AWARD NUMBER: W81XWH-22-1-0988

TITLE: The miR15a/16 Axis and Precision Gene Intervention to Provide a Novel Therapy for Lung Cancer

PRINCIPAL INVESTIGATOR: James Fluckey

CONTRACTING ORGANIZATION: Texas A & M University, College Station, TX

REPORT DATE: October 2023

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2023	Annual	01Sep2022-31Aug2023

4. TITLE AND SUBTITLE
The miR15a/16 Axis and Precision Gene Intervention to Provide a Novel Therapy for Lung Cancer

5a. CONTRACT NUMBER
W81XWH-22-1-0988

5b. GRANT NUMBER
GRANT13350075

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
James D. Fluckey, PhD
Peter Nghiem, PhD, DVM

E-Mail:jfluckey@tamu.edu

5d. PROJECT NUMBER
LC210172

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Texas A&M University
400 Harvey Mitchell Parkway
Suite 300
College Station, TX, Brazos
USA

8. PERFORMING ORGANIZATION REPORT NUMBER
M2203954

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)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

A recent advance in cell biology is the discovery of microRNA: small, non-coding nucleotide strands that have the potential to negatively regulate the expression of potentially hundreds of genes by the process of RNA interference. These miRNAs have been demonstrated to play a role in numerous cellular processes related to cancer, including cell differentiation, proliferation, and migration. The loss of expression of two specific and cotranscribed species, miR15a and miR16, has been identified in numerous cancers, including those of the lung, although their precise role in the initiation and progression of cancer has not been fully elucidated. miR15a/16 are responsible for the regulation of several well-defined oncogenic pathways. The goal of this proposal is to develop a highly specific therapy capable of fighting lung cancer, with the central hypothesis that loss of miR15a/16 is a triggering event in the progression of healthy cells to a cancerous state, and that rescue of miR15a/16 expression will effectively halt growth and metastasis in lung cancer cells. In order to do so, we propose two specific aims. Specific Aim 1 (SA1) will demonstrate the effect of targeted CRISPR/Cas9 transfection of miR15a/16 specific to SCLC and NSCLC in vitro. This aim will use the CRISPR/Cas9 system to insert a functional miR15a/16 sequence into the cancer cell genome, with the goal of fundamentally altering the nature of these malignant cells. To ensure cancer cell-specific targeting, vectors coding for the Cas9 enzyme and miR15a/sequence will be delivered using a lentiviral vector that has been conjugated to an antibody for MAGE-3, a cell surface protein that has been demonstrated to be uniquely expressed in both SCLC (H446) and NSCLC (A549), in order to promote fusion of the lentivirus with the cell membrane and subsequently insert a miR15a/16 sequence into the cancer cell genome using a CRISPR/Cas9 genetic editing technique, restoring production of these regulatory microRNA. The hypothesis of SA1 is that restored expression of miR15a/16 will result in cell-specific reductions in cancer growth and metastasis. Specific Aim 2 (SA2) seeks to elucidate the role of miR15a/16 in the initiation and progression from healthy cells to lung cancer. SA2 will also use CRISPR/Cas9 technology, in this case to insert sequences that are antisense to suppress miR15a/16 in in vitro models of healthy lung cells (BEAS-2B) to investigate the initiation and progression of otherwise healthy tissues to cancer. The hypothesis of SA2 is that loss of miR15a/16 will be a triggering event for the progression of healthy cells to lung cancer, and that miR15a/16 levels will be low in both tumors and serum. The proposed studies are closely aligned with the areas of emphasis outlined in the LCRP Strategic Plan, especially related to understanding mechanisms of initiation and progression to clinically significant lung cancer, identifying innovative strategies for prevention and treatment, developing predictive and prognostic markers of responders vs non-responders, and investigating treatment susceptibility or resistance in malignant lung cancer.

15. SUBJECT TERMS

None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	17	

TABLE OF CONTENTS

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

Underlying the development of cancer, including cancers of the lung, is the disruption of regulatory networks that govern cell growth, leading to the rampant proliferation characteristic of malignant tumors. The disruption of these networks itself can arise due to the loss of expression of critical tumor suppressing genes. A recent advance in cell biology is the discovery of microRNA: small, non-coding nucleotide strands that have the potential to negatively regulate the expression of potentially hundreds of genes by the process of RNA interference. The goal of this proposal is to develop a highly specific therapy capable of fighting lung cancer, with the central hypothesis that the loss of two key microRNAs, miR15a/16, is a triggering event in the progression of healthy cells to a cancerous state, and that rescue of miR15a/16 expression will effectively halt growth and metastasis in lung cancer cells. To address this hypothesis, specific aim 1 (SA1) will demonstrate the effect of targeted CRISPR/Cas9 transfection of miR15a/16 specific to SCLC and NSCLC in vitro. The first aim will use the CRISPR/Cas9 system to insert a functional miR15a/16 sequence into the cancer cell genome, with the goal of fundamentally altering the nature of these malignant cells. The delivery of the therapy to cancerous cells while simultaneously avoiding healthy cells is of paramount concern. To ensure cancer cell-specific targeting, vectors coding for the Cas9 enzyme and miR15a/sequence will be delivered using a lentiviral vector that has been conjugated to an antibody for MAGE-3, a cell surface protein that has been demonstrated to be uniquely expressed in both SCLC (H446) and NSCLC (A549) cells, and incorporating a fusogenic protein, to promote fusion of the lentivirus with the cell membrane, allowing delivery of the gene therapy to the cell. From there, using sgRNA specific for the AAVS1 safe-harbor site, the Cas9 vector will insert the miR15a/16 sequence into the cancer cell genome, restoring production of these regulatory microRNA. *The hypothesis of SA1 is that restored expression of miR15a/16 will result in cell-specific reductions in cancer growth and metastasis.* The second aim (SA2) seeks to elucidate the role of miR15a/16 in the initiation and progression from healthy cells to lung cancer. SA2 will also use CRISPR/Cas9 technology, but to insert sequences that are antisense to suppress miR15a/16 in in vitro models of healthy lung cells (BEAS-2B). Knockdown of miR15a/16 in this fashion will allow for exploration of the specific cellular events caused by reductions in these regulatory microRNA, and thus to investigate the initiation and progression of otherwise healthy tissues to cancer. Measurements of miR15a/16 expression will also be made in deidentified human samples of SCLC, NSCLC, and blood serum to further evaluate the importance of miR15a/16 during in vivo lung cancer progression. *The hypothesis of SA2 is that loss of miR15a/16 will be a triggering event for the progression of healthy cells to lung cancer, and that miR15a/16 levels will be low in both tumors and serum.*

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

Small cell lung cancer, non-small cell lung cancer, microRNA, CRISPR/Cas9, lentiviral vector, MAGE-3

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: Design and introduce microRNA genes into lung cancer cells using antibody-directed lentiviral approaches. Work to be conducted in calendar months 1 through 9. Work is still ongoing for this major task.

Milestone 1: Major Task 1: Subtask 0. Full institutional and DOD approval to analyze deidentified tissues obtained from CHTN. Approved August of 2022
Milestone 2: Major Task 1: Subtask 1. Complete development of custom lentivirus clones with MAGE-3 embedded antibodies and the CRISPR packages.
Milestone 3: Major Task 1: Subtask 2. Successful implementation of viruses in human cell lines. Successful implementation will be verified by analysis of miR15a/16 and the reporter gene constructs. Successfully transfected cells will be assayed for proliferation (via standard ATPlite methodology), invasive potential (via transwell migration assay), apoptosis (using Annexin V staining), and gene expression (via RT-PCR for mRNA content and Western blot for protein expression). These assays will also be performed in the control cells, with one-way ANOVA used to compare outcomes of these experiments between groups.

Major Task 2: Develop a miR-16/15a 'antisense' model using similar methodologies as above to assess their value as culprits leading to cancer in normal lung cell lines. Work to be conducted in calendar months 3-9. Most of the nonviral proof of concept work has been completed as of September 2023.

Major Task 3: To characterize tissues collected from normal and lung cancer patients obtained through the Collaborative Human Tissue Network. Work to be conducted in calendar months 6-12. This work is still on-going.

Milestone 4: Major Task 2: Subtask 3. Successful design of nonviral vector delivery of miR15a/16 antisense clones to normal BEAS-2B cells.
Milestone 5: Major Task 2: Subtask 4. Complete analyses related to the effect of antisense microRNA in normal lung BEAS-2B cells. Successfully transfected cells will be assayed for proliferation (via standard ATPlite methodology), invasive potential (via transwell migration assay), apoptosis (using Annexin V staining), and gene expression (via RT-PCR for mRNA content and Western blot for protein expression). These assays will also be performed in nontransfected control cells, with one-way ANOVA used to compare outcomes of these experiments between groups.
Milestone 6: Major Task 3: Subtask 5. Complete analyses of miR15a/16 status, with or without cancer in lung tissue or serum collected from CHTN.

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.

Major Task 1: Design and introduce microRNA genes into lung cancer cells using antibody-directed lentiviral approaches. Work to be conducted in calendar months 1 through 9. Work is still ongoing for this major task.

Milestone 1: Major Task 1: Subtask 0. Full institutional and DOD approval to analyze deidentified tissues obtained from CHTN. Approved August of 2022
Milestone 2: Major Task 1: Subtask 1. Complete development of custom lentivirus clones with MAGE-3 embedded antibodies and the CRISPR packages.

Our initial approach was to embed the MAGE-3 antibody into the viral protein coat, but with issues developing that virus, we diverted our focus to developing a less invasive viral vector with a mutant, non-infectious protein into the virus (which could be implemented in a number of cancer/disease states), and developed a conjugate virus approach using spycatcher/spytag. This has allowed us to conjugate any two antibodies to target our cancers by using one antibody directed toward the mutant protein in the virus and the other targeting the testes protein on the cancer cell. The viruses were custom designed and built in the PI's laboratory and are fully functional. We are still troubleshooting the spycatcher/spytag conjugate system (also being created in the PI laboratory) in test wells to ensure we can couple the virus to the cancer cells. We anticipate we will have fully functional targeted lentivirus design in the lab in early spring 2024.

Milestone 3: Major Task 1: Subtask 2. Successful implementation of viruses in human cell lines. Successful implementation will be verified by analysis of miR15a/16 and the reporter gene constructs. We have fully functional lentiviral vectors in our laboratory that are now being directed to cells via the conjugate antibody technique. We are hopeful that we will have systematic confirmation that this approach will be viable as a therapeutic delivery mechanism by the end of the 2023 calendar year. Following confirmation, we will complete the virus experiments proposed in this major task. That said, we have tested the impact of overexpression of miR15a/16 on lung cancer cells using nonviral vectors to demonstrate the efficacy of our microRNA approach.

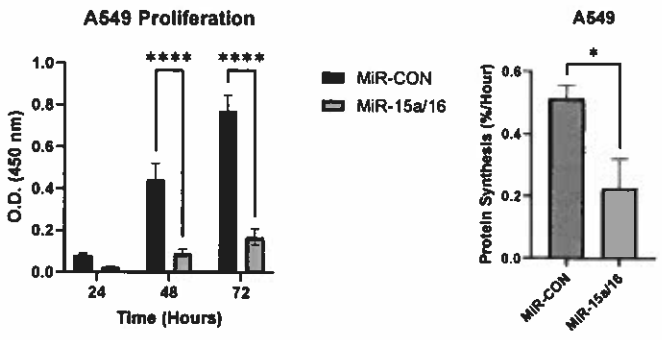

Figure 1. Proliferation and anabolic capacity assays on A549 hypotriploid alveolar basal epithelial cells with transient expression of miR15a/16 in cell culture. Expression of these specific microRNAs result in significant reductions of both proliferative capacity and the cellular ability to express proteins via protein anabolism. These findings indicate that miR15a/16 exert an anti-proliferative affect on lung cancer. * indicates $p<0.05$; **** indicates that $p<0.01$.

While experiments to optimize our cell-specific lentiviral vector are still ongoing, we show that a transient expression of miR15a/16 in both lung adenocarcinoma and squamous cell carcinoma result in reductions in cellular proliferation and protein synthesis rates, indicating that the miR15a/16 axis is indeed a regulator of anabolism in lung cancer. These results, completed in spring of 2022, are the first of their kind and demonstrate that these miRNA species represent a potentially viable therapeutic avenue in the treatment of aggressive cancer.

Major Task 2: Develop a miR-16/15a 'antisense' model using similar methodologies as above to assess their value as culprits leading to cancer in normal lung cell lines. Work to be conducted in calendar months 3-9. Most of the nonviral proof of concept work has been completed as of September 2023. Based on the outcomes shown above, we are grateful to the CDMP for allowing us to continue this work through a no cost extension. The viral experiments are still ongoing, and data will be analyzed soon. We anticipate that data outcomes will be available in very early Spring 2024.

Major Task 3: To characterize tissues collected from normal and lung cancer patients obtained through the Collaborative Human Tissue Network. This work is still on-going, but assessments of lung cancer and these microRNAs are now publicly available.

We have explored several databases that have focused on numerous microRNAs in a variety of cancers, with regard to lung cancer and miR15a and 16, there is an accumulating amount of data indicating that warrants further pursuit of our initial hypotheses.

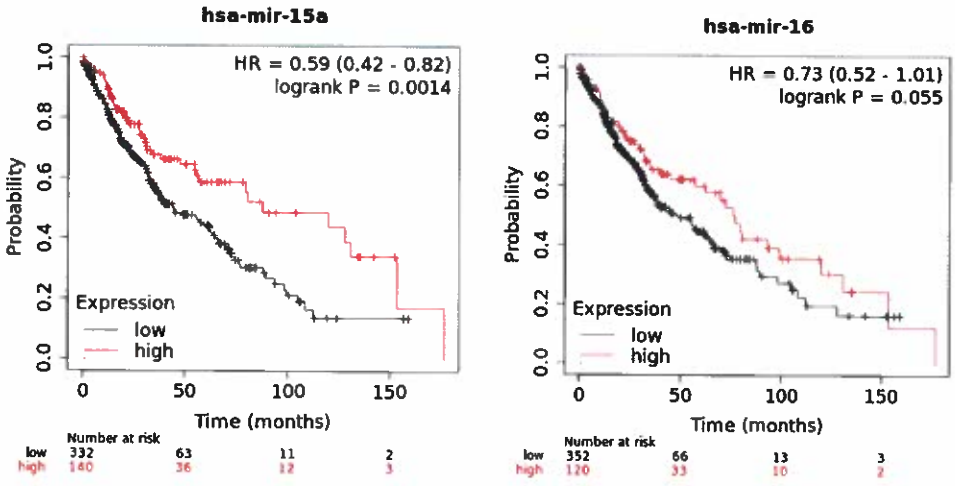

Figure 2. Samples collected in the TCGA database that are specific to lung cancer. These results show that the presence of these specific microRNAs may extend survival rates in patients with higher expression, supporting our hypothesis that virally-mediated expression of miR15a/16 could serve as a viable therapy for lung cancers.

We have performed an analysis of lung cancer samples in the TCGA database that revealed a divergent pattern of miR15a/16 expression in lung cancer. Of particular interest and contrary to our hypothesis, miR15a was higher in lung adenocarcinoma, with no difference in miR16 expression between normal and pathological samples, and no statistically significant difference in patient mortality between high and low miR15a/16 expressing tumors (results not shown). On the other hand, while miR-16 expression was unchanged in lung squamous cell carcinoma when compared to normal tissues, miR15a expression was significantly reduced. In squamous cell carcinoma, high miR15a expression was associated with a significantly reduced hazard ratio for mortality in lung cancer patients, with the same analysis for miR16 narrowly failing to rise to the level of significance (p=0.055).

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 at this time.

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.

As indicated above, with the no cost extension that was granted, we plan to confirm and validate the lentivirus therapeutic approach designed to implement our genetic overexpression of miR15a/16 in lung cancer cells. This approach, using cell-specific antibody-directed techniques, should prove as a useful tool to slow or stop the progression of cancers of the lung.

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).

Although still in its preliminary stages, we have demonstrated that the overexpression of these microRNAs profoundly impact the proliferation and growth rates of cancers involving the lung. We are excited to continue these experiments that are focused on the development and implementation of viral therapies that specifically target and permanently alter the genetic behavior of these cancerous cells. We are hopeful that the preliminary results arising from this project will stimulate development of targeted therapies using viral vectors and genetic interventions that will not affect normal, healthy cells in the organism.

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 at this time, but the proposed lentiviral approach (which can serve as a 'large cargo' transport device, coupled with conjugate antibody targeting techniques, should prove useful in a variety of diseases that can be treated through genetic interventions.

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 at this time, but we are confident that results from this project will catapult the use of similar therapeutic constructs for the treatment of disease.

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:*

Nothing to Report. The conjugated antibody design, which is a slightly different approach compared to the original proposal, is completely consistent with the use of antibody-directed therapies and lentiviruses.

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.

The major delays were largely due to antibody methodologies in the development of the virus package. This has since been resolved, and we now have viable antibodies capable of infecting and inserting genomic DNA into the host cell. Once we have the conjugate antibody technique validated, we anticipate that the project can be completed prior to the end of the no cost extension period.

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. All methodologies were consistent with the proposed budget, and did not come at any additional costs.

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. This project was determined as Exempt by the IRB of TAMU at the onset of the project.

Significant changes in use or care of vertebrate animals

N/A.

Significant changes in use of biohazards and/or select agents

We sought approval from the IBC of TAMU to begin manufacturing our own spycatcher/spytag in accordance with NIH guidelines for intentions to manufacture proteins using bacteria.

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 at this time.

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 at this time.

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: James D. Fluckey
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0003-1231-0412
Nearest person month worked: 1

Contribution to Project: The PI has had direct oversight on the project for design, implementation and assessment of all experiments.
Funding Support: Institutional support

Name: Peter Nghiem
Project Role: Co-investigator
Researcher Identifier (e.g. ORCID ID): 0000-0002-8796-8123
Nearest person month worked: 0.6

Contribution to Project: Dr. Nghiem has been instrumental in the design and implementation of the CRISPR/Cas9 intervention studies.
Funding Support: Institutional support

Name: Patrick Ryan
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 6

Contribution to Project: Mr. Ryan is the senior PhD student on this project. He is responsible for day-to-day activities on the project, implementation of the study design, experimental procedures and analyses.
Funding Support: N/A

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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) 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.