AWARD NUMBER: W81XWH-19-1-0234

TITLE: Non-Invasive Immune Monitoring Biomarkers using Plasma microRNAs in VCA

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

CONTRACTING ORGANIZATION: Johns Hopkins University

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

# REPORT DOCUMENTATION PAGE

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| **1. REPORT DATE** | **2. REPORT TYPE** | **3. DATES COVERED** |
|---|---|---|
| JULY 2020 | Annual Report | 15cJUN 2019 - 14cJUN 2020 |

**4. TITLE AND SUBTITLE**

Non-Invasive Immune Monitoring Biomarkers using Plasma microRNAs in VCA

**5a. CONTRACT NUMBER**
W81XWH-19-1-0234

**5b. GRANT NUMBER**
Log: RT180159

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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

Johns Hopkins University
Department of Plastic and
Reconstructive Surgery, VCA
lab, 720 Rutland Ave,
Baltimore 21205

**8. PERFORMING ORGANIZATION REPORT NUMBER**

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

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

**10. SPONSOR/MONITOR'S ACRONYM(S)**

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**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**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** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | USAMRMC |
| Unclassified | Unclassified | Unclassified | Unclassified | 12 | **19b. TELEPHONE NUMBER** *(include area code)* |

**Standard Form 298 (Rev. 8-98)**
**Prescribed by ANSI Std. Z39.18**

## TABLE OF CONTENTS

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

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

VCA, immunosuppression, miRNA, non-invasive, monitoring

**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.*

| **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 | 25 |
| ***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 | 25 |
| ***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 | |
| ***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 | 30 |
| ***Subtask 4.2 –*** Collect samples; monitor kinetics of plasma expression of miRNAs; evaluate and validate in any of miRNAs in human samples | 15-17 | |
| ***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?**

*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.*

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.

**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.**

During this reporting period. We have performed total 13 transplantation in syngeneic setting to monitor kinetics of plasma expression of miRNAs after different transplantation. Below is the table summering the animal assignment till 4/14/2020 Currently, newly added animals will be monitored and eventually harvest when reach designated endpoint.

| | POD2 | POD5 | POD7 | POD21 | POD50 | POD70 |
|---|---|---|---|---|---|---|
| Group 1 (Skin) | | | | | | |
| Group 2 (Heart) | | +1 (harvested) | | | | |
| Group 3 (Hindlimb) | +3(harvested) | +4(harvested) | +4(harvested) | +2(harvested) | | |

**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.**

During this reporting period. We have performed total 4 transplantation in allogenic setting to monitor kinetics of plasma expression of miRNAs after different transplantation. Below is the table summering the animal assignment till 4/14/2020 Currently, newly added animals will be monitored and eventually harvest when reach designated endpoint.

| | POD2 | POD5 | POD7 | POD21 | POD50 | POD70 |
|---|---|---|---|---|---|---|
| Group 4 (Skin) | | | | | | |
| Group 5 (Heart) | | | | +4(harvested) | | |
| Group 6 (Hindlimb) | | | | | | |

**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.*

We plan to continue performing surgeries to fill up remaining groups under both Major task 1 and Major task 2. And sacrifice animals for miRNA examination and histopathological evaluation. IRB approval under Major task 4 is pending.

***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).*

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 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.*

Nothing to report

**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).*

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. 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".*

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?**
*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.*

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