
Actual Expenditure: 102,925.40