

AWARD NUMBER: W81XWH-19-1-0234

TITLE: Noninvasive Immune Monitoring Biomarkers Using Plasma microRNAs in VCA

PRINCIPAL INVESTIGATOR: Byoung Chol Oh, D.V.M., Ph.D.

CONTRACTING ORGANIZATION: Johns Hopkins University, Baltimore, MD

REPORT DATE: July 2021

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

# REPORT DOCUMENTATION PAGE

*Form Approved*  
*OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

<b>1. REPORT DATE</b> July 2021			<b>2. REPORT TYPE</b> Annual		<b>3. DATES COVERED</b> 15Jun2020-14Jun2021	
<b>4. TITLE AND SUBTITLE</b>  Noninvasive Immune Monitoring Biomarkers Using Plasma microRNAs in VCA					<b>5a. CONTRACT NUMBER</b> W81XWH-19-1-0234	
					<b>5b. GRANT NUMBER</b> Log: RT180159	
					<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b> Byoung Chol Oh, D.V.M., Ph.D.  E-Mail: boh3@jhmi.edu					<b>5d. PROJECT NUMBER</b>	
					<b>5e. TASK NUMBER</b>	
					<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  Johns Hopkins University Department of Plastic and Reconstructive Surgery, VCA lab, 720 Rutland Ave, Baltimore 21205					<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012					<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for Public Release; Distribution Unlimited					<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>14. ABSTRACT</b> 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.						
<b>15. SUBJECT TERMS</b> None listed.						
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b> USAMRMC	
<b>a. REPORT</b>	<b>b. ABSTRACT</b>	<b>c. THIS PAGE</b>			<b>19b. TELEPHONE NUMBER</b> (include area code)	
Unclassified	Unclassified	Unclassified	Unclassified	9		

## TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	7
5. Changes/Problems	7
6. Products	7
7. Participants & Other Collaborating Organizations	8
8. Special Reporting Requirements	9
9. Appendices	9

## 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 (%)
<b>Subtask 1.1-</b> Receive Institutional Animal Care and Use Committee (IACUC) and DoD Animal Care and Use Review Office (ACURO) approvals	0-4	100
<b>Subtask 1.2 -</b> 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	70
<b>Subtask 1.3-</b> Evaluate histopathological changes (H&E) and inflammatory infiltration (IHC or IF for CD3, CD4 and CD8 (T cells)) on indicated time points.	5-6	
<b>Milestone(s):</b> <i>To determine the base line of plasma expression of miRNAs after surgical inflammation in different transplantations</i>	6	
Major Task 2: Investigate whether profile of plasma miRNAs differ in allotransplantation setting	Months	
<b>Subtask 2.1-</b> 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	70
<b>Subtask 2.2 -</b> Evaluate histopathological changes (H&E) and inflammatory infiltration (IHC or IF for CD3, CD4 and CD8 (T cells)) on indicated time points.	7-9	
<b>Milestone(s):</b> <i>To assess distinct differences in mechanisms of plasma expression of miRNAs observed in different setting of combination and grafts.</i>	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	

<b>Subtask 3.1</b> - 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	70
<b>Subtask 3.2</b> - Evaluate histopathological changes (H&E) and inflammatory infiltration (IHC or IF for CD3, CD4 and CD8 (T cells)) on indicated time points.	9-14	
<b>Milestone(s):</b> <i>Identify pattern and sensitivity of plasma expression of miRNAs to be advanced of clinical signs</i>	14	
<b>Major Task 4: Correlation and validation in any of miRNAs in human samples will be verified any signature identified in Aim 1 and 2.</b>	Months	
<b>Subtask 4.1</b> - Receive IRB and DoD Human Research Protection Office approvals	0-6	100
<b>Subtask 4.2</b> – Collect samples; monitor kinetics of plasma expression of miRNAs; evaluate and validate in any of miRNAs in human samples	15-17	
<b>Subtask 4.3</b> - Final data analysis and interpretation. Prepare reports and manuscripts for submission	17-18	
<b>Milestone(s):</b> <i>Identify pattern and sensitivity of plasma expression of miRNAs to be advanced of clinical signs from human samples</i>	18	

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

<p>1) <b>Major Activities:</b></p> <ul style="list-style-type: none"> <li>• Major Task 1: Investigate plasma expression of miRNAs after transplantation</li> <li>• Major Task 2: Investigate whether profile of plasma miRNAs differ in allotransplantation setting</li> </ul> <p>2) <b>Specific Objectives:</b></p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• 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.</li> </ul>
--

**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.**

We have performed total 39 transplantation in syngeneic setting to monitor kinetics of plasma expression of miRNAs after different transplantation. Below is the table summering the animal assignment till 04/14/2021.

		POD2	POD5	POD7	POD21
Group 1	Skin			+4	+4
Group 2	Heart	+4	+4	+4	+4
Group 3	Hind limb	+3	+4	+4	+4

**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.**

We have performed total 36 transplantation in allogenic setting to monitor kinetics of plasma expression of miRNAs after different transplantation. Below is the table summering the animal assignment till 04/14/2021.

		POD2	POD5	POD7
Group 4	Skin	+4	+4	+4
Group 5	Heart	+4	+4	+4
Group 6	Hind limb	+4	+4	+4

We have performed total 6 miRNA extraction in order to optimize condition of miRNA expression and comparison to monitor kinetics of plasma expression of miRNAs after different transplantation.

#	Plasma - Sample ID	RNA Concentration (ng/ul)	Total amount/sample (ng/ul)	260/280	260/230
1	miRNA_c-2-7-2	11.1	222	1.11	0.14
2	miRNA_c-2-7-3	14.2	284	0.98	0.19
3	miRNA_c-1-5-3	5.7	114	1.08	0.09
4	miRNA_c-1-5-4	11.2	224	1.19	0.18
5	miRNA_ctrol no TX-1	30.9	618	1.24	0.29
6	miRNA_ctrol no TX-2	52.6	1052	1.22	0.33

**Major Task 3, 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.**

We have performed total 96 transplantation in allogenic setting to monitor kinetics of plasma expression of miRNAs after different transplantation. Below is the table summering the animal assignment till 04/14/2021.

		POD2	POD5	POD7	POD21	POD50	POD70
Group 7	Skin	+4	+4	+4	+4	+4	+4
Group 8	Heart	+4	+4	+4	+4	+4	+4
Group 9	Hind limb	+4	+4	+4	+4	+4	+4
Group 10	Hind limb	+4	+4	+4	+4	+4	+4

• **What opportunities for training and professional development has the project provided?**

Nothing to report

• **How were the results disseminated to communities of interest?**

Nothing to report

• **What do you plan to do during the next reporting period to accomplish the goals?**

We have done performing surgeries and sample collection to fill up remaining groups under both **Major task 1** and **Major task 2**. We will continue for miRNA examination and histopathological evaluation. We received IRB and HRPO approvals for Major task 4.

#### 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:	2
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: Closed Phase II: Novel Super-cooling of Genitourinary Cells and Tissues for Transplant Sponsor: X-Therma / Prime: DOD Award: W81XWH-16-C-0212 Role: Co- I Effort: 2.16CM Date: 10/01/2018 - 05/24/2020
Oh, Byoung Chol	Change: Received Human iPSC-derived EGFR+ functional Schwann Cells to Enhance Nerve Regeneration Sponsor: TEDCO Award: 2020-MSCRFL-5414 Role: PI Effort: 2.4CM Date: 06/30/2020 – 06/29/2022
Oh, Byoung Chol	Change: Received - 1R43A1155196 - A Novel and Clinically Feasible Co-therapy of Deceased Donor Bone Marrow Combine Role: Co-I Effort: .60CM Date: 02/15/2021 – 06/30/2022
Gerald Brandacher	Change: Closed Phase II: Novel Super-cooling of Genitourinary Cells and Tissues for Transplant Sponsor: X-Therma / Prime: DOD Award: W81XWH-16-C-0212 Role: PI Effort:1.08CM Date: 10/01/2018 - 05/24/2020
Gerald Brandacher	Change: Closed Phase II: Non-Toxic, Highly-Effective Bioinspired Cryoprotectants Sponsor: X-Therma Role: PI Effort:.12CM Date: 11/01/2019 – 10/31/2020
Gerald Brandacher	Change: Closed Engineering a Hybrid Thymus to Unravel the Tolerogenic Properties of Vascularize Sponsor: CDMRP Award: W81XWH-16-1-0708 Role: Co-I Effort:.12CM

	Date 09/30/2016 – 06/29/2020
Gerald Brandacher	Change: Received Human iPSC-derived EGFR+ functional Schwann Cells to Enhance Nerve Regeneration Sponsor: TEDCO Award: 2020-MSCRFL-5414 Role: Co-I Effort: 12CM Date: 06/30/2020 – 06/29/2022
Gerald Brandacher	Change: Received - R43HL152941- Feasibility of expanding ischemia time for hearts destined for transplantation Role: PI Effort: 1% Date: 01/01/2021 – 08/31/2022
Gerald Brandacher	Change: Received - Replacing Sutures for Microvascular and Vascular Anastomosis Role: Co-I Effort: 1% Date: 01/13/2021 – 12/13/2021
Gerald Brandacher	Change: Received - 1R43A1155196 - A Novel and Clinically Feasible Co-therapy of Deceased Donor Bone Marrow Combine Role: PI Effort: 3% Date: 02/15/2021 – 06/30/2022
Gerald Brandacher	Changes: Received – “C00069632-2 Quantitative Ambulatory Assessment and Prognosis of the Impact of Severe Upper...” Role: PI Effort: 2% Dates: 03/01/2021 – 02/29/2024

- **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