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TITLE: Noninvasive Immune Monitoring Biomarkers Using Plasma microRNAs in VCA

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CONTRACTING ORGANIZATION: Johns Hopkins University

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

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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) has become a viable alternative to reconstruct complex defects. In the emerging field of VCA, a critical component in the success of the graft is careful maintenance of immunosuppression. Over-immunosuppression results in chronic infections and the accumulation of dangerous side effects. On the other hand, insufficient immunosuppression can lead to acute or chronic rejection episodes and the loss of graft function or even the graft itself. When embarking on innovative new tolerance induction protocols, or attempting to wean patients from conventional therapies the ability to monitor the immunological status of an allograft is of critical importance. Confirmation of clinical rejection still requires the use of an invasive biopsy which is not ideal for routine monitoring. There exists a need for non-invasive technologies which can detect changes in the immunological status of the graft prior to obvious clinical manifestations of inflammation and tissue damage. MicroRNAs (miRNAs) are single-stranded non-coding RNAs and exist in various tissues, organs and even in blood. The objective of this application is to develop some tissue specific miRNAs without the requirement for invasive tissue biopsy. Herein, Specific Aim1 propose to investigate whether profile of miRNAs differ between donor grafts. miRNAs including let 7a/7c, miR-125b, miR-146, miR-150, miR-181a, miR-155, miR-144, miR-29, miR-21, miR-192, miR-142-5p and miR-223 will be compared to histopathological changes and clinical outcomes between groups. In Specific Aim2, mechanistic trends of expression in miRNAs within long term surviving and tolerant recipients will be tested. In Specific Aim3, correlation and validation in any of miRNAs in human samples will be verified any signature identified in Aim 1 and 2.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
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1. Introduction
Non-invasive biomarkers using miRNA could allow for detection of rejection prior to clinical manifestation and for fine-tuning of individual immunosuppression. Purpose of this study is that some miRNAs could provide specific and sensitive immune biomarker profiles to allow for improved monitoring and diagnosis of rejection without the need for invasive tissue biopsies and advance of clinical signs of rejection or permanent tissue damages in VCA.

2. Keywords
VCA, immunosuppression, miRNA, non-invasive, monitoring

3. Accomplishment

- **What were the major goals of the project?**
  Major Goals: Subtask 1: Obtain IACUC and ACURO approval for the mouse studies.

Major Task 1: Investigate plasma expression of miRNAs after transplantation	Months	Completion (%)
Subtask 1.1- Receive Institutional Animal Care and Use Committee (IACUC) and DoD Animal Care and Use Review Office (ACURO) approvals	0-4	100
Subtask 1.2 - Perform murine skin, heart and hind limb transplantation (Groups 1, 2, and 3); monitor kinetics of plasma expression of miRNAs after different transplantation.	5-6	80
Subtask 1.3- Evaluate histopathological changes (H&E) and inflammatory infiltration (IHC or IF for CD3, CD4 and CD8 (T cells)) on indicated time points.	5-6	
Milestone(s): *To determine the base line of plasma expression of miRNAs after surgical inflammation in different transplantations*	6	
Major Task 2: Investigate whether profile of plasma miRNAs differ in allotrasplantation setting	Months	
Subtask 2.1- Perform murine skin, heart and hind limb transplantation (Groups 4, 5, and 6); monitor kinetics of plasma expression of miRNAs after different transplantation.	7-9	80
Subtask 2.2 - Evaluate histopathological changes (H&E) and inflammatory infiltration (IHC or IF for CD3, CD4 and CD8 (T cells)) on indicated time points.	7-9	
Milestone(s): *To assess distinct differences in mechanisms of plasma expression of miRNAs observed in different setting of combination and grafts.*	9	
Major Task 3: Investigate the mechanistic trends and correlation with rejection between plasma expression in miRNAs in setting of long term surviving treatment*	Months	

Subtask 3.1 - Perform murine skin, heart and hind limb transplantation (Groups 7, 8, 9 and 10); monitor kinetics of plasma expression of miRNAs after different transplantation.	9-14	80
Subtask 3.2- - Evaluate histopathological changes (H&E) and inflammatory infiltration (IHC or IF for CD3, CD4 and CD8 (T cells)) on indicated time points.	9-14	
Milestone(s): *Identify pattern and sensitivity of plasma expression of miRNAs to be advanced of clinical signs*	14	
Major Task 4: Correlation and validation in any of miRNAs in human samples will be verified any signature identified in Aim 1 and 2.	Months	
Subtask 4.1- Receive IRB and DoD Human Research Protection Office approvals	0-6	100
Subtask 4.2 – Collect samples; monitor kinetics of plasma expression of miRNAs; evaluate and validate in any of miRNAs in human samples	15-17	50
Subtask 4.3 - Final data analysis and interpretation. Prepare reports and manuscripts for submission	17-18	
Milestone(s): *Identify pattern and sensitivity of plasma expression of miRNAs to be advanced of clinical signs from human samples*	18	

- **What was accomplished under these goals?**

1) **Major Activities:**
   - Major Task 1: Investigate plasma expression of miRNAs after transplantation
   - Major Task 2: Investigate whether profile of plasma miRNAs differ in allotrasplantation setting

2) **Specific Objectives:**
   - Major Task 1, Subtask 1.2 : Perform murine skin, heart and hind limb transplantation (Groups 1, 2, and 3); monitor kinetics of plasma expression of miRNAs after different transplantation.
   - Major Task 2, Subtask 2.1: Perform murine skin, heart and hind limb transplantation (Groups 4, 5, and 6); monitor kinetics of plasma expression of miRNAs after different transplantation.

<u>Major Task 1, Subtask 1.2</u>: Perform murine skin, heart and hind limb transplantation (Groups 1, 2, and 3); monitor kinetics of plasma expression of miRNAs after different transplantation.

<u>Major Task 2, Subtask 2.1</u>: Perform murine skin, heart and hind limb transplantation (Groups 4, 5, and 6); monitor kinetics of plasma expression of miRNAs after different transplantation.

We have selected miRNA probes in order to optimize condition of miRNA expression and comparison to monitor kinetics of plasma expression of miRNAs after different transplantation. Below is the table exampling miRNA probes.

MiRCury Assay cat. no.	Plate No.	Row	Column	miRNA ID
YP02105294	1	A	1	
YP00205411	1	A	2	
YP00205986	1	A	3	
YP00204272	1	A	4	
YP00205400	1	A	5	
YP02105524	1	A	6	
YP02104818	1	A	7	
YP00205173	1	A	8	
YP02104706	1	A	9	
YP02115408	1	A	10	
YP00205586	1	A	11	
YP00205315	1	A	12	
YP00203907	1	A	13	U6 snRNA miRCURY LNA miRNA PCR Assay
YP00203952	1	A	14	cel-miR-39-3p miRCURY LNA miRNA PCR Assay
YP02119305	1	A	15	
YP00204772	1	A	16	
YP00204063	1	A	17	
YP00206026	1	A	18	
YP00204306	1	A	19	
YP00204715	1	A	20	
YP00204337	1	A	21	
YP00203950	1	A	22	UniSp2 miRCURY LNA miRNA PCR Assay
YP00203953	1	A	23	UniSp4 miRCURY LNA miRNA PCR Assay
YP02119288	1	A	24	UniSP3 miRCURY LNA miRNA PCR Assay
YP02105294	1	B	1	
YP00205411	1	B	2	
YP00205986	1	B	3	
YP00204272	1	B	4	
YP00205400	1	B	5	
YP02105524	1	B	6	
YP02104818	1	B	7	
YP00205173	1	B	8	
YP02104706	1	B	9	
YP02115408	1	B	10	
YP00205586	1	B	11	
YP00205315	1	B	12	
YP00203907	1	B	13	U6 snRNA miRCURY LNA miRNA PCR Assay
YP00203952	1	B	14	cel-miR-39-3p miRCURY LNA miRNA PCR Assay

-
- **<u>Major Task 3, Subtask 3.1</u>: Perform murine skin, heart and hind limb transplantation (Groups 7, 8, 9, and 10);**
- **monitor kinetics of plasma expression of miRNAs after different transplantation.**

We have approved NCE as of Apr 4th, 2022, We have placed order customized miRNA array kits and we are collecting materials to perform quality control of the RNA isolation and cDNA synthesis steps of miRNA PCR experiments. We will utilize the remaining funds in accordance with the proposed SOW to finalize our specific aims.

4. Impact

- **What was the impact on the development of the principal discipline(s) of the project?**
  Nothing to report
- **What was the impact on other disciplines?**
  Nothing to report
- **What was the impact on technology transfer?**
  Nothing to report
- **What was the impact on society beyond science and technology?**
  Nothing to report

5. Changes/Problems

- **Changes in approach and reasons for change**
  Nothing to report
- **Actual or anticipated problems or delays and actions or plans to resolve them**

Actual or anticipated Problems or delays

1. With the pandemic of COVID-19, certain delay is anticipated in the upcoming reporting period.

Course of actions planned to mitigate problems

1. Currently focus is laid on anticipating future experiments and coordinating experimental plans, supplies and resources in order to allow a timely re-start of the *in-vivo* and *in-vitro* experiments.

- **Changes that had a significant impact on expenditures**
  Nothing to report
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  Nothing to report
- **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

- **Publications, conference papers, and presentations**
  Nothing to report
- **Website(s) or other Internet site(s)**
  Nothing to report

- **Technologies or techniques**
  Nothing to report
- **Inventions, patent applications, and/or licenses**
  Nothing to report
- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name: Byoung Chol Oh, D.V.M., Ph.D.
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: .60
Contribution to Project: Dr. Oh has performed work regarding the establishment and completion of the IACUC protocol as well as the submission of the ACURO protocol.

Name: Gerald Brandacher, M.D.
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: .12
Contribution to Project: Dr. Brandacher has supervised work regarding the establishment and completion of the IACUC protocol as well as the submission of the ACURO protocol.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Name (First and Last)	Current Support Changes
Oh, Byoung Chol	Change: Effort Ended Feasibility of expanding ischemia time for hearts destined for transplantation Sponsor: Tissue Testing Technologies LLC/ Prime: NIH Award: R43HL152941 Role: Co- I Effort: .60 CM Date: 01/01/2021 – 08/31/2021
Oh, Byoung Chol	Change: Closed Autologous Hematopoietic Stem Cell Transplantation to Prevent Antibody-Mediated Sponsor: DoD Award: W81XWH-16-1-0664 Role: Co- I Effort: .60 CM Date: 09/15/2016 – 09/14/2021

Oh, Byoung Chol	Change: Closed Multiparametric Bioreactor for Functional Preservation of Vascularized Composite Sponsor: DoD Award: W81XWH-17-1-0287 Role: Co- I Effort: 1.20CM Date: 09/30/2017 – 09/29/2021
Oh, Byoung Chol	Change: Received Building upon current practices by perfusion and mechanism-based s... Sponsor: DoD Award: W81XWH-21-1-0689 Role: Co- I Effort: .96 CM Date: 09/25/2021 – 09/24/2024
Oh, Byoung Chol	Change: Received Using Bioinspired Next-Generation Cryoprotectants to Advance Ex… Sponsor: DoD Award: W81XWH-21-1-0734 Role: Co- I Effort: 1.20 CM Date: 09/30/2021 – 09/29/2024
Oh, Byoung Chol	Change: Received Myocardial-associated B lymphocytes and inflammatory injury Sponsor: National Heart Lung and Blood Institute Award: R01HL160716 Role: Co- I Effort: .12 CM Date: 01/01/2022 – 12/31/2025
Gerald Brandacher	Change: Closed Replacing Sutures for Microvascular and Vascular Anastomosis Sponsor: TEDCO Award: Project #0920-003_02 Role: Co-I Effort:.12CM Date: 01/13/2021-12/13/2021
Gerald Brandacher	Change: Closed Autologous Hematopoietic Stem Cell Transplantation to Prevent Antibody-Mediated Sponsor: DoD Award: W81XWH-16-1-0664 Role: PI Effort: .60 CM Date: 09/15/2016 – 09/14/2021
Gerald Brandacher	Change: Closed Multiparametric Bioreactor for Functional Preservation of Vascularized Composite Sponsor: DoD Award: W81XWH-17-1-0287 Role: PI Effort: 1.20CM Date: 09/30/2017 – 09/29/2021
Gerald Brandacher	Change: Received Building upon current practices by perfusion and mechanism-based s... Sponsor: DoD Award: W81XWH-21-1-0689 Role: PI Effort: .60 CM Date: 09/25/2021 – 09/24/2024
Gerald Brandacher	Change: Received Using Bioinspired Next-Generation Cryoprotectants to Advance Ex… Sponsor: DoD Award: W81XWH-21-1-0734 Role: PI Effort: .60 CM Date: 09/30/2021 – 09/29/2024

Gerald Brandacher	Change: Received Myocardial-associated B lymphocytes and inflammatory injury Sponsor: National Heart Lung and Blood Institute Award: R01HL160716 Role: Co- I Effort: .12 CM Date: 01/01/2022 – 12/31/2025
Gerald Brandacher	Change: Received Isochoric Pressure Based Preservation of Cells, Tissues and Organs Sponsor: Sylvatica Biotech Inc. Prime: NIH Award: 5R44AI145782 Role: PI Effort: .45 CM Date: 01/01/2022 – 03/31/2023

- **What other organizations were involved as partners?**
  Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

9. APPENDICES

ACURO, Animal Care and Use Review Office
H&E, Hematoxylin and eosin
IACUC, Institutional Animal Care and Use Committee
miRNA, micro Ribonucleic acid
VCA, vascularized composite allotransplantation