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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

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6. AUTHOR(S)
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5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

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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TABLE OF CONTENTS

	Page
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	13
5. Changes/Problems	13
6. Products	13
7. Participants & Other Collaborating Organizations	14
8. Special Reporting Requirements	15
9. Appendices	15

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	100
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	100
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	100
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 allotransplantation setting
- Major Task 3: Investigate the mechanistic trends and correlation with rejection between plasma expression in miRNAs in setting of long term surviving treatment

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 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.
- Major Task 3, 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.

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 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 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 graphs of miRNA profiles by groups. We are under analysis of miRNA profiling and correlation to rejection episodes identifying by histology samples.

Orthotopic hind limb transplantation

Group	Donor1	Recipient	Treatment	Plasma/Tissue collection (POD3)	Specification
HL-1	C57BL/6 (n=8)	C57BL/6 (n=16)	None	2, 5, 7, 21	Syngeneic controls
HL-2	Balb/c (n=6)	C57BL/6 (n=12)	None	2, 5, 7	Allogeneic controls
HL-3	Balb/c (n=12)	C57BL/6 (n=24)	CoB*	2, 5, 7, 21, 50, 70	Treated groups
HL-4	Balb/c (n=12)	C57BL/6 (n=24)	TBI + CoB**	2, 5, 7, 21, 50, 70	Treated groups

Heterotopic Heart transplantation

Group	Donor	Recipient	Treatment	Plasma/Tissue collection (POD3)	Specification
H-1	C57BL/6 (n=16)	C57BL/6 (n=16)	None	2, 5, 7, 21	Syngeneic controls
H-2	Balb/c (n=12)	C57BL/6 (n=12)	None	2, 5, 7	Allogeneic controls
H-3	Balb/c (n=12)	C57BL/6 (n=24)	CoB*	2, 5, 7, 21, 50, 70	Treated groups
H-4	Balb/c (n=12)	C57BL/6 (n=24)	TBI + CoB**	2, 5, 7, 21, 50, 70	Treated groups

Full Thickness Skin transplantation

Group	Recipient	Treatment	Plasma/Tissue collection (POD3)	Specification
SK-1	C57BL/6 (n=12)	None	5, 7, 21	Syngeneic controls
SK-2	C57BL/6 (n=8)	None	5, 7	Allogeneic controls
SK-3	C57BL/6 (n=20)	CoB*	5, 7, 21, 50, 70	Treated groups
SK-4	C57BL/6 (n=21)	TBI + CoB**	5, 7, 21, 50, 70	Treated groups

Figure 1 mRNA expression profile of Group1

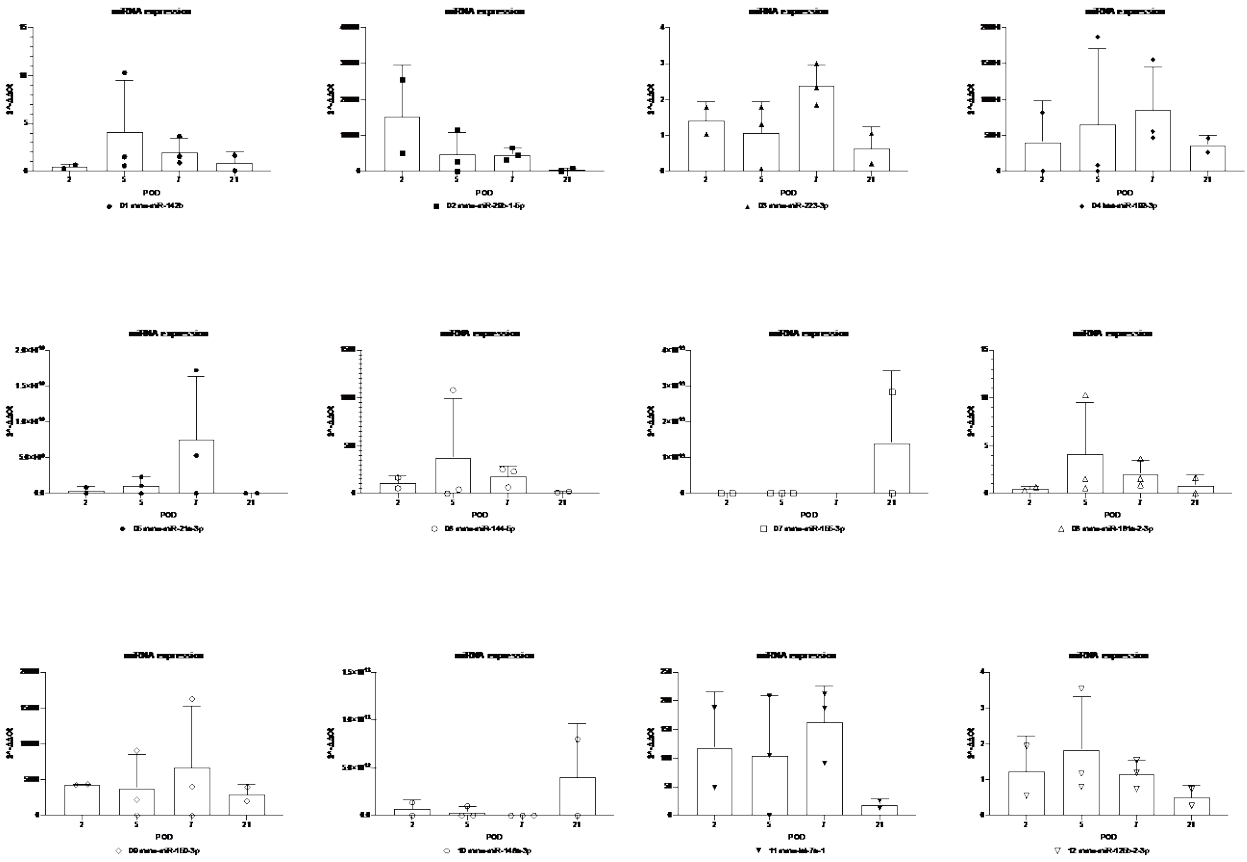


Figure 2 mRNA expression profile of Group2

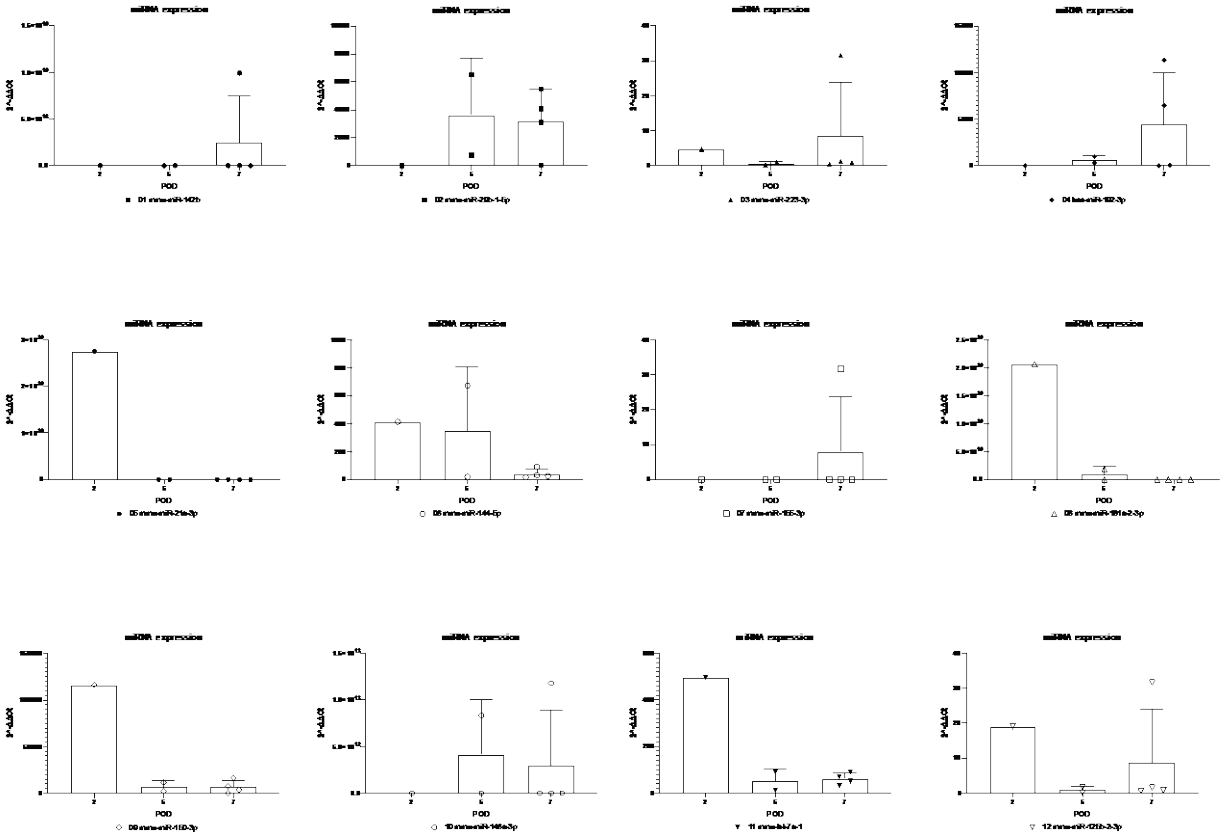


Figure 3 mRNA expression profile of Group3

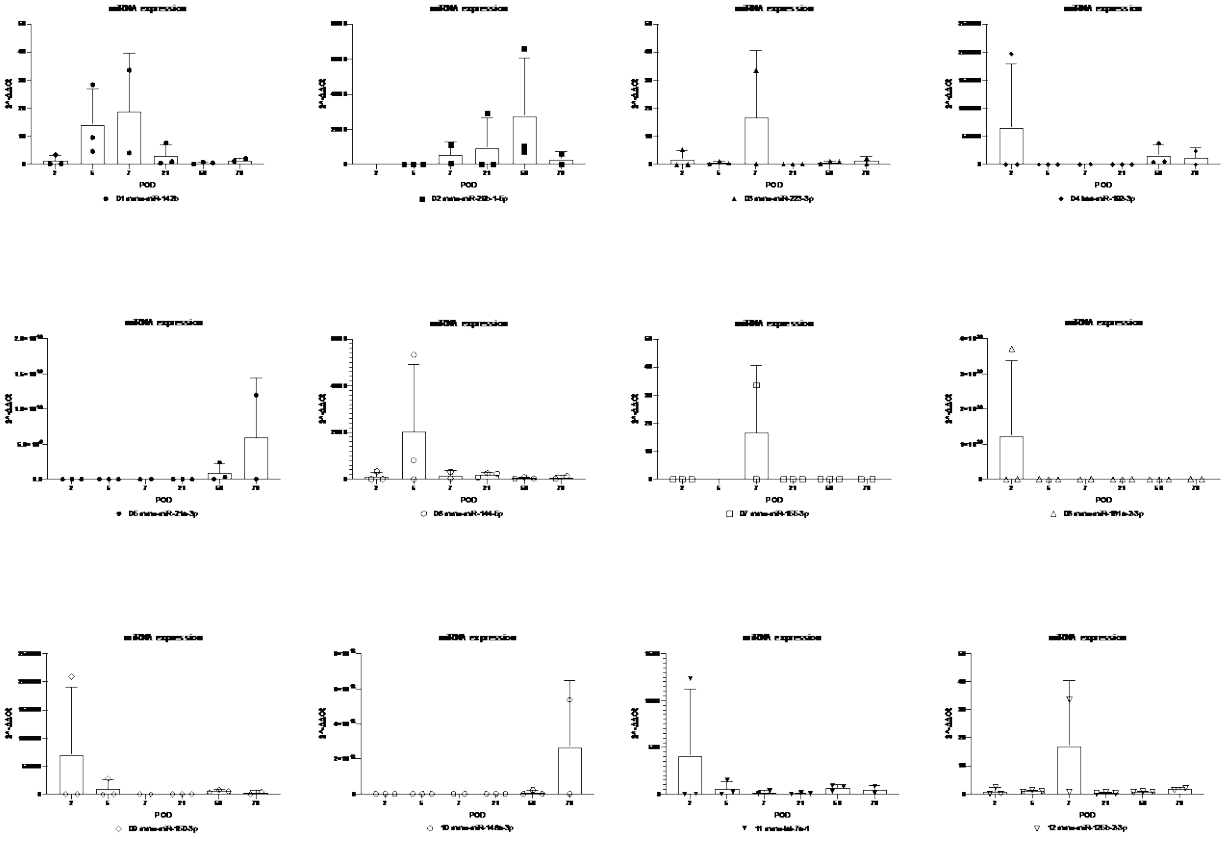


Figure 4 mRNA expression profile of Group4

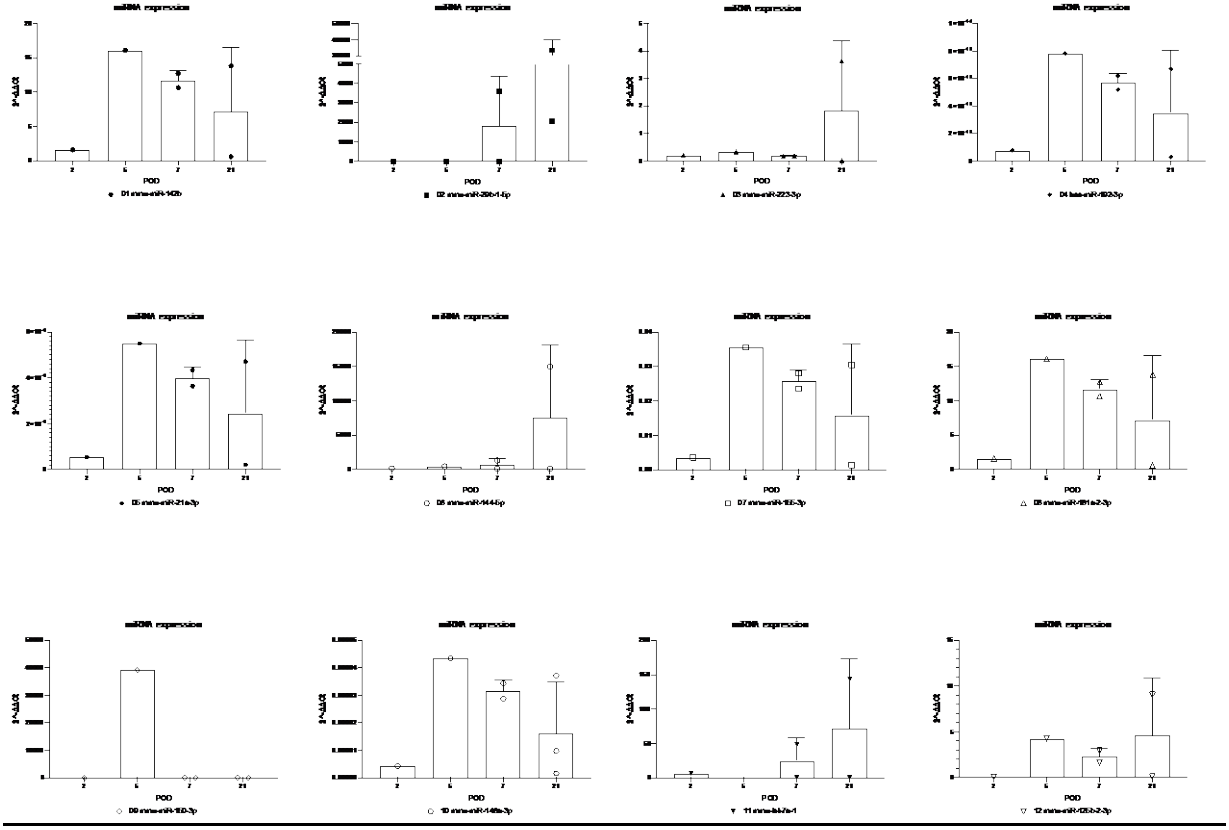


Figure 5 mRNA expression profile of Group5

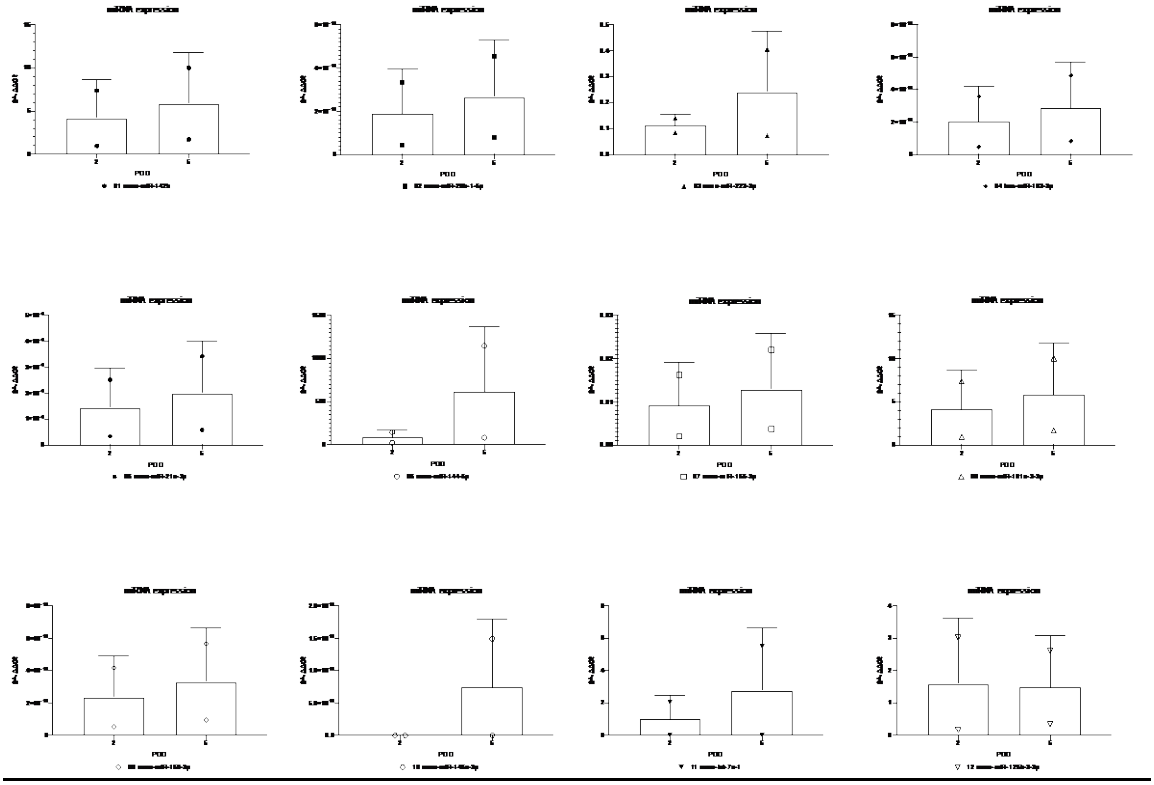


Figure 6 mRNA expression profile of Group6

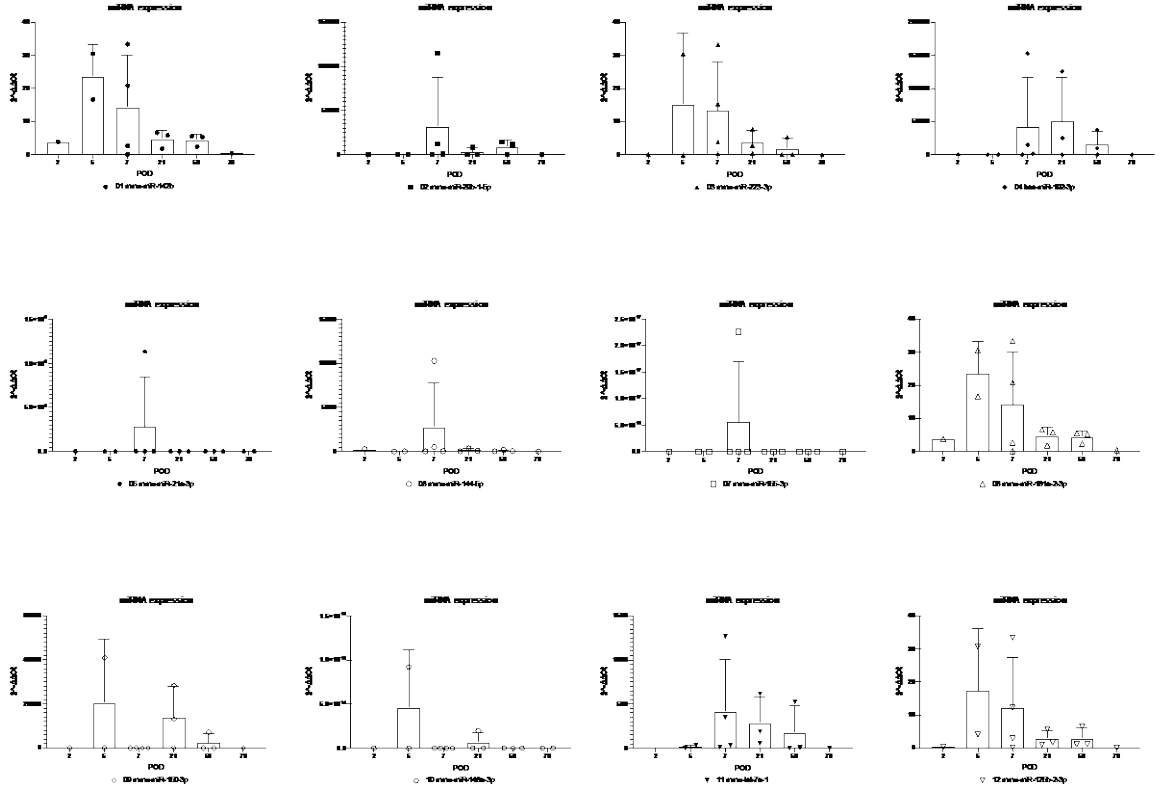


Figure 7 mRNA expression profile of Group7

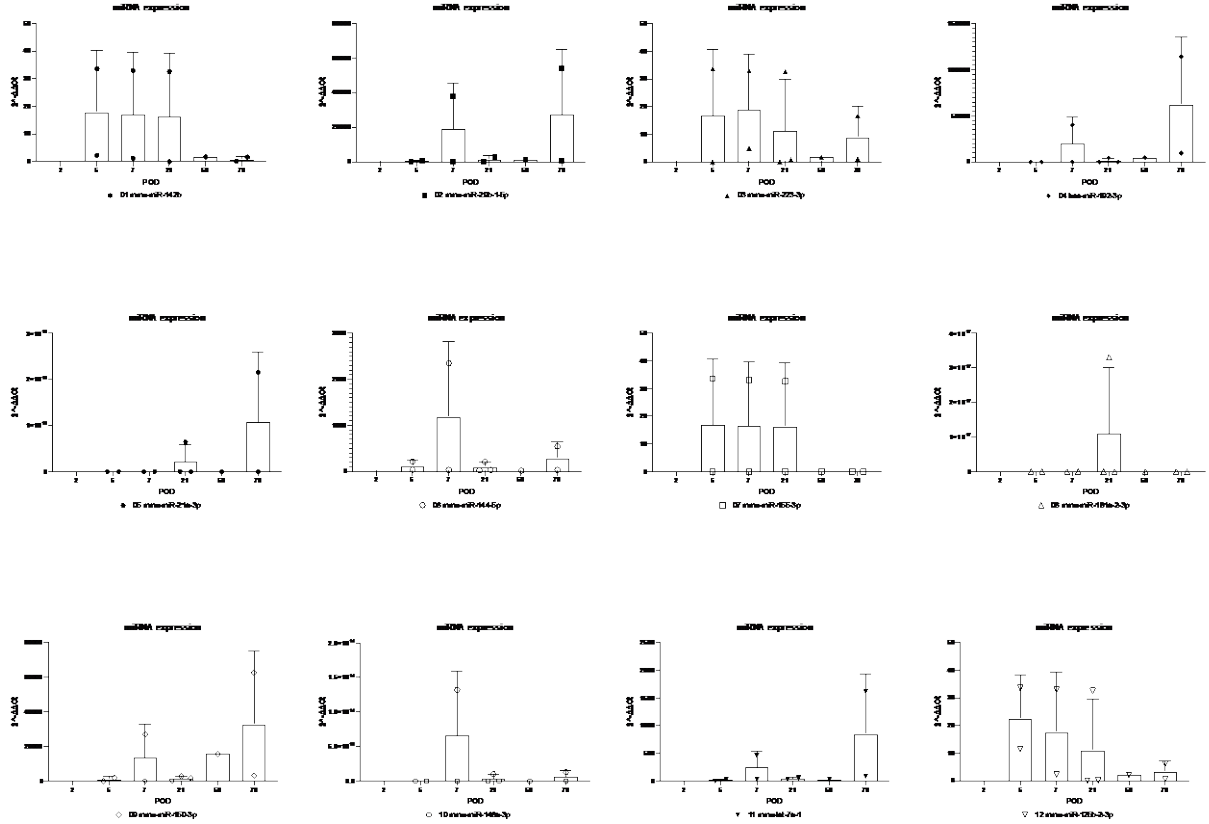


Figure 8 mRNA expression profile of Group8

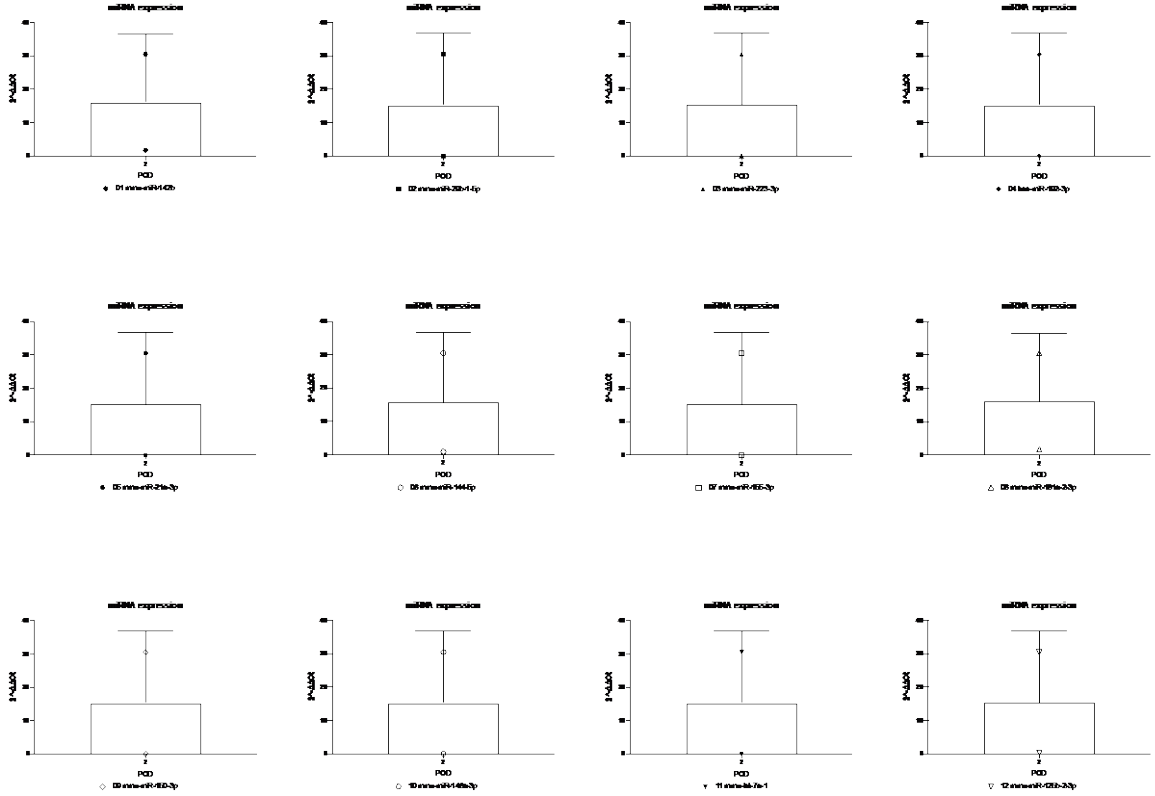


Figure 9 mRNA expression profile of Group9

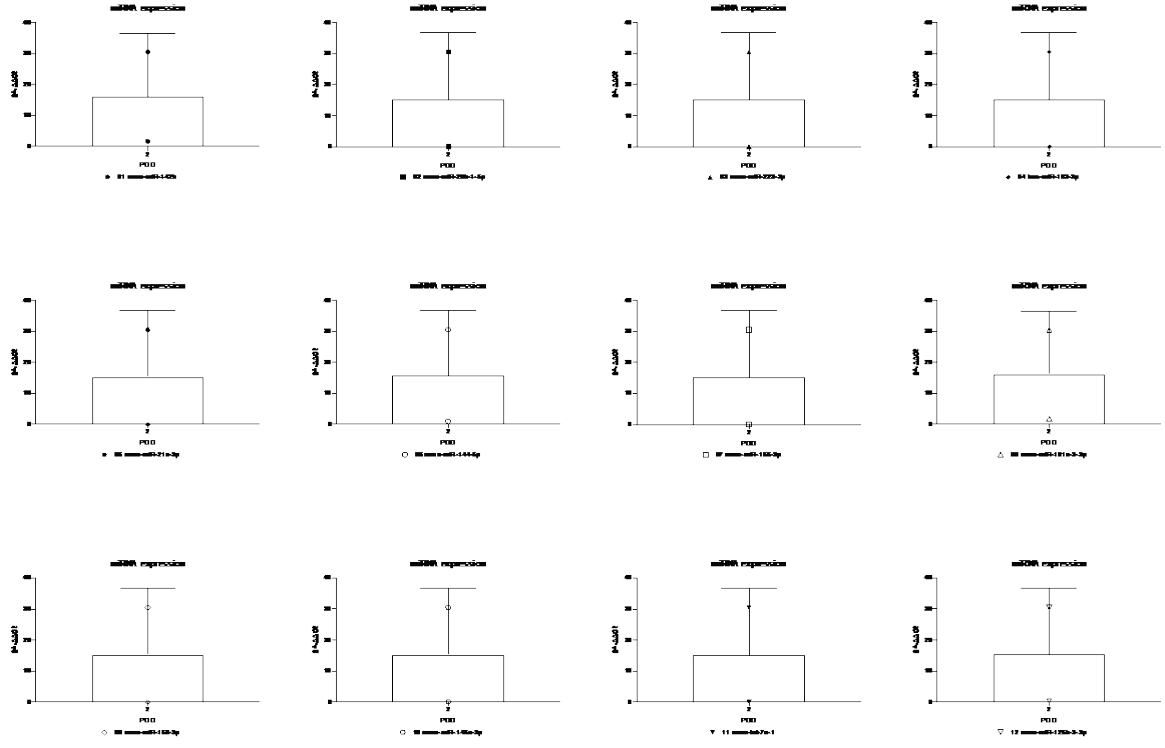


Figure 10 mRNA expression profile of Group10

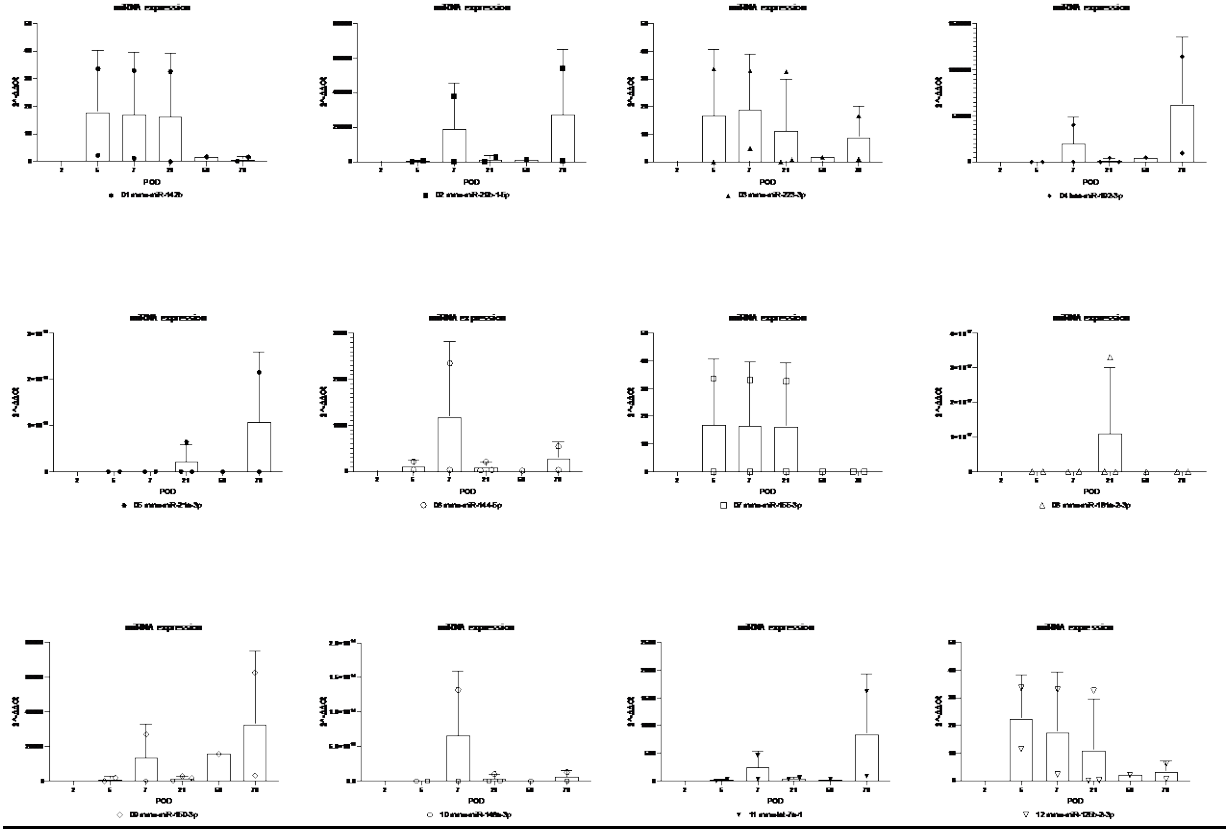


Figure 11 mRNA expression profile of Group11

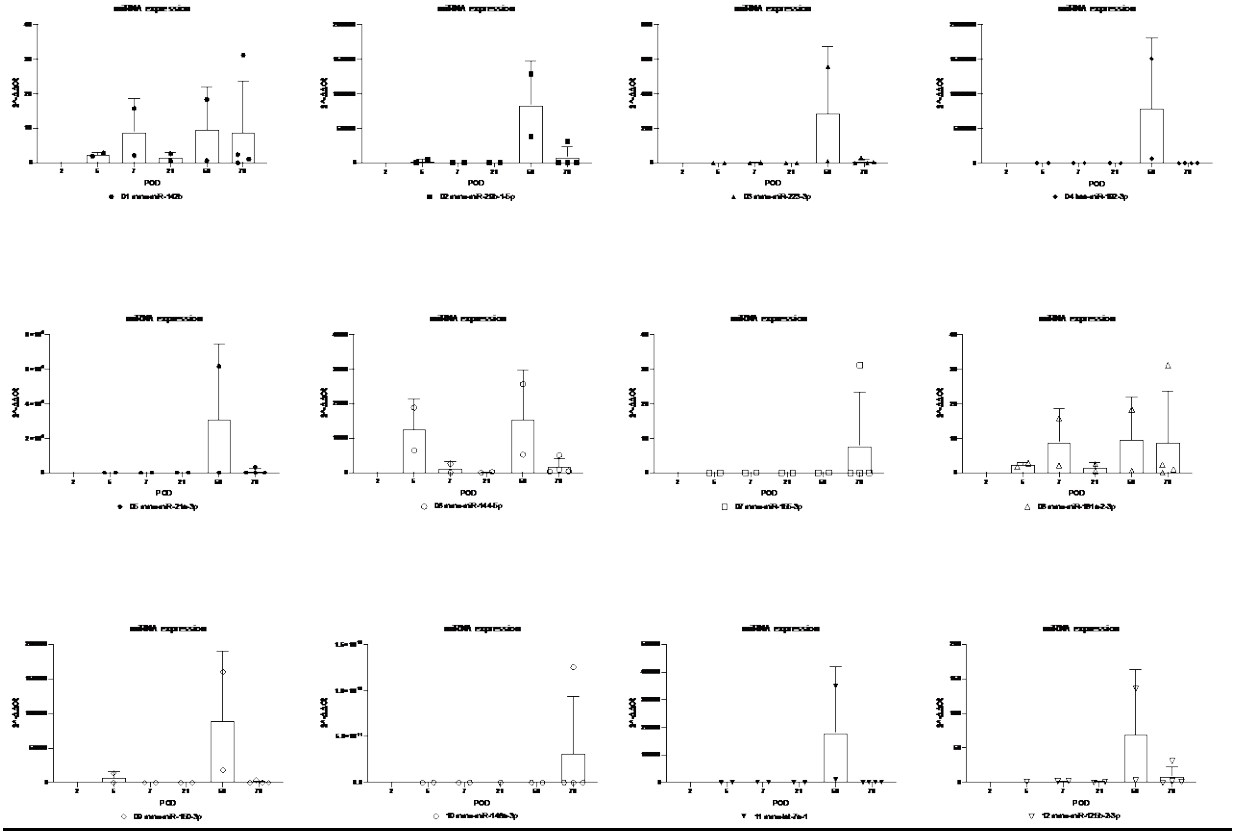
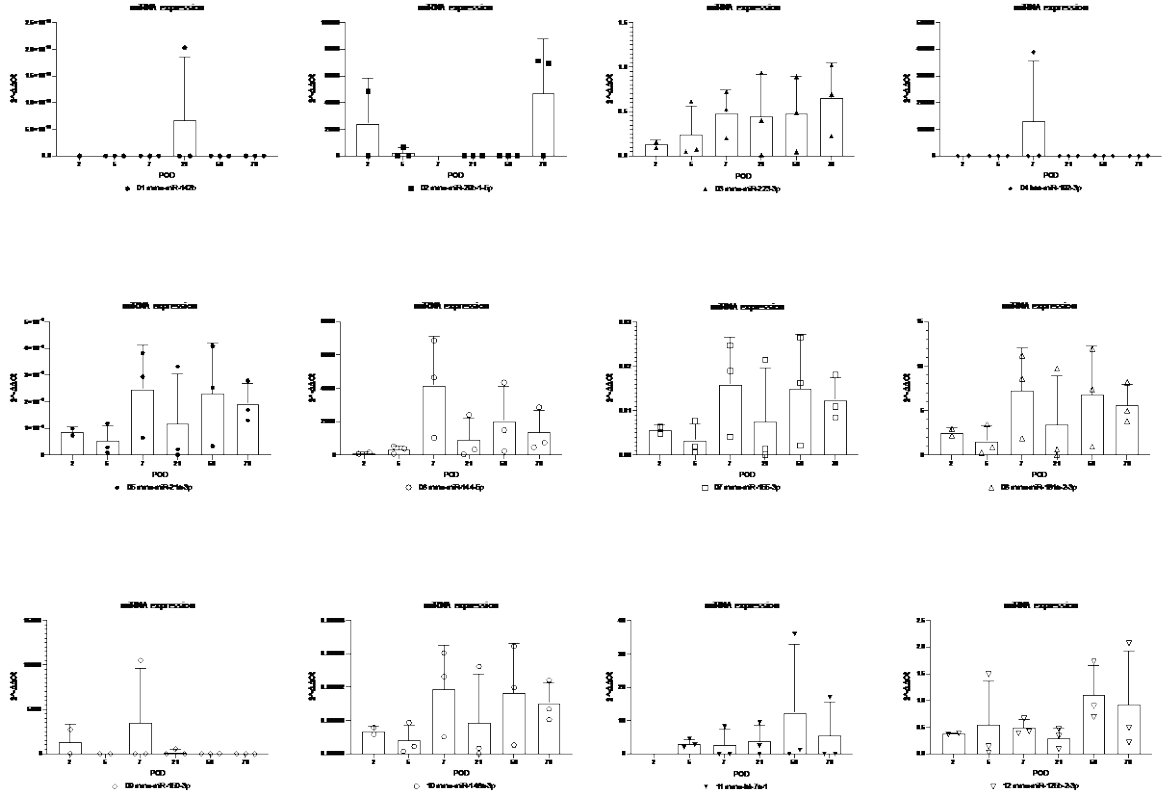


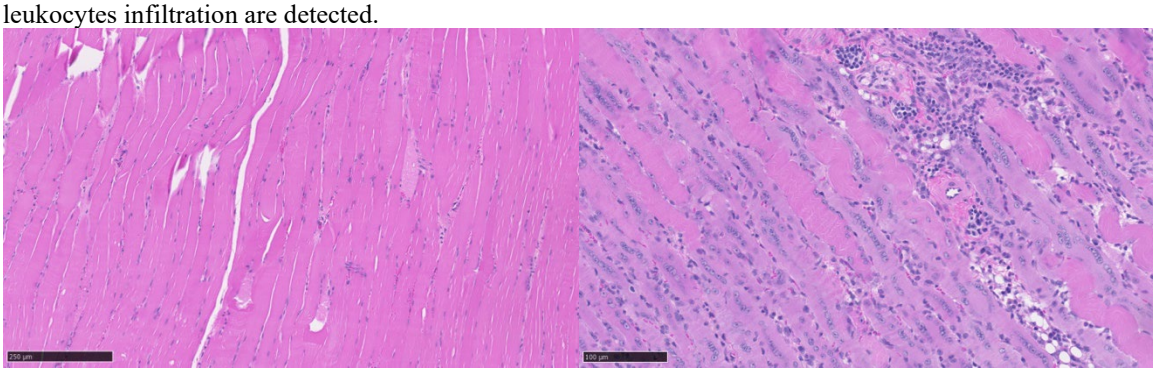
Figure 12 mRNA expression profile of Group12



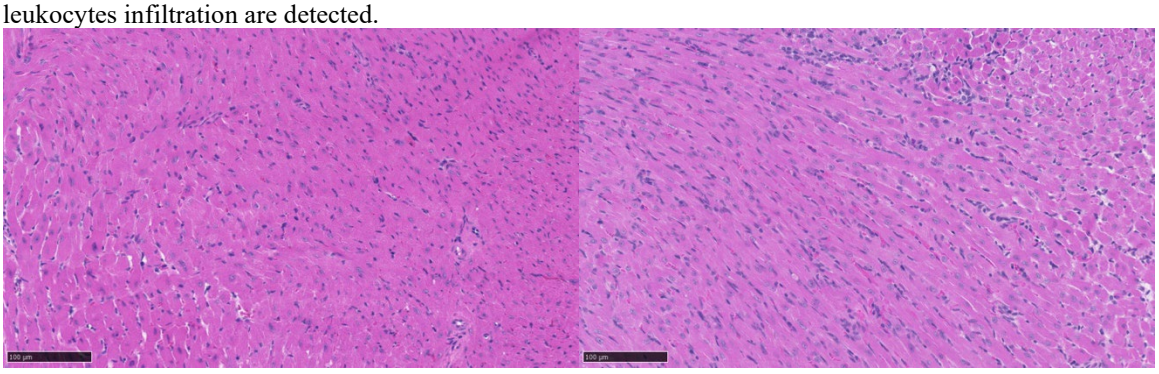
<u>Major Task 3, Subtask 3.2</u>- - Evaluate histopathological changes (H&E) and inflammatory infiltration (IHC or IF for CD3, CD4 and CD8 (T cells)) on indicated time points.

Histopathological changes (H&E) of representative samples from selected groups

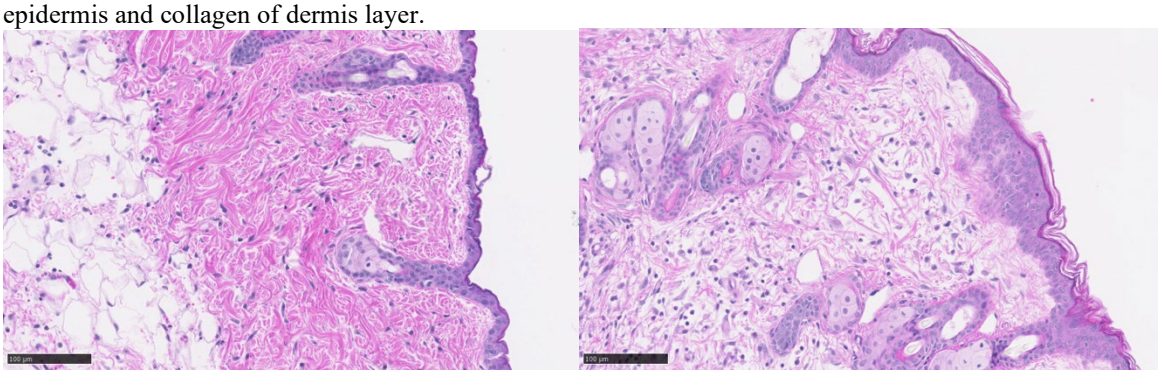
Hindlimb muscle sample from grafts that are free of rejection (Left). Hindlimb muscle sample from grafts that has rejection(Right), leukocytes infiltration are detected.



Cardiac muscle sample from grafts that are free of rejection (Left). Cardiac muscle sample from grafts that has rejection (Right), leukocytes infiltration are detected.



Skin sample from grafts that are free of rejection (Left). Skin sample from grafts that has rejection (Right), disruption of continuity of epidermis and collagen of dermis layer.



- **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?**
  None applicable

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**

  Nothing to report

- **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: .12
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 (through 12/31/2022)
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.

Name: Amy Bodine .
Project Role: Animal Research Specialist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: .60 (through 05/17/2023)
Contribution to Project: She has work regarding sample process.

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

Byoung Oh	Changes: Ended W81XWH-19-1-0234 Non-Invasive Immune Monitoring Biomarkers using Plasma microRNAs in VCA Role : PI Effort: 5% Dates: 06/15/2019 – 06/14/2023
Byoung Oh	Changes: Ended - Human iPSC-derived EGFR+ functional Schwann Cells to Enhance Nerve Regeneration Role: PI Effort:15% Dates:06/30/2020 – 06/29/2023
Byoung Oh	Changes: Ended - A Novel and Clinically Feasible Co-therapy of Deceased Donor Bone Marrow Comb. Role: Co - I Effort: 5% Dates: 07/01/2020 – 06/30/2023
Byoung Oh	Changes: Received Extended Limb Preservation Employing an Optimization Strategy for Stabilization Role: Co-I

	Effort: 5% Dates 07/01/2022 – 03/31/2024
Byoung Oh	Changes: Received - W81XWH2210726 A Novel Universal and Portable Normothermic Machine Perfusion Device for Vascularized Composite Allografts Role: Co-I Effort: 10% Dates 09/30/2022 – 09/29/2025
Byoung Oh	Changes: Received Engineering thymic selection to control the development of alloreactive T cells and promote VCA acceptance Role: Co-I Effort: 10% Dates 09/30-2022 – 09/29/2025
Byoung Oh	Changes: Received Fertility Protection in Cancer: Recovery of Whole Ovaries enabled by Next... Role: Co-I Effort: 2% Dates 01/01/2023 – 07/31/2023
Byoung Oh	Changes: Received HT94252310593- Immunogenicity in subzero stored VCA Role: PI Effort: 20% Dates 09/01/2023 – 02/28/2025
Byoung Oh	Changes: Received- Use of extracellular vesicles from metabolically engineered Mesenchymal Stem cell Role: Collaborator Effort: 2% Dates 09/01/2023 – 05/31/2025

- **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

Non-Invasive Immune Monitoring Biomarkers using Plasma microRNAs in VCA

RT180159: Restorative Transplantation Research
W81XWH-19-1-0234

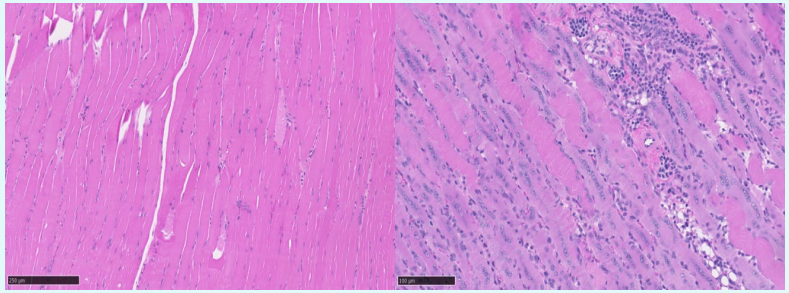
PI: Byoung Chol Oh, D.V.M., Ph.D **Org:** Johns Hopkins University, School of Medicine **Award Amount:** $199,482

Study/Product Aim(s)

- Specific Aim 1: To investigate plasma expression of miRNAs after transplantation
- Specific Aim 2: To investigate whether profile of plasma miRNAs differ in allotrasplantation setting and to Investigate the mechanistic trends and correlation with rejection between plasma expression in miRNAs in setting of long term surviving treatment
- Specific Aim 3: To correlation and validation in any of miRNAs in human samples will be verified any signature identified in Aim 1 and 2.

Approach

The objective of this application is to develop some tissue specific miRNAs without the requirement for invasive tissue biopsy. The proposal involves investigators with previous experience in the development and clinical translation of various tolerance protocols from laboratory animals to humans, resulting in numerous novel clinical transplantation trials and surgical/immunological expertise in VCA in animals and humans.



Hindlimb muscle sample from grafts that are free of rejection (Left). Hindlimb muscle sample from grafts that has rejection(Right), leukocytes infiltration are detected.

Timeline and Cost

Activities CY	19	20
Aim 1: Major Task 1, 2		
Aim 2: Major Task 3		
Aim 3: Major Task 4		
Estimated Budget ($K)	**$121,582**	**$76,947**

Updated: 10/12/2023

Goals/Milestones

CY19 Goal – Obtain regulatory approval from IACUC and ACURO
☑IACUC approval: completed #MO19M172
☑ACURO approval: Completed

CY20 Goals – 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.
Comments/Challenges/Issues/Concerns

Budget Expenditure to Date

Actual Expenditure: $190,943.15 through 06/14/2023