

AWARD NUMBER: W81XWH-12-1-0573

TITLE: Prediction of Response to Therapy and Clinical Outcome through a Pilot Study of Complete Genetic Assessment of Ovarian Cancer

PRINCIPAL INVESTIGATOR: Ursula Matulonis, MD

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute
Boston, MA 02215

REPORT DATE: December 2015

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel 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.					
1. REPORT DATE December 2015		2. REPORT TYPE Final		3. DATES COVERED 30-SEP-2012 TO 29-SEP-2015	
4. TITLE AND SUBTITLE Prediction of Response to Therapy and Clinical Outcome through a Pilot Study of Complete Genetic Assessment of Ovarian Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-12-1-0573	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Ursula Matulonis, MD E-Mail:Ursula_matulonis@dfci.harvard.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Dana-Farber Cancer Institute 450 Brookline Avenue, YC 1421 Boston MA 02215-5450				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel 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 In this project low grade serous cancer, clear cell, endometrioid (low grade and high grade),and mucinous cancers will be interrogated and subjected to somatic mutation profiling using genome sequencing and array comparative genomic hybridization (aCGH) to explore copy number alterations (CNA). To date, over 200 subjects have enrolled and pathology has identified blocks for DNA extraction. The Study remains important as nothing has been published on the molecular alternation of the different histological subtypes of ovarian cancer.					
15. SUBJECT TERMS Ovarian Cancer, histological subtypes, somatic mutations					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 12	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

<u>Table of Contents</u>	<u>Page</u>
I. INTRODUCTION	2
II. BODY OF WORK	2
III. KEY RESEARCH ACCOMPLISHMENTS	7
IV. REPORTABLE OUTCOMES	8
V. CONCLUSIONS	9
VI. REFERENCES	10

I INTRODUCTION

Several key advancements have shaped the understanding of ovarian cancer genomics as well as exploiting and translating these discoveries into therapeutic advances for ovarian cancer patients. Although once considered a single entity, ovarian cancer can be now subdivided into different histological subtypes that have different identifiable risk factors, molecular compositions, clinical features, and, more recently, treatments. These histologies include high grade serous carcinoma (HGSC) and low grade serous carcinoma (LGSC), as well as endometrioid, clear cell, and mucinous carcinoma (1-3).

The most common histologic subtype of ovarian cancer, HGSC, has undergone the most extensive genomic investigation and is characterized by underlying deficiencies in DNA repair, presence of BRCA mutations in some tumors, and evidence homologous recombination deficiency (HRD) abnormalities in about 50% of HGSC cases (1-3). Oral poly (ADP ribose) polymerase (PARP) inhibitors exploit the HRD abnormalities of HGSC for their efficacy, leading to the United States Food and Drug Administration approval of the oral PARP inhibitor olaparib. The approval of olaparib in 2014 was the first targeted and personalized therapy for ovarian cancer, specifically for women with recurrent ovarian cancer who have a germline *BRCA* mutation.

The overarching goal of this Department of Defense (DOD) project has been to comprehensively genetically characterize ovarian cancer at our institution in order to identify targetable and actionable mutations.

II. BODY OF WORK:

Body of work:

Year 1:

During year 1, the following was intended to be accomplished:

1) Specific aim 1 will be started:

- 75 tumor samples (formalin fixed paraffin embedded) from different histologic subtypes of ovarian cancer will be pulled from the Department of Pathology at the Brigham and Women's Hospital. These histologic subtypes will include: high grade serous, low grade serous, clear cell, high grade endometrioid, and mucinous cancer.
- Clinical data will be extracted for each case.
- Areas of highest percentage of tumor will be assessed by a gynecologic oncology pathology, circled and cored.
- Somatic mutation assessment, copy number variation and analysis as well as gene expression

profiling will be started on these samples.

Progress:

Specific Aim 1 was started. Specific aim 1 is as follows:

Specific aim 1: Newly diagnosed epithelial ovarian cancer patients and active patients with recurrent cancer undergoing treatment at the DFCI will have their cancers tested for cancer gene mutations as well as copy number alterations. This project seeks to identify the molecular and genetic abnormalities of ovarian cancer in order to better choose and select rationale therapies for women with ovarian cancer.

Protocol 11-104, which is an Institutional Review Board (IRB)-approved Dana-Farber/Harvard Cancer Center (DF/HCC) research protocol, enrolled patients and subjected cancer samples to a test called OncoPanel (4). Clinical research staff from the Dana-Farber Cancer Institute (DFCI) Gynecologic Oncology program supported by this grant consented patients to 11-104. OncoPanel is a cancer genomic assay that detects somatic mutations, copy number variations and structural variants in tumor DNA extracted from fresh, frozen or formalin-fixed paraffin-embedded samples. DNA was isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500 sequencer (4). This genetic profiling is performed within the Department of Pathology at the Brigham and Women's Hospital in Boston MA. In addition, the results of OncoPanel are result and available for viewing in a restricted website that the patient's attending physician can access. This is a research test and it is not billed to patients. Through this protocol 11-104, clinical data extraction was also started for cases.

The statistician on this grant, William Barry, Ph.D, assisted in all aspects of statistical analysis of this project.

Year 2:

During year 2, the following will be accomplished:

1) Specific aim 1 will be completed.

2) Specific aim 2 will be started:

-- Our analysis will begin by looking within each genomic data type to search for additional common robust subtypes and to explore whether any correlation exists between the OncoMap genomic alterations or copy number alterations and the expression-based subtypes.

-- Additional subtypes will be looked for defined by molecular profiles by extending our analysis beyond single mutational events and looking for common-pathway mutations that might indicate commonly disrupted processes within subsets of HGSC patients.

-- Dr. Quakenbush and his group will then investigate clinical correlation between outcome and results of the OncoMap, aCGH, and expression profiling.

Progress:

Specific aim 1 was completed and Specific aim 2 was started.

Specific aim 1: This task was completed and over 1000 samples of ovarian cancer have been identified and genomically characterized.

Since this grant's inception, more than 1000 patients with ovarian cancer have been enrolled into 11-104 and consented by our data managers in the Gynecologic Oncology program. Through a DF/HCC IRB request placed by Dr. Matulonis for 11-104 ovarian cancer data, data retrieval and analysis was initiated in 2015 with the assistance of William Barry, PhD, the statistician on this project. This project included Elizabeth Stover, M.D., Ph.D, currently an Instructor of Medicine at Harvard Medical School who joined this project in April 2015 and has been on faculty in the DFCI Gynecologic Oncology program since July 2016.

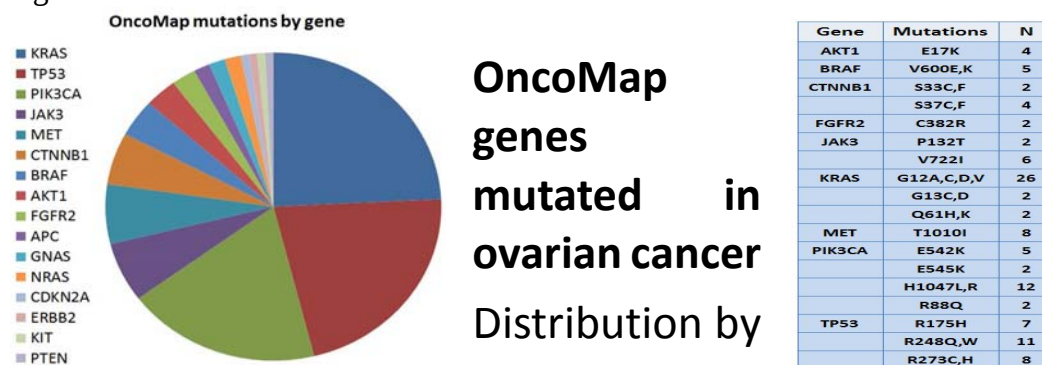
This grant has led to several publications and has been significantly instrumental for our program's development of precision medicine for our patients with ovarian cancer which are listed in Section IV (REPORTABLE OUTCOMES). In addition, the de-identified genomic information generated from this grant can be accessed other research investigators at DFCI for collaborative studies across cancer types; these investigators must submit a formal request to access this data to the DF/HCC IRB to access this information and also receive permission from Dr. Matulonis who is the gynecologic oncology user committee lead.

Accessible clinical information as per DF/HCC IRB approved protocol 11-104 includes ovarian cancer stage, histology, type of initial treatment (upfront surgical debulking versus neoadjuvant therapy), progression free survival, time to first recurrence, and responses to administered treatments will provide critical outcome data for current and future abstracts and projects.

Specific aim 2:

We have started specific aim 2 and 302 patients were tested for hotspot mutations in 41 genes by OncoMap. Most tumors were primary tumors, with 8% metastatic. Histologic types included 89% adenocarcinoma and its subtypes, 4% sex cord stromal, 2% borderline, and ~1.5% each carcinosarcoma, germ cell, and other. 128 mutations were found in 102 patients; most patients had 1 mutation (see below Figure 1).

Figure 1:



OncoMap mutations identified two or more times in the OncoMap cohort of 128 mutations (N=number of mutations). The OncoMap platform is designed to genotype known hotspot mutations, so all have biologic relevance. Several mutations are potentially targetable with small molecule inhibitors including BRAF V600E, MET T1010I, and PIK3CA mutations. Overall, approximately 1/4 of patients exhibit a potentially targetable alteration using this panel of oncogenic mutations.

Mutations found in specific ovarian cancer histologies:

We also identified specific mutations for specific histologic subtypes:

Table 1

Histology	Most frequently altered genes
High grade serous	TP53, BRCA1/2, ARID1A/B, KMT2D, APC, CREBBP, PRKDC
Low grade serous	KRAS
Endometrioid	ARID1A/B, PTEN, BRCA1/2, APC, KMT2D, ATRX, NF1, CTNNB1, MTOR, PIK3CA
Clear Cell	ARID1A/B, PIK3CA, BRCA2, ATM, SETD2, ATRX, MTOR, PIK3R1
Mucinous	KRAS, TP53, CDKN2A, SMAD4, TERT
Carcinosarcoma	TP53, RB1, APC
Granulosa cell	ATM, KMT2D, EP300, FANCD2
Sertoli Leydig cell	DICER1

Copy number variants: In addition, 219 patients were analyzed for copy-number variations (CNV) in OncoPanel genes. >12,000 total CNV were reported in the cohort (Figure 2). Single-copy deletions (n=5558) and copy-number gains (low amplification) (n=6635) were numerous. 188 highly amplified genes were reported from 64 patients, and 130 2-copy deletions from 33 patients, largely high-grade serous carcinoma and carcinosarcoma.

Figure 2:

Frequent CNV events

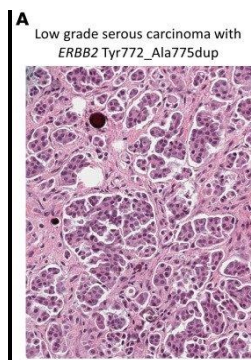
Most frequent highly-amplified and 2-copy deleted genes

Highly amplified	N	2-copy deletion	N
MECOM	11	STK11	5
CCNE1	9	CDKN2B	4
AKT2	7	GNA11	4
FGFR1	7	RB1	4
PIK3CA	7	FGFR3	3
PRKCI	7	FLCN	3
MYC	6	MAP3K1	3
ERBB2	5	NF1	3
KRAS	5	PHOX2B	3
		PIK3R1	3
		PTEN	3

Specific actionable mutations have been found:

Sholl et al (Section IV: REPORTABLE OUTCOMES) Rare activating ERBB2 mutations in ovarian cancer. A patient with a low grade serous cancer, heavily pretreated for her recurrent cancer, had her recurrent tumor analyzed and identified was an *ERBB2* Tyr772_Ala775dup mutation (Figure 3, from Sholl et al). Demonstrating this mutation resulted in the use of trastuzumab and navelbine with anti-cancer response radiographically and symptomatic improvement (less pain) for over 2 years.

Figure 3: Histology from a 48 yo patient with low grade serous carcinoma



III. KEY RESEARCH ACCOMPLISHMENTS:

- 1) Genotyping and NGS of cancer-associated genes in formalin-fixed tissue from ovarian tumors are feasible and can identify potentially clinically actionable alterations.
- 2) Known and novel single nucleotide variants and copy-number variations were identified in each histologic subtype.

IV. REPORTABLE OUTCOMES

Ritterhouse L, Nowak J, Strickland K, Garcia E, Jia Y, Lindeman N, MacConaill L, Konstantinopoulos P, Liu J, Berkowitz R, Nucci M, Crum C, Matulonis U, Sholl L, Howitt B. Morphologic Correlates of Molecular Alterations in Extra-uterine Müllerian Carcinomas, *Mod Pathol*. 2016 Aug;29(8):893-903

Stover, Elizabeth; Garraway, Levi, and Matulonis Ursula. Somatic mutations and copy number variations in cancer-associated genes in 569 ovarian cancer patients, accepted for a poster American Association of Cancer Research Annual meeting, 2016

Stover, Elizabeth; Garraway, Levi, and Matulonis Ursula. Somatic alterations in the PTEN-PI3K-AKT-mTOR pathway in a cohort of 379 ovarian cancer patients. Submitted to American Society of Clinical Oncology Annual meeting, 2016.

Stover, Elizabeth; Garraway, Levi, and Matulonis Ursula. Somatic mutations and copy-number variations in ovarian cancers via targeted sequencing of exons of 300 cancer-associated genes, including multiple DNA damage repair genes. 11th Biennial Ovarian Cancer Research Symposium on September 12-13th, 2016 at the University of Washington in Seattle, WA.

Sholl LM, Do K, Shivdasani P, Cerami E, Dubuc AM, Kuo FC, Garcia EP, Jia Y, Davineni P, Abo RP, Pugh TJ, van Hummelen P, Thorner AR, Ducar M, Berger AH, Nishino M, Janeway KA, Church A, Harris M, Ritterhouse LL, Campbell JD, Rojas-Rudilla V, Ligon AH, Ramkissoon S, Cleary JM, Matulonis U, Oxnard GR, Chao R, Tassell V, Christensen J, Hahn WC, Kantoff PW, Kwiatkowski DJ, Johnson BE, Meyerson M, Garraway LA, Shapiro GI, Rollins BJ, Lindeman NI, MacConaill LE. Institutional implementation of clinical tumor profiling on an unselected cancer population. *JCI Insight*. 2016 Nov 17;1(19):e87062.

Strickland KC, Howitt BE, Shukla SA, Rodig S, Ritterhouse LL, Liu JF, Garber JE, Chowdhury D, Wu CJ, D'Andrea AD, Matulonis UA, Konstantinopoulos PA. Association and prognostic significance of BRCA1/2-mutation status with neoantigen load, number of tumor-infiltrating lymphocytes and expression of PD-1/PD-L1 in high grade serous ovarian cancer. *Oncotarget*. 2016 Mar 22;7(12):13587-98.

V. CONCLUSIONS:

1) Both copy number and mutational data are now available for accessing on over 1000 ovarian cancer patients as of February 2016. Importantly, this genomic information is available to other research investigators at DFCI for collaborative studies who are IRB approved to access this information and provided in a de-identified manner.

2) For the ovarian cancer cohort, this data has now been accessed and several abstracts and manuscripts are in process. Dr. Elizabeth Stover, formerly a fellow at DFCI, has joined Dr. Matulonis' group at DFCI and has joined our faculty in July 2016; this work is a crucial part of her academic development.

3) Identification of important aberrant pathways found in ovarian cancer has led to new treatment strategies for ovarian cancer treatments such as HER2 mutations.

VI. REFERENCES

- 1) Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011;474:609-15.
- 2) Berns EM, Bowtell DD. The changing view of high-grade serous ovarian cancer. *Cancer Res* 2012;72:2701-4.
- 3) Liu J, Matulonis UA. New strategies in ovarian cancer: translating the molecular complexity of ovarian cancer into treatment advances. *Clin Cancer Res.* 2014 Oct 15;20(20):5150-6.
- 4) Wagle N, Berger MF, Davis MJ, Blumenstiel B, Defelice M, Pochanard P, Ducar M, Van Hummelen P, Macconail LE, Hahn WC, Meyerson M, Gabriel SB, Garraway LA. High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. *Cancer Discov.* 2012 Jan;2(1):82-93.