

AWARD NUMBER: W81XWH-19-1-0169

TITLE: Rational Development of Immune Therapy for Low-Grade Ovarian Serous Carcinoma to Overcome MEK Inhibitor Resistance

PRINCIPAL INVESTIGATOR: Kwong Kwok Wong

CONTRACTING ORGANIZATION: The University of Texas MD Anderson Cancer Center

REPORT DATE: July 2020

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

<b>1. REPORT DATE</b> July 2020			<b>2. REPORT TYPE</b> Annual		<b>3. DATES COVERED</b> 6/1/2019 to 5/31/2020	
<b>4. TITLE AND SUBTITLE</b>  Rational Development of Immune Therapy for Low-Grade Ovarian Serous Carcinoma to Overcome MEK Inhibitor Resistance					<b>5a. CONTRACT NUMBER</b>	
					<b>5b. GRANT NUMBER</b> W81XWH-19-1-0169	
					<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b> Kwong Kwok Wong, PhD  E-Mail: <a href="mailto:kkwong@mdanderson.org">kkwong@mdanderson.org</a>					<b>5d. PROJECT NUMBER</b>	
					<b>5e. TASK NUMBER</b>	
					<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  The University of Texas MD Anderson Cancer Center      1515 Holcombe Boulevard, Houston, TX77030					<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012					<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
					<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for Public Release; Distribution Unlimited						
<b>13. SUPPLEMENTARY NOTES</b>						
<b>14. ABSTRACT</b> Low-grade serous ovarian cancer accounts for a relatively large percentage of epithelial ovarian cancers in young women. The exact biological pathways that underlie this disease are elusive. Moreover, chemotherapy and hormonal therapy are relatively ineffective and have generally failed to reduce high morbidity and mortality. Because the Ras/Raf/MEK/ERK pathway is frequently activated in LGSC, targeting this pathway has been examined as an avenue for potential treatment. In the phase II GOG0239 clinical trial, 52 patients were treated with the MEK inhibitor (MEKi), selumetinib. Of these, one had a complete response, seven had a partial response, and 34 had stable disease. Unfortunately, all the patients with stable disease eventually developed resistance to MEKi and died of the disease. This study is investigating the immune pathway and how the immune pathway responds to MEK inhibitor in low-grade ovarian serous carcinoma (LGSC). The immune profile of low-grade serous cancer is largely unknown, and how the immune profile will be affected by currently targeted therapy in clinical trial using MEK inhibitors is also unclear. By deciphering how targeted therapy may affect the immune system in LGSC, rational design of clinical trials by combining targeted therapy and immunotherapy will be possible to improve low-grade ovarian serous cancer patient survival.						
<b>15. SUBJECT TERMS</b>						
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>  Unclassified	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b> USAMRMC	
<b>a. REPORT</b>  Unclassified	<b>b. ABSTRACT</b>  Unclassified	<b>c. THIS PAGE</b>  Unclassified			<b>19b. TELEPHONE NUMBER (include area code)</b>	

## TABLE OF CONTENTS

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

## Introduction

LGSC accounts for a relatively large percentage of epithelial ovarian cancers in young women. The exact biological pathways that underlie this disease are elusive. Moreover, chemotherapy and hormonal therapy are relatively ineffective and have generally failed to reduce high morbidity and mortality. Because the Ras/Raf/MEK/ERK pathway is frequently activated in LGSC, targeting this pathway has been examined as an avenue for potential treatment. In the phase II GOG0239 clinical trial, 52 patients were treated with the MEK inhibitor (MEKi), selumetinib. Of these, one had a complete response, seven had a partial response, and 34 had stable disease. Unfortunately, all the patients with stable disease eventually developed resistance to MEKi and died of the disease. This study is investigating the immune pathway and how the immune pathway responds to MEK inhibitor in low-grade ovarian serous carcinoma (LGSC). The immune profile of low-grade serous cancer is largely unknown, and how the immune profile will be affected by currently targeted therapy in clinical trial using MEK inhibitors is also unclear. By deciphering how targeted therapy may affect the immune system in LGSC, rational design of clinical trials by combining targeted therapy and immunotherapy will be possible to improve low-grade ovarian serous cancer patient survival.

## Keywords

Low-grade ovarian serous carcinoma, MEK inhibitor, trametinib, immune profile, RNAseq, mouse model

## Accomplishments

For the first year of this study, the following are our major goals of the project as stated in the approved SOW.

	Timeline	Percentage of Completion
Specific Aim 1. To identify immunogenic suppressive signals as potential biomarkers for MEK inhibitor resistance in LGSC.		
Major Task 1	Months	
a. Submission of institution's IRB approval and related material for DoD's HRPO approval.		100%
b. Receive HRPO approval or exempt finding before initiating Human Anatomical Substances related studies.	1-18	100%
Subtask 1: To determine the immune profiles in ovarian low-grade serous carcinoma patient samples.		50%
Specific Aim 2. To determine the role of MHC-I and B7-H4 on immune response and tumor progression of LGSC.		
Major Task 2	Months	
Subtask 1: To determine how MEK inhibitor treatment affects CD8+ T-cell recognition of ovarian cancer by up-regulation of MHC-I.	1-24	25%
Specific Aim 3. To determine the efficacy of combining MEK inhibitors and immune checkpoint therapy using syngeneic LGSC mouse models.		
Major Task 3	Months	
a. Submission of institution approved animal protocols and related material for DoD's ACURO approval.		100%
b. Receive ACURO approval before initiating animal experiments.	1-24	100%
Subtask 1: To determine the changes in immune profiles of low-grade OSC in mouse models after MEKi treatment.		25%

For task 1, we have generated RNAseq data for low-grade serous ovarian carcinoma frozen tissues from 14 patients and has generated a tissue microarray from FFPE paraffin blocks from 70 patients. We are in the process of analyzing the RNAseq data to identify immunogenic suppressive signals. We will also perform multiplexing immunofluorescent staining using the tissue microarray to validate potential immunogenic suppressive biomarkers in the coming year.

For task 2, we have generated syngeneic mice that will develop low-grade ovarian serous carcinoma in approximately 15 weeks of age. We have treated two pairs of mice with trametinib (MEK inhibitor) or vehicle control daily for three days. Mice were euthanized and tumor tissues collected for FFPE, DNA/RNA processing. The tumor size from trametinib treated mice appeared to be smaller than the vehicle control at the testing dose of 2mg/kg. We will breed more mice for an expanded treatment study to determine how MEK inhibitor treatment might affect CD8+ T-cell recognition of ovarian cancer and the expression of MHC-I in the coming year. We have also generated MEK inhibitor resistant low-grade serous ovarian cancer cell lines to decipher the MEKi adaptive response (a poster presentation was given at the International Conference on Molecular Targets and Cancer Therapeutics; 2019 Oct 26-30; Boston).

For task 3, we are in process of expanding the mice colony. We have optimized the dose of trametinib to have an impact on low-grade serous ovarian carcinoma growth and have extracted RNA to determine gene expression profile change by RNAseq analysis. We will expand our mouse colony for treatment experiment and test if MEKi treatment will affect the expression of B7H4 to justify the use of combination treatment in the coming years.

## Impact

We will have a better understanding of the immune pathways, and immune response changes as a result of MEK inhibitor (MEKi) treatment in low-grade serous ovarian cancer (LGSC). Whether B7-H4 can be a biomarker for MEKi response and survival will be determined. This could immediately lead to a rational design of immunotherapy clinical trial by combining MEKi and anti-B7-H4 for LGSC. Potential long-term impact: A novel immunotherapy targeting B7-H4 in combination with MEKi targeted therapy could improve survival of patients with LGSC.

## Changes/Problems

Due to the COVID-19 pandemic, our research lab was shut down and animal experiments were terminated from March to June. In addition, our institution has imposed hiring freeze in March and the postdoc who supposed to start on April 1 will be delayed to September 1st. Thus, the animal experiments were delayed. We are starting to re-establish the mouse colony after the lab was reopened on June 22<sup>nd</sup> 2020. During the time the lab was closed, we are working on writing the manuscript on “ The Adaptive response of low-grade serous ovarian cancer cell to MEK inhibitor” and a review article on “The Challenge to overcome MEK inhibitor resistance”.

## Products

### Conference Presentation:

Kwong-Kwok Wong, Yvonne T Tsang, Michelle Chen, Eucharist H Kun, David M Gershenson. Adaptive responses of low-grade ovarian cancer cell lines to MEK inhibitor [abstract]. In: Proceedings of the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics; 2019 Oct 26-30; Boston, MA. Philadelphia (PA): AACR; Mol Cancer Ther 2019;18(12 Suppl):Abstract nr C089. doi:10.1158/1535-7163.TARG-19-C089

## Participants & Other Collaborating Organizations

Name:	Kwong Wong, PhD
Project Role:	Principal Investigator

Researcher Identifier (ORCID ID): <https://orcid.org/0000-0002-0375-6669>  
Nearest person month worked: 2  
Contribution to Project: Dr. Wong has performed work in the area of experimental design, mouse experiments and data analysis.

Name: Yvonne Tsang, PhD  
Project Role: Research investigator  
Researcher Identifier (employee ID): 159154  
Nearest person month worked: 7  
Contribution to Project: Dr. Tsang has performed work in the area of RNAseq analysis and immune histochemistry to identify immunogenic suppressive signals.  
Funding Support: This funding and MD Anderson Ovarian Cancer Moon Shots Program funding

Name: Eucharist Kun  
Project Role: Research Assistant  
Researcher Identifier (employee ID): 252838  
Nearest person month worked: 5  
Contribution to Project: Mr. Kun has performed work in the area of mouse experiments.  
Funding Support: Low-grade ovarian serous carcinoma philanthropic fund

#### Special Reporting Requirements

Not applicable

#### Appendices

Not applicable