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TITLE: Evaluation of Biomarkers Predictive of Benefit From 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 year. We have optimized the assays that will be used to study the immunophenotypic characteristics of the paired tumor samples. This is a necessary step prior to moving forward with the goals for year two of this grant. Additionally, we have compiled the cohort of paired tumor samples and have begun reviewing them to determine which samples have sufficient tumor to include in the study. We have accrued patients with NSCLC and untreated brain metastases to the clinical trial with pembrolizumab and have obtained both blood and tumor tissue samples from these patients. As this is a career development grant, the PI has had the opportunity to learn the laboratory skills necessary to complete this project.					
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	Percent
	(months)	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.	1-18	50%
Specific Aim 2: To determine tissue- and blood-based biomarkers predictive of response to immunotherapy		
Major Task 3: Tissue-based predictive biomarker evaluation	12-21	10%
Major Task 4: Blood-based predictive biomarker evaluation	12-24	10%

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 immunophenotypic patterns at various sites of disease. We proposed two aims to achieve these objectives:

Specific Aim 1: 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, including CNS metastases compared to other distant sites of disease and primary versus metastatic disease sites.

During the first few months of this grant period, I worked closely with Drs. Harriet Kluger and Lucia Jilaveanu to learn the laboratory techniques necessary to carry out the tasks required for completion of the proposed studies. Assays to use immunofluorescence to study immune markers on lung cancer tumor samples were

optimized. Specifically, antibodies were validated and optimized using lung cancer control arrays including assays for the following molecules:

Markers expressed on tumor cells: B7H1 (PD-L1), B7H2, B7H3, B7H4

Markers expressed on lymphocytes: CD3, CD4, CD8, FOXP3, TIM3, CTLA4

Additionally, we have made significant progress towards building a tissue microarray of paired tumor samples. We have screened several hundred cases of patients with lung cancer in the Yale pathology archives to find those with paired samples available, and have spent a considerable amount of time tracking down these samples. This has proven to be challenging as many samples are insufficient for our purposes (i.e. tissue was obtained via fine needle aspiration and is unable to be cored) or the tissue has already been exhausted. To date we have identified over 100 patients who have paired samples in the Yale pathology archives, and I have begun the process of reviewing the slides to determine which samples have sufficient tissue for our purposes and to identify the appropriate site for coring of the tissue. We expect that the tissue microarray will be commissioned within the next few weeks once the cohort is finalized.

Specific Aim 2: 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. Work on this aim of the grant was intended to be performed during Year 2, but we have already started work on these studies during Year 1.

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. We have screened 61 patients with lung cancer for possible participation. Of these patients, 33 have been enrolled and treated with pembrolizumab on the trial, and another 2 patients are currently undergoing the screening process. Of the 33 enrolled patients, we have obtained tumor

tissue taken just prior to the start of pembrolizumab from 30 patients. The proposed plan for these samples was to create a tissue microarray (TMA) to study biomarkers predictive of response to immunotherapy. However, it has become clear that the majority of the tissue samples are extremely small and therefore it is not possible to create a TMA. Instead we plan to analyze these samples using the same immunohistochemistry method (AQUA) as initially proposed, but perform the studies on individual slides taken from the tumor samples instead of on a TMA. These assays have been optimized and we believe this will yield equivalent data compared to using a TMA.

Additional progress has included a review performed by myself and a pathologist of H&Es from all of the pre-treatment tumor samples to determine tumor content and adequacy of tissue for correlative studies. Only 1 sample was exhausted and another 13 had low tumor content. Of these 14 samples with low or no tumor cells, 10 have additional samples taken prior to the start of the trial (although not immediately pre-treatment); 2 of these have been reviewed and have adequate tumor for analysis, and an additional 8 samples are pending review. Only 4 patients out of the total 30 reviewed thus far have low tumor content in one specimen and no other specimen available. The trial remains open and we are continuing to accrue patients and obtain pre-treatment tumor tissue for analysis.

We have also made some progress towards Aim 2b which includes investigation of blood-based predictive biomarkers in patients on the previously-mentioned trial of pembrolizumab. We have compiled blood samples from 24 patients on this trial thus far. Blood was collected at multiple timepoints and we currently have a total of 40 samples.

In summary, we have made substantial progress towards the goals proposed in this grant. The conditions necessary for the assays involved have been established and the compilation of cases for the TMAs is nearly complete. We have adjusted our laboratory technique to account for challenges we have faced, and we continue to accrue to the clinical trial to increase our sample size.

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 year 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 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.

During this grant period I have also worked closely with my collaborators Drs. Harriet Kluger and Lucia Jilaveanu to learn the laboratory techniques necessary for 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 and the IASLC World Conference on Lung Cancer.

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:

- Finalize the paired samples cohort and create a tissue microarray (Aims 1a and 1b).
- Begin staining the clinical trial tissue samples (Aim 2a).
- Begin assays on the 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?**

During this reporting period, we published our results from the interim analysis of the clinical trial of patients with brain metastases from lung cancer or melanoma treated with pembrolizumab. 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.

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**

- *Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

We have experienced delays with several aspects of this project. For Aim 1, it has taken a longer period of time than expected to compile a cohort of patients with paired tumor samples to create a TMA. The reasons for this include many patients with insufficient tumor tissue for coring due to small biopsy samples, and also tissue samples that were previously exhausted. We have screened several hundred patients to find cases that are acceptable for our purposes. Although our progress has been delayed, we are now nearing completion of finalizing the cohort and expect to be ready to commission the TMA in the coming weeks.

An additional delay is due to clinical trial accrual being slower than anticipated, likely due to FDA approval of pembrolizumab (and other immunotherapies) and fewer patient referrals for clinical trials. Difficulties with this trial are not unique as there has been declining accrual for other clinical trials at Yale and also across the country. Fortunately, accrual continues at a steady rate, albeit lower than initially anticipated. We have screened 61 patients with lung cancer for possible participation in the trial, and of these patients 33 have been enrolled and treated with pembrolizumab so far. We continue to work on enhancing accrual by communicating with our referring physicians and reaching out to the community to enhance enrollment. We have weekly calls with community oncologists to discuss possible clinical trial patients and we also plan to personally visit community practices to educate oncologists about this trial. Because Aim 2 requires accrual to the clinical trial, progress on this aim has been delayed. To overcome this problem, we plan to start 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. In fact, we have already begun to compile and review the tumor and blood samples from the current patients on the trial ahead of when we initially proposed to begin this aspect of the project.

c. **Changes that had a significant impact on expenditures**

Due to the delays described above, we did not use all of the funds during the current year. These funds will be used during year 2.

d. **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.**

Goldberg SB, Gettinger SN, Mahajan A, Chiang AC, Herbst RS, Sznol M, Tsiouris AJ, Cohen J, Vortmeyer A, Jilaveanu L, Yu J, Hegde U, Speaker S, Madura M, Ralabate A, Rivera A, Rowen E, Gerrish H, Yao X, Chiang V, Kluger HM. Pembrolizumab for patients with melanoma or non-small cell lung cancer and untreated brain metastases: early analysis of a non-randomised, open-label, phase 2 trial. *Lancet Oncol.* 2016 Jun 3.

pii: S1470-2045(16)30053-5. doi: 10.1016/S1470-2045(16)30053-5. [Epub ahead of print] **PMID:** 27267608.

- Federal support acknowledged.

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

Nothing to report.

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

*IASLC World Conference on Lung Cancer 2015, Denver, CO. Oral Abstract: “A Phase II trial of pembrolizumab for untreated brain metastases from non-small cell lung cancer.”

*American Society of Clinical Oncology Annual Meeting 2015, Chicago, IL. Poster Presentation: “Activity and safety of pembrolizumab in patients with metastatic non-small cell lung cancer with untreated brain metastases.”

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:	<i>Sarah Goldberg</i>
Project Role:	<i>Principal Investigator</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	<i>5</i>
Contribution to Project:	<i>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.</i>

Funding Support:	<i>NIH/NCI</i> <i>The Hope Foundation</i> <i>AstraZeneca</i> <i>Boehringer Ingelheim</i>
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- 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 reported at the time of award activation:

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	Active
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	Active
NIH/NCI	R01 PAR-14-166	Validation of Quantitative 11C-Erlotinib PET for Imaging EGFR-Mutant Lung Cancer	Active	Active
Boehringer Ingelheim	Not applicable	A Randomized Phase II/III Trial of Afatinib Plus Cetuximab Versus Afatinib Alone in Treatment Naïve Patients with Advanced, EGFR Mutation Positive Non-Small Cell Lung Cancer	Active	Active
NIH/NCI	1UM1CA186689-01	ViKTriY Early Clinical Trials Consortium (ECTC)	Active	Active

Additional funding support activated during the course of the current award:

Funding Agency	Award Number	Project Title	Award Activation Date
NIH/NCI	P50 CA196530-01	Targeting the EGF Receptor Pathway in Lung Adenocarcinomas	09/01/2015

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