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TITLE: Molecular Signature of Acute Rejection in Clinical Face Transplants

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RECIPIENT: Brigham and Women's Hospital

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Rejection is the primary barrier to broader implementation of vascularized composite allografts. Diagnosis of rejection currently relies on histological assessment of skin biopsies according to the 2007 Banff criteria. The purpose of this study was to systemically interrogate the molecular mechanism underlying clinical facial allograft rejection. We also compared it to solid organ transplant rejection and non-transplant inflammatory skin diseases.
Grade 1 rejection did not differ significantly from non-rejection, suggesting that it does not represent a pathologic state and that watchful waiting is warranted. Grade 2 rejection had evidence of Th1 activation and upregulation of T cell co-stimulatory as well as co-inhibitory pathways. IFNγ-driven cytotoxicity, tissue injury and upregulation of immunoregulatory pathways were present in Grade 3 rejection. Rejection of VCA and solid organ transplants had both distinct and common features. VCA rejection was uniquely associated with upregulation of immunoregulatory genes and induction of lipid antigen-presenting CD1 proteins. These genes were unique to rejection and not upregulated in non-transplanted individuals with inflammatory skin diseases. Our findings suggest that the distinct features of VCA rejection reflect the unique immunobiology of skin and that enhancing cutaneous immunoregulatory networks may be a useful strategy in combating rejection.

15. SUBJECT TERMS
Molecular analysis, Face transplants, Acute rejection

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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 non-rejecting 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 publicly 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 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 2. Current percentage of completion 100%.
Task 2. To determine the molecular phenotype of rejection in facial allografts using biopsies from 7 face transplant patients. Estimated completion at month 9. Current percentage of completion 100%.
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 100%.
Task 4: To compare molecular signature of acute rejection in facial allografts with kidney allografts using publicly available gene expression datasets. Estimated completion at month 12. 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.

We studied archived formalin-fixed paraffin-embedded skin biopsies from 7 face transplant recipients using NanoString gene expression profiling. Histological analysis of these biopsies were performed using the 2007 Banff classification (Grade 0 = no rejection; Grade 1 = mild rejection; Grade 2 = moderate rejection; Grade 3 = severe rejection). Immunofluorescence staining of skin biopsies was used to validate our gene expression findings at the protein level.

Findings

Severe acute cellular rejection in face transplants has a distinct gene expression signature

We compared the gene expression profiles of Grade 0 biopsies with those obtained during Grade 3 rejection to identify the molecular changes associated with severe acute cellular rejection (ACR). Unsupervised principal component analysis (PCA) clustered Grade 3 biopsies separately from Grade 0 biopsies along the first principal component (Figure 1).

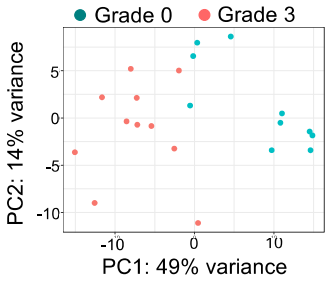

Figure 1. Unsupervised principal component analysis clustered Grade 3 rejection biopsies separately from Grade 0 samples.

Subsequent differential expression analysis revealed that a total of 202 genes were differentially expressed in Grade 3 rejection compared to Grade 0 (log2 fold change > 1; adjusted p-value < 0.05). The top 50 differentially expressed genes (DEGs) are shown in Figure 2. The single most upregulated gene was *GZMB* (log2 fold change = 3.41 compared to Grade 0). Many of the top up-regulated genes encode for proteins associated with T cell infiltration (e.g. *CD3G, CD8A*), interferon-gamma signaling and effects (e.g. *STAT1*), and effector molecules (e.g. *PRF1, GNLY, GZMA, GZMB, GZMK*). Interestingly, Grade 3 ACR biopsies had increased expression of T cell co-inhibitory receptor genes, including *LAG3*, and genes associated with immunoregulation (e.g. *IDO1*), suggesting that regulatory processes are induced within face transplants during severe rejection.

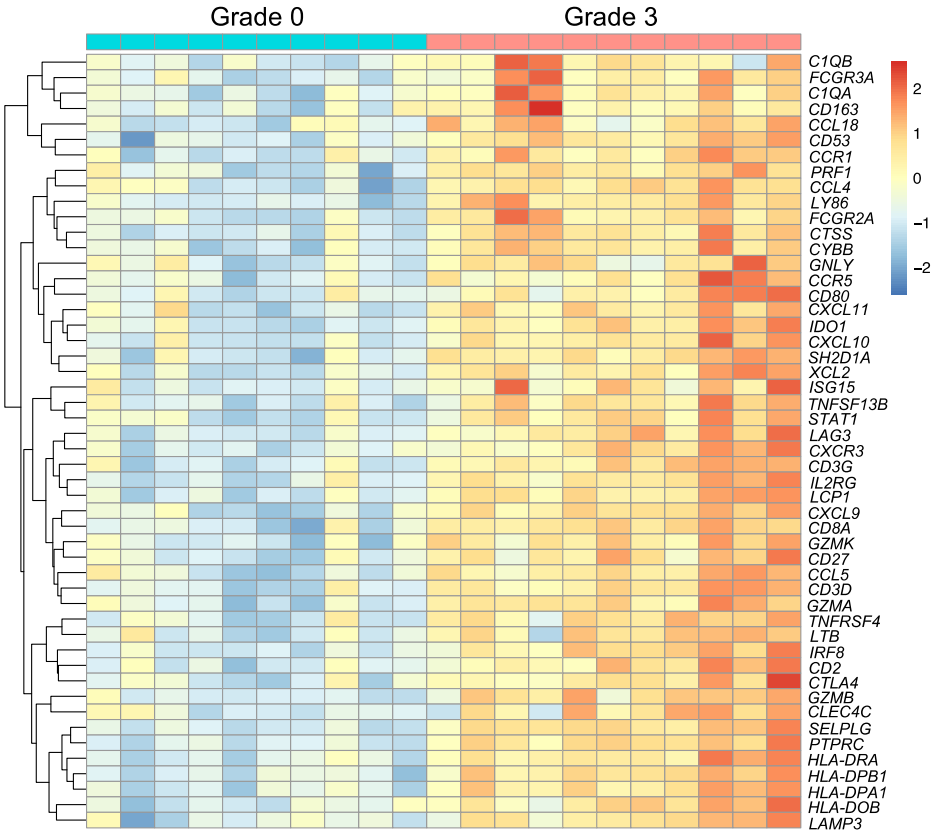

Figure 2. Heatmap of the top 50 genes differentially expressed in Grade 3 compared to Grade 0 biopsies (log2 fold change>1; adjusted p value <0.05). Each column represents a facial allograft biopsy. The cell color represents normalized levels from high (red) to middle (yellow) to low (blue). Gene expressions row scaled.

Mild ACR and non-rejection samples are not significantly different

Mild ACR (Grade 1) is the most commonly reported rejection grade in face transplants. According to the 2007 Banff classification, the difference between no rejection (Grade 0) and mild rejection (Grade 1) is: 'rare perivascular inflammation' versus 'mild perivascular inflammation' respectively. The terms 'rare' and 'mild' are not defined by an objective set of parameters. Healthy adult human skin contains 1 million memory T cells/cm^2 and many of these T cells are located in a perivascular distribution under non-inflamed conditions. The perivascular T cell presence in face transplant skin biopsies could therefore represent either normal skin resident T cell presence or true pathogenic immune activation. To discriminate between these possibilities, we carried out an unsupervised PCA of normalized gene expression counts of biopsies collected during non-rejection, and mild (Grade 1), moderate (Grade 2) and severe (Grade 3) ACR. There was no clear separation between mild ACR and non-rejection biopsies (Figure 3). Subsequent differential expression analysis between non-rejection and Grade 1 biopsies revealed no DEGs (Figure 4, left panel), suggesting that there is no significant difference between non-rejection and Grade 1 ACR at the molecular level.

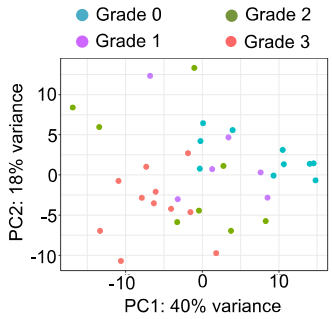

Figure 3. Unsupervised principal component analysis clustered all Grade 3 samples separately from Grade 0 biopsies, but Grade 1 and Grade 2 biopsies were molecularly heterogeneous.

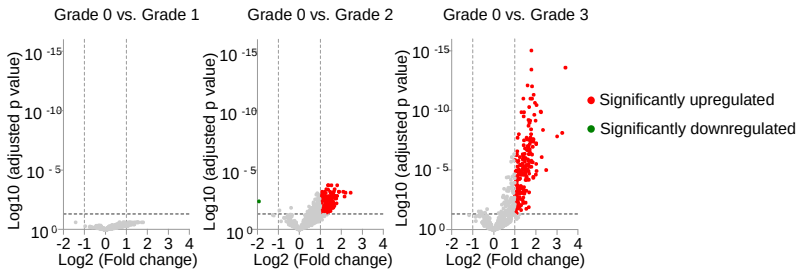

Figure 4. Differentially expressed genes in Grade 1, 2 and 3 rejections in relation to Grade 0 samples.

Progression to severe ACR involves a stepwise, coordinated induction of antigen presenting cells and T cell-associated genes

Although there were no DEGs in Grade 1 ACR compared to Grade 0, the number of DEGs identified increased with the transition to Grade 2 and Grade 3 (153 DEGs in Grade 2 compared to Grade 0; 202 DEGs in Grade 3 compared to Grade 0) (Fig. 4 middle and right panels). There was a stepwise increase in the expression of genes related to leukocyte trafficking, T cell infiltration, T cell co-stimulation, IFNγ signaling, antigen presentation, Th1 polarization, and effector molecules (Figure 5a-g).

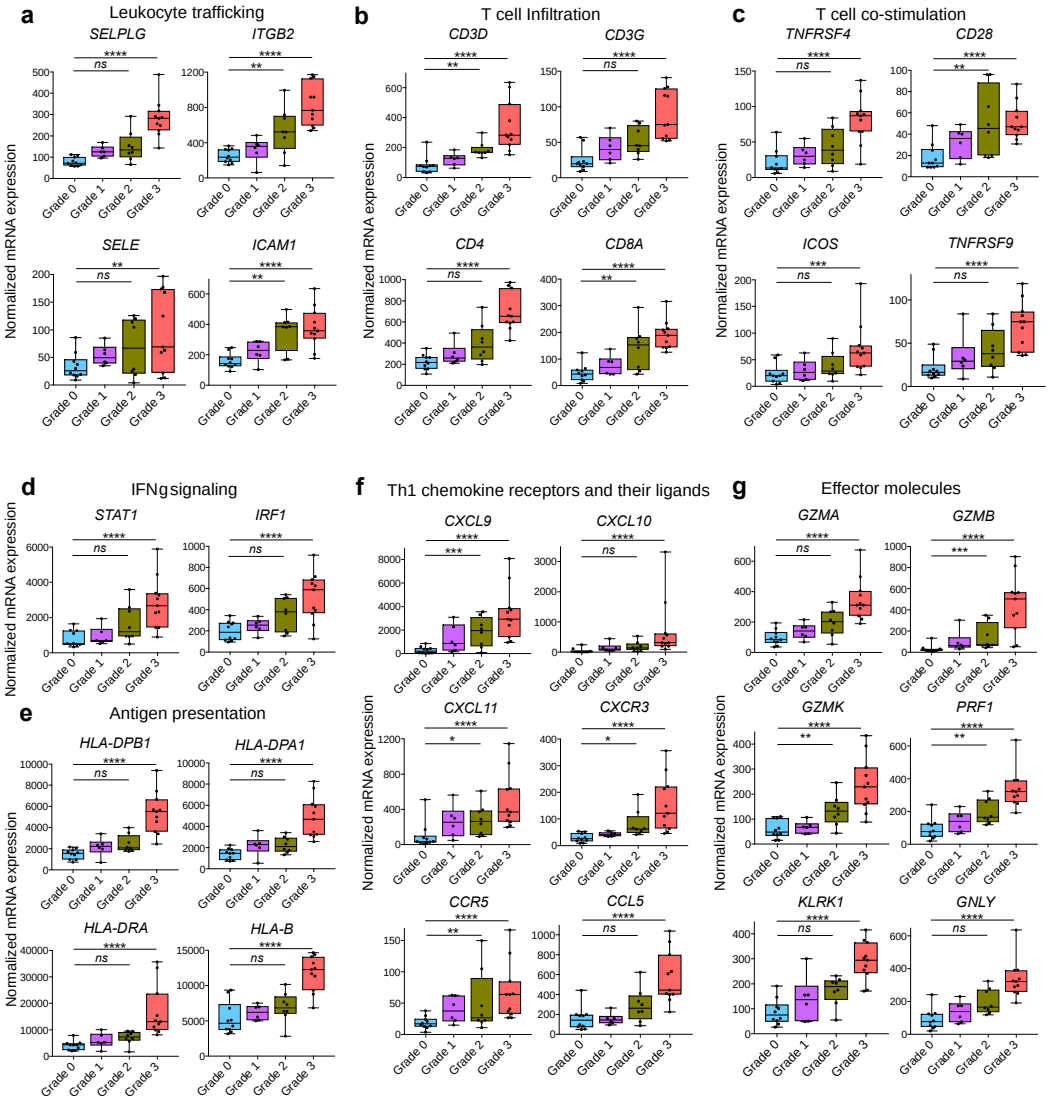

Figure 5. Box plots of normalized expression values of genes associated with a, leukocyte trafficking; b, T cell infiltration; c, T cell co-stimulation; d, IFNγ signaling; e, antigen presentation; f, Th1 polarization; g, effector molecules. Horizontal lines represent median values, with whiskers extending to the farthest data points. * indicates adjusted p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001; ****, p ≤ 0.0001. ns, not significant.

Remarkably, there was progressive upregulation of genes associated with pro-inflammatory immune activation (Figure 5) as well as genes associated with anti-inflammatory immune checkpoints and immunoregulation (Figure 6) with increasing severity of rejection.

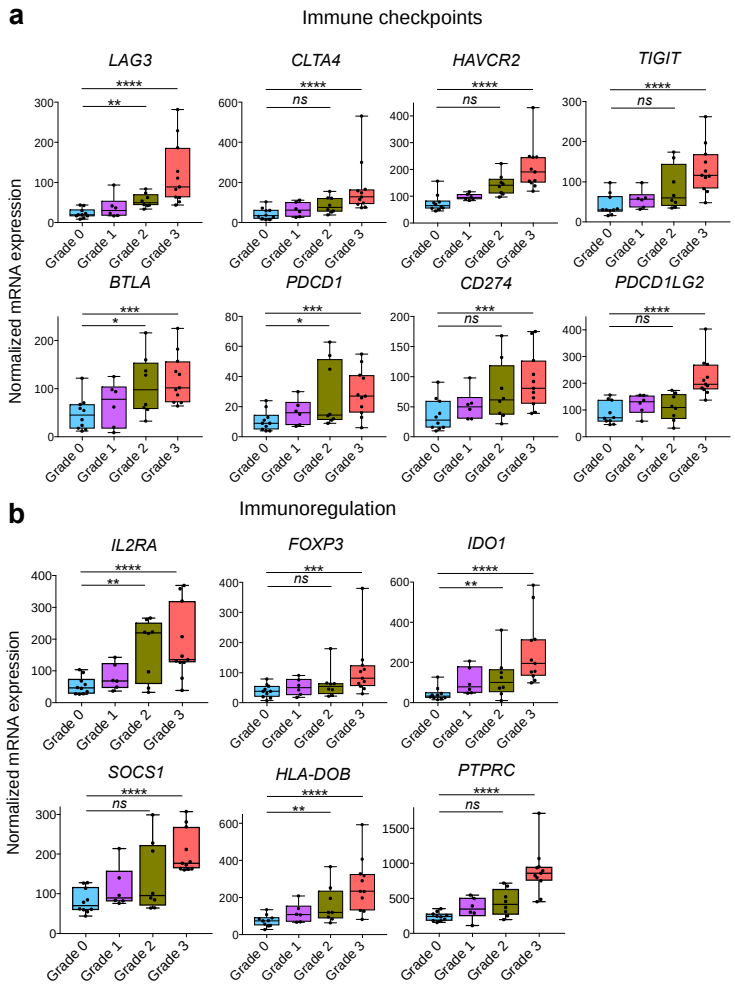

Figure 6. Box plots of normalized expression values of genes associated with a, immune checkpoint genes; b, genes associated with regulation of the immune response. Horizontal lines represent median values, with whiskers extending to the farthest data points. * indicates adjusted $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$. ns, not significant.

Severe ACR is characterized by expression of effector molecules and further induction of immunoregulatory pathways

Keratinocyte cell death (apoptosis, dyskeratosis and/or keratinolysis) is the primary histologic feature that distinguishes severe from moderate ACR according to the Banff classification. We hypothesized that Grade 3 biopsies displayed an increased expression of cytotoxic effector genes, in agreement with the cell death observed histologically. To examine this, we identified Grade 3-*specific* genes. A set of 107 genes was uniquely differentially expressed in Grade 3 ACR (but not in Grade 2) when compared to Grade 0 (Figure 7).

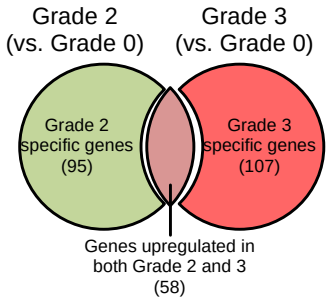

Figure 7. Venn diagram showing shared and unique differentially expressed genes in Grade 2 and Grade 3 ACR biopsies (when compared to Grade 0 samples).

Among these were seven genes associated with cytotoxicity (*GZMA*, *GZMH*, *GZMM*, *GNLY*, *KLRB1*, *KLRD1*, *KLRK1*) (Figure 8). Immunofluorescence analysis of skin biopsies confirmed the infiltration of granzyme expressing CD3-positive T cells within the allograft during Grade 3 rejection, validating our gene expression findings at the protein level (Figure 9).

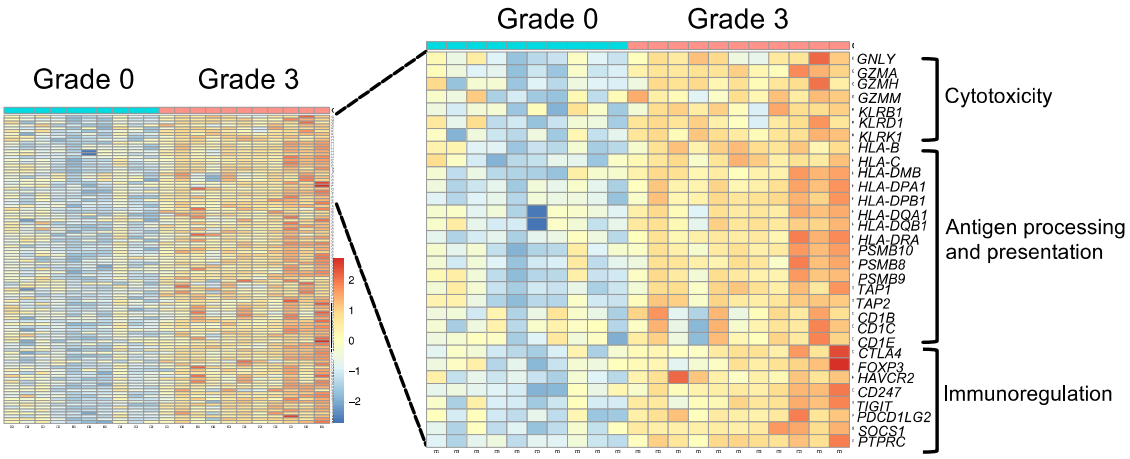

Figure 8. Heatmap of 107 genes that are uniquely differentially expressed in Grade 3 ACR, which included genes associated with cytotoxicity, antigen processing and presentation and immunoregulation. Each column represents a biopsy. Gene expression row scaled.

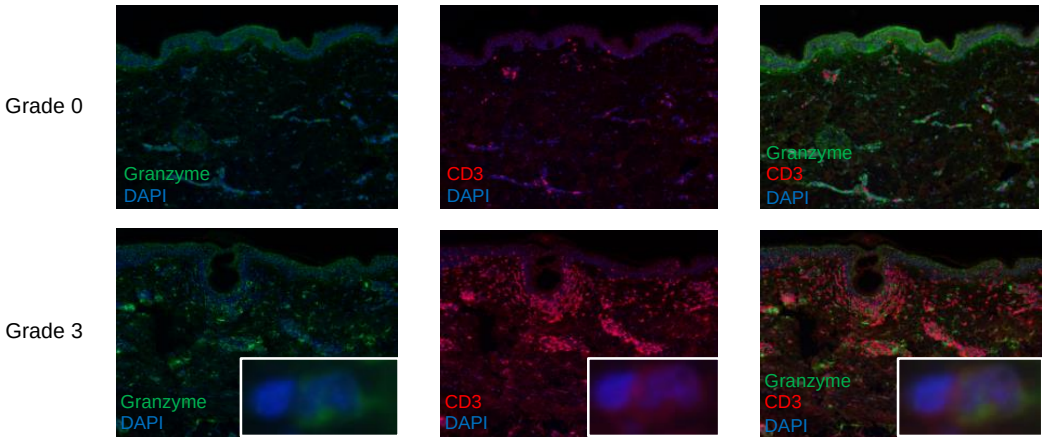

Figure 9. Immunofluorescence staining for granzyme and CD3 in skin biopsies collected during Grade 3 and Grade 0. Granzyme is shown in green, CD3 in red and DAPI in blue. Merged images are shown in the right column. Original magnification, x 10. Higher magnification (x 40) images are shown in small insets. DAPI, 4', 6- diamidine-2'-phenylindole dihydrochloride.

Face transplant rejection has both distinct and shared gene signatures compared to solid organ transplant rejection

To compare the gene expression signature of face transplant ACR with molecular signatures of acute rejection in human SOTs, we undertook a scoping review of SOT literature utilizing the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Studies measuring mRNA levels in the biopsy specimens across three types of human solid organ transplants (kidney, liver, heart) during established acute rejection and non-rejection were evaluated (Figure 10).

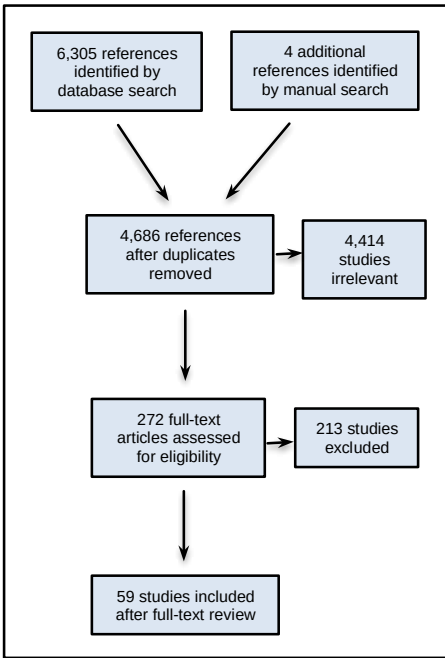

Figure 10. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of selection of solid organ transplant studies.

166/202 genes upregulated in face transplant ACR were shared with genes previously reported to be increased in SOT biopsies during acute rejection (Figure 11). Common elements included leukocyte trafficking, T cell infiltration, Th1 polarization, T cell co-stimulation, T cell co-inhibition, antigen processing and presentation, and effector molecules. We identified 36 genes that were unique to face transplant rejection, including genes associated with skin homing, APC and innate cell adhesion, type I interferon signaling, immunoregulation, co-stimulation and lipid presentation (Figure 11). These findings suggest that acute cellular rejection of face and solid organ transplants has both common and distinct features.

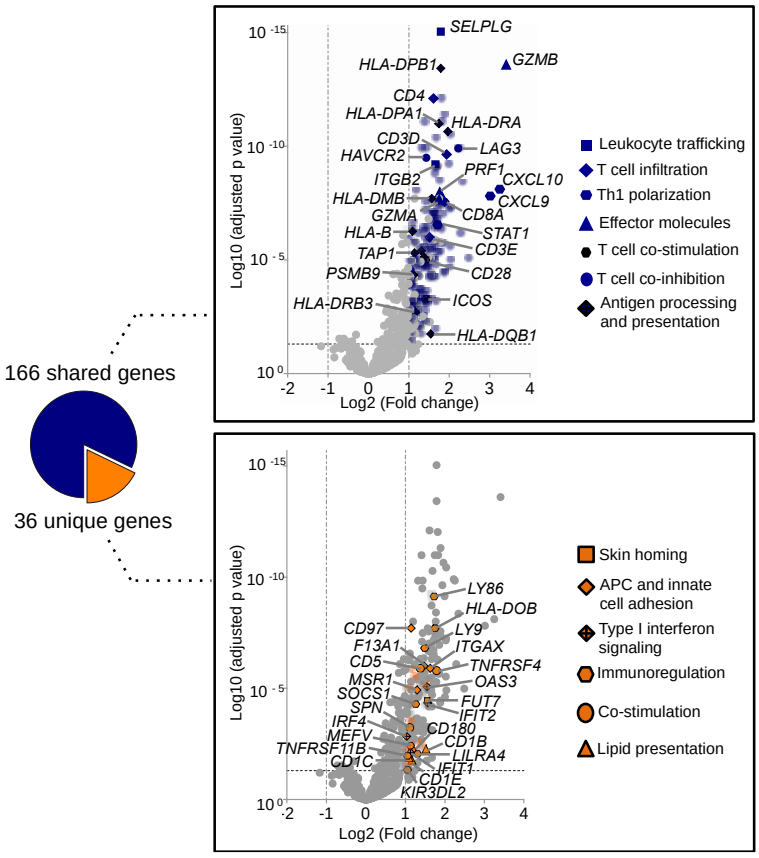

Figure 11. Volcano plots showing differentially expressed genes (DEGs) between Grade 3 rejection biopsies versus Grade 0 non-rejection biopsies. Genes shared with solid organ transplant rejection are shown in blue, and genes unique to face transplant rejection are shown in orange. Each dot represents an individual gene. Horizontal dashed lines represent an adjusted p value cutoff of $-\log_{10}$ (0.05); vertical dashed lines represent $\log_2$ fold change of -1 and +1.

Face transplant rejection has unique gene signature that are not upregulated in other inflammatory skin diseases

We next compared the gene expression profiles of face transplant skin biopsies collected during rejection with facial skin biopsies from non-transplanted patients with rosacea and delayed-type hypersensitivity (DTH) reaction. We selected to compare with these two T-cell mediated skin pathologies because they represent the two most common differential diagnoses in transplant skin biopsies when there is clinical suspicion of rejection in our cohort of face transplant patients. Healthy skin samples from non-transplanted patients who underwent facelift surgeries were used as additional controls.

Unsupervised principal component analysis clustered both rosacea and DTH samples separately from rejection samples (Figure 12).

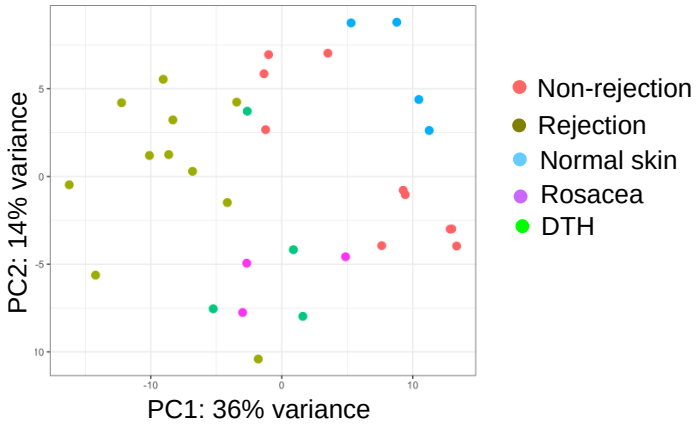

Figure 12. Unsupervised principal component analysis clustered biopsies from patients with rosacea and delayed type hypersensitivity reaction (DTH) separately from rejection biopsies.

Subsequent differential expression analysis revealed that 260 genes were significantly upregulated and 22 genes downregulated in rejection compared to normal skin, while 115 genes were upregulated and 30 genes downregulated in rosacea, and 137 genes upregulated and 22 genes downregulated in DTH, compared to normal skin (Figure 13).

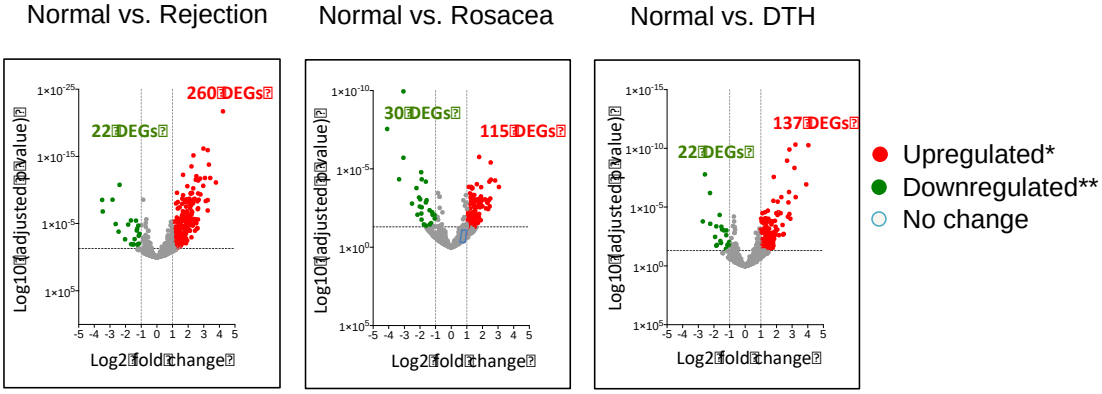

Figure 13. Differentially expressed genes (DEGs. Log2 fold change>1; adjusted p value <0.05) in rejection, rosacea and DTH, in relation to normal facial skin.

We next studied whether the 36 genes upregulated in face transplant rejection (but not in solid organ transplant rejection) represent unique characteristics of face transplant rejection or are common to other type of skin inflammation. Unsupervised hierarchical clustering showed that all except one biopsy from rosacea clustered separately from rejection biopsies, suggesting that upregulation of these genes was unique to face transplant rejection and not a feature common to other types of skin inflammation (Figure 14).

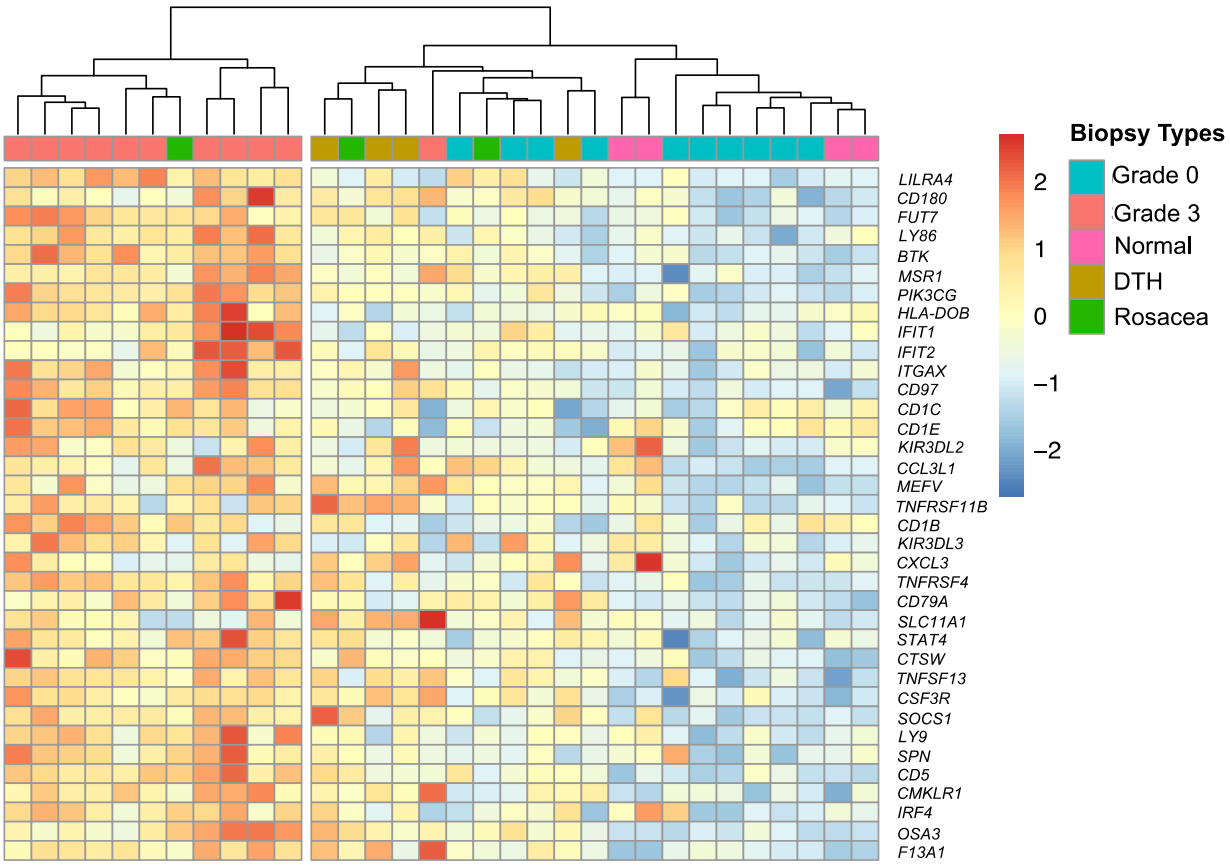

Figure 14. Heatmap of normalized expression values for thirty-six genes upregulated in face transplant rejection that have not been reported in solid organ transplant rejection. Each column represents a biopsy sample. Gene expression row scaled. The cell color represents normalized levels from high (red) to middle (yellow) to low (blue). The degree of relatedness is represented by the dendrogram at the top of the panel (unsupervised hierarchical clustering). All except one biopsy collected from non-transplanted patients with rosacea clustered separately from rejection (Banff Grade 3) samples.

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 study found that:

1). There were no significant differences between non-rejection and mild rejection (Grade 1). We propose that Grade 1 rejection does not represent a pathologic state and can be managed with watchful waiting. This represents a significant change in clinical practice. Rejection is currently treated with systemic pulsed steroids and/or increases in maintenance immunosuppressive medications, therapies that put patients at increased risk for infection or cancer.

2). In Grade 2 rejection, there were features of T cell signaling and Th1 polarization, but no tissue damage. There was also upregulation of immunoregulatory pathways and immune checkpoint molecules. We hypothesize that it is the induction of these diverse immunoregulatory pathways that prevents cytotoxic tissue damage in Grade 2 rejection, at a point in time when T cells are responding to allostimulatory antigen presenting cells. These results suggest that therapies that enhance skin associated immunoregulatory pathways may be useful in face transplantation to combat rejection.

3). There was marked upregulation of cytotoxicity genes in Grade 3 biopsies that corresponded to the tissue injury observed histologically. Immunoregulatory pathways continued to be induced but were clearly no longer adequate to prevent tissue damage. Induction of genes associated with IFNγ signaling suggests that IFNγ is the principal cytokine driving human face transplant rejection.

4). There were gene signatures that were unique to face transplant rejection and not observed in rejection of solid organ transplants. These included genes associated with skin homing, immunoregulation, APC presence, adhesion and function, co-stimulation and lipid antigen presenting CD1 molecules. These genes were unique to rejection and not upregulated in other skin inflammation unrelated to transplantation.

These results demonstrate that the transplantation of skin containing VCAs is both a challenge and an opportunity. These tissues are heavily populated by donor immune cells but also have complex immunoregulatory networks that could be leveraged to combat rejection. Further studies on the details of these immunoregulatory pathways may lead to novel therapies for rejection in patients who have received these life-changing transplants.

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.

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 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).*

  Win T.S., Dyring-Andersen B., Lopdrup R., Teague J.E., Barrera V., Ho Sui S., Tasigiorgos S., Murakami N., Chandraker A., Tullius S.G., Pomahac B., Riella L.V, Clark R.A. Immunoregulatory and lipid presentation pathways are upregulated in human face transplant rejection. *Science Translational Medicine* (Submitted). Acknowledgement of federal support (yes).

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.*

1. Comparison with solid organ transplants reveals distinct face transplant rejection gene signature that reflects the unique immunobiology of skin.
   American Transplant Congress (Boston, June 2019)

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

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 worked on experimental procedures and data analysis.

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.*