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TITLE: Evaluation of Biomarkers Predictive of Benefit from the PD-1 Inhibitor MK-3475 in Patients with Non-Small Cell Lung Cancer and Brain Metastases

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14. ABSTRACT
Immunotherapies inhibiting the Programmed Death-1 (PD-1) axis can result in dramatic responses and durable benefit in patients with non-small cell lung cancer (NSCLC). However, the overall response rate is only 20-30% and there is no clearly-defined biomarker that predicts which patients are most likely to benefit. Moreover, patients with NSCLC and brain metastases represent a population for which there are limited treatment options, and these patients are typically excluded from immunotherapy clinical trials or require local therapy prior to study enrollment. Therefore we are conducting a trial of the PD-1 inhibitor pembrolizumab (MK-3475) in patients with NSCLC and untreated brain metastases. The objective of this proposal is to study the immunophenotypic characteristics of primary lung tumors, brain metastases and extra-cerebral metastases with the goal of determining the variability across sites, and to study tumor- and blood-based biomarkers to establish predictors of immunotherapy benefit. We hypothesize that identifying biomarkers predictive of benefit to immunotherapy in patients with NSCLC and brain metastases will result in improved patient outcomes. We have made progress towards these goals in several areas over the last two years. Over the first year of the grant, we optimized the assays to be used to study, compiled the cohort of paired tumor samples, accrued patients with NSCLC and untreated brain metastases to the clinical trial with pembrolizumab, and obtained both blood and tumor tissue samples from these patients. Additionally, the PI had the opportunity to learn the laboratory skills necessary to complete this project. Over the course of the past year, we have finalized the paired tumor sample cohort and the tissue microarray is currently under construction. We have begun to analyze the tissue and blood samples from patients on the clinical trial with pembrolizumab to determine biomarkers predictive of treatment response. Accrual to the clinical trial has continued and we have worked to enhance accrual by opening the trial in several additional locations.

15. SUBJECT TERMS
NSCLC, Immunotherapy, PD-1, PD-L1, Brain metastases, Biomarker

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1. **INTRODUCTION:**

Lung cancer is the leading cause of cancer death in the United States, resulting in more than 160,000 deaths each year. The majority of patients with lung cancer have non-small cell lung cancer (NSCLC) and present with disease at an advanced stage when cure is not possible. Approximately 30% of these patients develop brain metastases at some point during their clinical course. Typically these patients have more limited survival than patients without brain metastases, and many undergo surgery or radiation therapy that can have lasting neurologic toxicity. In recent years we have seen dramatic responses to a new class of therapeutics that target the immune system, specifically with agents targeting the PD-1 axis. Among these agents, the PD-1 inhibitor pembrolizumab has been found to be a safe and effective treatment for a subset of patients with NSCLC. Although the overall response rate is 20-30% with the PD-1 agents, it is unknown whether these agents benefit patients with brain metastases and there is no clearly defined predictive biomarker that determines which patients are most likely to benefit from treatment. We have designed and are conducting an investigator-initiated trial at our institution of the PD-1 inhibitor pembrolizumab (MK-3475) in patients with untreated brain metastases from NSCLC (NCT 02085070). The tumor biopsy specimens and blood samples from patients on the trial form the basis for this proposal with the goal of identifying predictive biomarkers for response to PD-1 inhibitors in patients with NSCLC and untreated brain metastases. Additionally, among the putative predictive biomarkers, it is unknown whether expression is consistent at various sites of disease, including in the CNS, where the tumor microenvironment may alter marker expression. Understanding biomarker variability is critical as we explore which patients derive benefit from treatment.

2. **KEYWORDS:**

- NSCLC
- Immunotherapy
- PD-1
- PD-L1
- Brain metastases
- Biomarker

3. **ACCOMPLISHMENTS:**

a. **What were the major goals of the project?**

The major goals of the project are to identify biomarkers that are predictive of response to PD-1 inhibitors in patients with NSCLC and untreated brain metastases, as well as to determine whether biomarker expression is consistent at various sites of disease.

Completion dates and estimates of the percentage of completion for each of the major tasks in the Statement of Work are as follows:

	Timeline (months)	Percent accomplished
Major Task 1: Obtain HRPO approval	1-6	Completed
Specific Aim 1: To examine the immunophenotype variability in lung cancer		
Major Task 2: Analyze the immunophenotypic pattern in CNS metastases compared to other distant sites of disease and primary versus metastatic disease sites.	1-28	70%
Specific Aim 2: To determine tissue- and blood-based biomarkers predictive of response to immunotherapy		
Major Task 3: Tissue-based predictive biomarker evaluation	12-32	50%
Major Task 4: Blood-based predictive biomarker evaluation	12-35	50%

b. **What was accomplished under these goals?**

The objective of this grant is to identify biomarkers predictive of benefit to immunotherapy in patients with NSCLC and brain metastases and delineate immunphenotypic patterns at various sites of disease. We proposed two aims to achieve these objectives:

<u>Specific Aim 1</u>: To examine the immunophenotype of NSCLC and the variation at different sites of disease. To achieve this goal we planned to study expression of checkpoint stimulators and inhibitors in both tumor cells and tumor infiltrating lymphocytes (TILs) at various sites of disease to determine the variability across tumor sites.

During the first year of this grant, I learned the laboratory techniques necessary to carry out the tasks required for completion of the proposed studies, optimized the assays to use immunofluorescence to study immune markers, and started the process of building a tissue microarray of paired tumor samples.

Over the last year of the grant, we have finalized the review of all potential tissue samples and confirmed the cohort of paired samples to be included in the tissue microarray. Because other data has emerged on biomarker expression at different sites of disease but the brain remains an understudied organ in this area of research, we focused our efforts on collecting brain metastasis samples and were successful at finding 72 cases of brain metastases from lung cancer. Since not all of these cases had a paired sample from the same patient, we included several cases with tissue from a brain metastasis only and other cases with lung tissue only. These tissues are not from the same patients so marker expression will be compared from the different sites of disease to assess for differential patters from the various organs. See Table 1 for details of the samples that will be included in the TMA. The TMA has been commissioned and is currently in the Pathology Department being built.

Table 1. Summary of lung cancer tissue samples to be included in the Tissue Microarray

Sample Type	**Number**
Paired Brain – Other metastatic site	33
Brain only	39
Primary lung	20
Paired metastatic sites	10

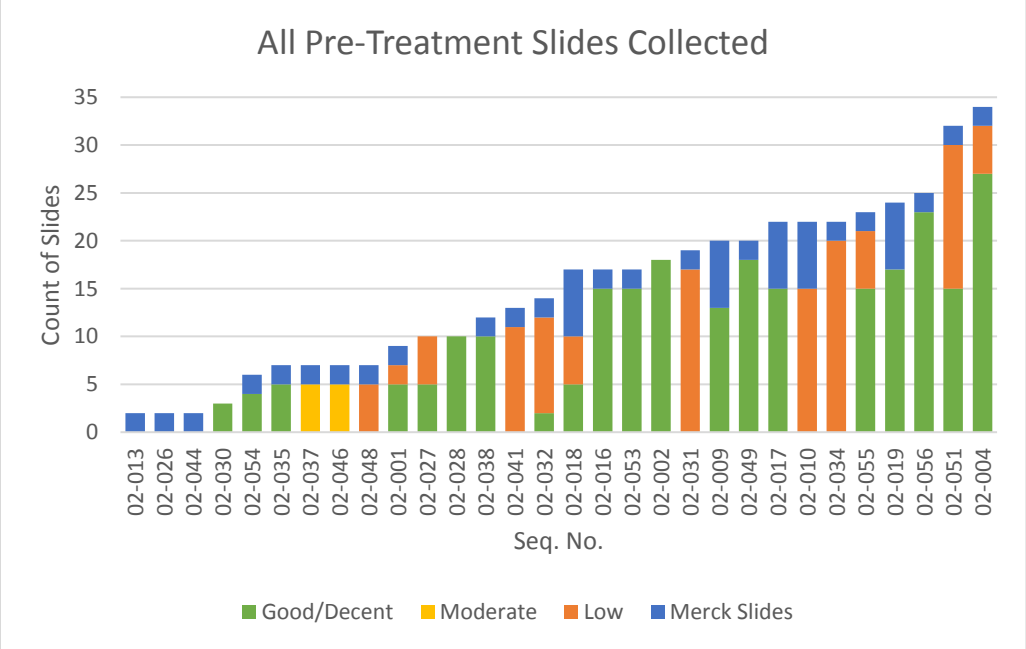
<u>Specific Aim 2</u>: To determine tissue- and blood-based biomarkers predictive of response or resistance to the PD-1 inhibitor pembrolizumab in patients with NSCLC and untreated brain metastases treated on a prospective Phase II clinical trial. We proposed to study the immunophenotypic pattern of NSCLC tumor samples including T-cell infiltration and immune marker expression as well as blood-based biomarkers.

Tissue and blood samples for these studies are obtained from patients on the clinical trial open at Yale "A phase 2 study of MK-3475 in patients with metastatic melanoma and non-small cell lung cancer with untreated brain metastases." Since the trial opened in

March 2014, we have made significant progress accruing patients, although accrual remains slower than anticipated due to the approval of pembrolizumab by the FDA and ability of patients to receive drug as standard-of-care. In total, we have enrolled and treated 38 patients with pembrolizumab on the trial (5 over the last year), and another patient is currently undergoing the screening process. In the past year we have opened the trial at two additional sites in an effort to increase accrual. We are also planning to open at a third site in the near future. The trial remains open and we are continuing to accrue patients.

During this last year, we have begun to analyze pre-treatment tumor tissue from patients on this trial. We have reviewed the tissue samples to determine the quality of the tumor tissue and found that most cases had sufficient tissue for correlative studies (see Figure 1 below). Additional slides that were initially sent to Merck for PD-L1 testing as part of the clinical trial that were not used for their assay were obtained to use for our analysis.

Figure 1. Number of pre-treatment slides available for correlative analyses from patients on the clinical trial of pembrolizumab



Thus far, 18 tissue samples have begun to be analyzed. We initially planned to perform quantitative immunofluorescence (QIF) as the sole method of determine predictive biomarkers from tumor tissue, however given limitations from this method we decided to also perform transcriptomic analysis using the NanoString nCounter Gene Expression

Assay. This was chosen based on the small amount of genetic material needed (10 ng ≈ 1 slide) and the potential for detecting expression of a large number of genes (~800). Examples of some of the results obtained thus far can be found in Figures 2 and 3 below; these data have not yet been analyzed by a bioinformatician but demonstrate the power of this assay in analyzing tumor samples.

Figure 2. Transcriptome analysis of immune pathway genes in patients with brain metastases treated on the trial of pembrolizumab. Patients are divided by response in the brain, body and overall.

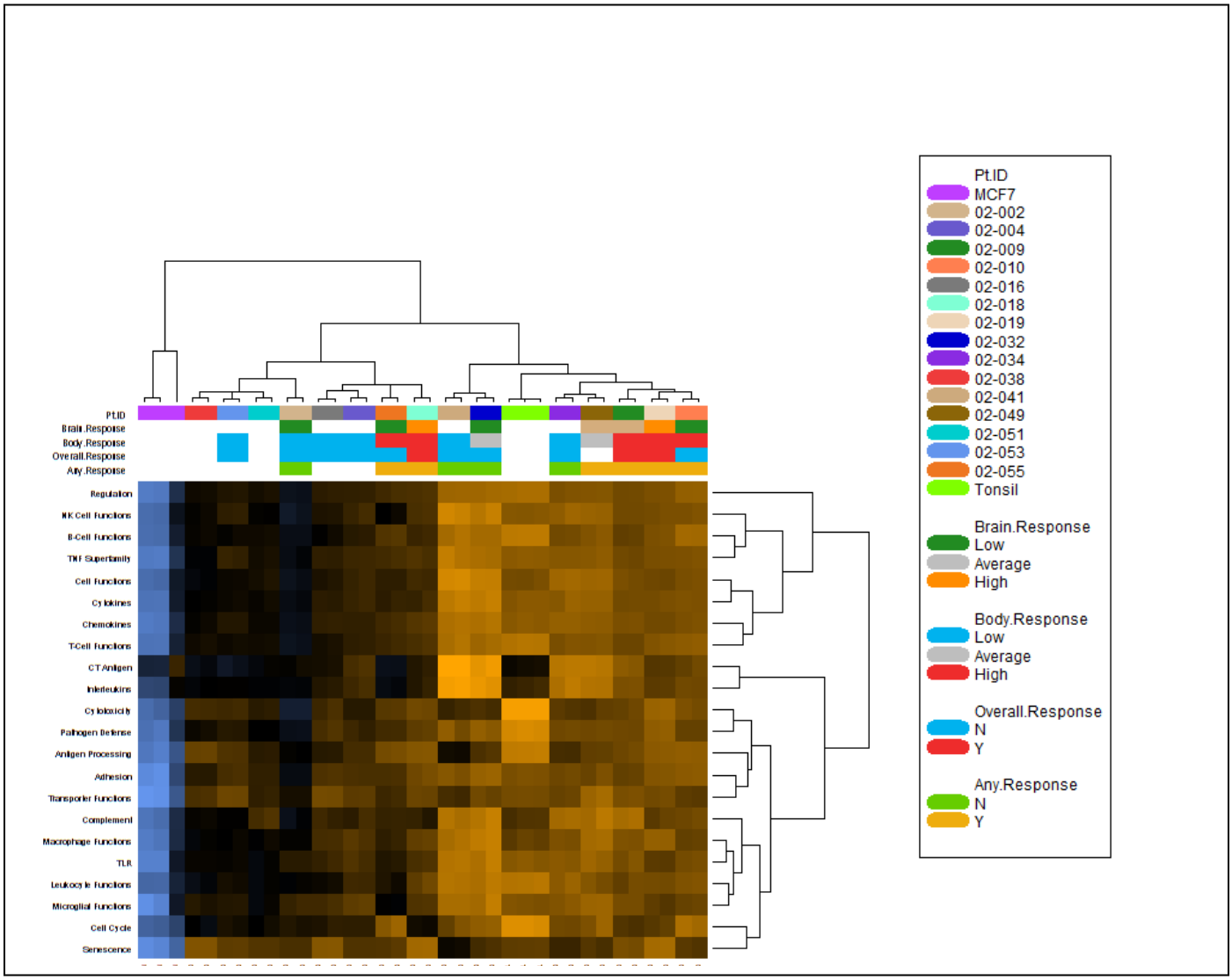
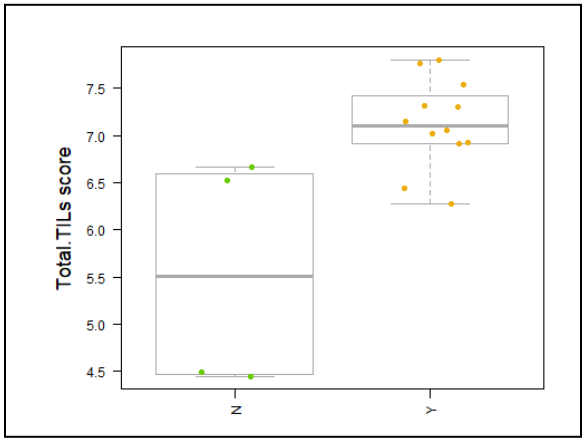
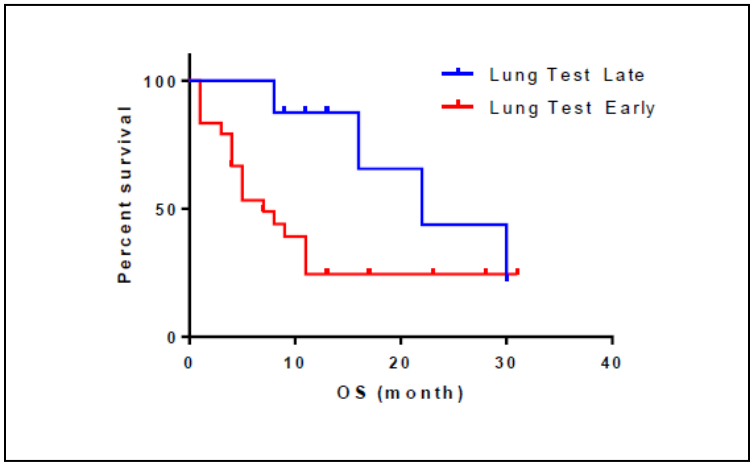


Figure 3. Transcriptome analysis of Tumor Infiltrating Lymphocytes (TILs) in patients with brain metastases treated on trial with pembrolizumab, divided by whether they achieved a response to therapy.



We have also made progress towards Aim 2b which includes investigation of blood-based predictive biomarkers in patients on the previously-mentioned trial of pembrolizumab. Blood was collected at multiple timepoints, including prior to the start of study treatment and throughout the course of treatment on trial. Over the last year we subjected 32 pre-treatment samples to a mass-spectrometry-based proteomic test to determine whether we could predict clinical outcomes based on the results. The test divides patients into groups that potentially could predict for better or worse outcomes. We found that there was a trend towards predicting overall survival (see Figure 4 below) but it was not statistically significant, HR 0.357 (95% CI 0.118-1.081, p=0.0624), possibly because of the small sample size. We are currently preparing an abstract to submit to the Society for Immunotherapy of Cancer Conference with this data.

Figure 4. Overall survival in patients with brain metastases treated with pembrolizumab, divided into predictive groups based on a mass-spectrometry-based proteomic test.



In summary, we have made substantial progress towards the goals proposed in this grant during the first 2 years. We are on track for completing the grant in the remaining year.

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

I have had ample opportunity for training and professional development during the first two years of this project. I have dedicated 40% of my effort to this project as well as additional time to other research projects. Dedicated time at this early stage of my career is invaluable and has allowed me the opportunity to work towards my research goals in a mentored setting.

I have worked closely with my mentor Dr. Roy Herbst and have learned a great deal from his guidance. We continue to meet weekly during a one-on-one session to discuss my research progress and goals. Throughout this grant period he has guided me in my translational and clinical projects and has taught me a great deal about lung cancer research. Additionally, I have attended weekly meetings with the thoracic research team which includes participation by basic scientists, clinical researchers, and research staff.

I have also continued to work closely with my collaborators Drs. Harriet Kluger and Lucia Jilaveanu on the basic science aspects of this project. I meet frequently with Drs. Kluger and Jilaveanu to carry out the tasks for this grant and to learn the skills required.

I have had many opportunities for professional development during this grant period. I have participated in weekly translational lung cancer meetings at Yale and weekly Cancer Center Grand Rounds. I have attended the ASCO Annual Meeting, the IASLC World Conference on Lung Cancer, and the IASLC Targeted Therapies in Lung Cancer Meeting.

d. How were the results disseminated to communities of interest?

Nothing to report.

e. What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period in the coming 3 months we plan to do the following:

- Begin testing immune marker expression on the tissue microarray (Aims 1a and 1b).
- Continue biomarker analysis on the clinical trial tissue samples (Aim 2a).
- Assay post-treatment blood specimens from the clinical trial (Aim 2b).
- Continue clinical trial accrual (Aims 2a and 2b).
- Continue mentorship by Dr. Herbst on clinical and translational research.

4. **IMPACT:**

a. **What was the impact on the development of the principal discipline(s) of the project?**

The results from the clinical trial of patients with brain metastases from lung cancer or melanoma treated with pembrolizumab was published during the first year of this grant. This trial was the first of its kind to demonstrate that immunotherapy can be effective in the brain. Prior to this study, patients with untreated brain metastases were typically excluded from clinical trials with immunotherapy agents. The knowledge gained from our study is likely to make an impact on the field of oncology as we now know that patients with brain metastases can benefit from immunotherapy. During the last year, we have begun to explore biomarkers from tumor tissue and blood that may be predictive of which patients are most likely to benefit from such treatment.

b. **What was the impact on other disciplines?**

Nothing to report

c. **What was the impact on technology transfer?**

Nothing to report

d. **What was the impact on society beyond science and technology?**

Nothing to report

5. **CHANGES/PROBLEMS:**

a. **Changes in approach and reasons for change**

Nothing to report.

b. **Actual or anticipated problems or delays and actions or plans to resolve them**

During year one of the grant we experienced delays with several aspects of this project which have been previously discussed. Over the last year, we have not additional issues but we have continued to have delays due to slow accrual to the clinical trial. This is likely due to FDA approval of pembrolizumab (and other immunotherapies) and fewer patient referrals for clinical trials. Accrual has continued but at a lower rate than initially anticipated. To date we have enrolled and treated 38 patients with pembrolizumab, and our goal is 44. Because Aim 2 requires accrual to the clinical trial, progress on this aim has been delayed. To overcome this problem, we have started analyzing the tumor and blood samples that we have thus far instead of waiting until full trial accrual to begin the analysis. Once the trial is fully accrued we will analyze the remainder of the samples. We continue to work on enhancing accrual by communicating with our referring physicians and reaching out to the community to enhance enrollment. We have monthly calls with community oncologists to discuss possible clinical trial patients and recently met with community practices to educate oncologists about this trial. We have also opened the trial at two community practices and plan to open it at a third practice in the coming months.

Changes that had a significant impact on expenditures

Due to the delays described above, we did not use all of the funds during the prior two years. These funds will be used during the remaining year of the grant.

c. **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report.

6. **PRODUCTS:**

a. **Publications, conference papers, and presentations**

- **Journal publications.**

  Nothing to report.

- **Books or other non-periodical, one-time publications.**

  Nothing to report.

- **Other publications, conference papers, and presentations.**

  Yale Cancer Center Grand Rounds, March 2017. "Systemic therapy for brain metastases in non-small cell lung cancer."

b. **Website(s) or other Internet site(s)**

Nothing to report.

c. **Technologies or techniques**

Nothing to report.

d. **Inventions, patent applications, and/or licenses**

Nothing to report.

e. **Other Products**

Nothing to report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

a. **What individuals have worked on the project?**

Name:	*Sarah Goldberg*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*5*
Contribution to Project:	*Dr. Goldberg proposed the work for this project and is responsible for overseeing the studies performed. She has accrued patients to the clinical trial, identified cases to include in the cohort, and has gathered blood and tumor tissue that will be analyzed.*
Funding Support:	*NIH/NCI* *Boehringer Ingelheim*

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

Updates to funding support status since the last reporting period:

Funding Agency	Award Number	Project Title	Status at time of award activation	Current status
AstraZeneca	Not applicable	Evaluation and identification of biomarkers in non-small cell lung cancer	Active	Completed 5/14/2016
AstraZeneca	Not applicable	Evaluation and identification of MEK-related biomarkers in non-small cell lung cancer	Active	Completed 12/17/2016
The Hope Foundation	Not applicable	A Phase II/III randomized trial of afatinib with or without cetuximab in treatment-naïve patients with advanced EGFR-mutant non-small cell lung cancer	Active	Completed 11/30/2016

c. **What other organizations were involved as partners?**

Nothing to report

8. **SPECIAL REPORTING REQUIREMENTS**

a. **COLLABORATIVE AWARDS:** Not applicable

b. **QUAD CHARTS:** Not applicable

9. **APPENDICES:** Not applicable