AWARD NUMBER: W81XWH-17-1-0075

TITLE: Real-Time Assessment of Homologous Recombination Deficiency During Ovarian Cancer Treatment

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REPORT DATE: July 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release:

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REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2018	Annual	1 Jul 2017 - 30 Jun 2018

4. TITLE AND SUBTITLE

Real-Time Assessment of Homologous Recombination Deficiency During Ovarian Cancer Treatment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0075

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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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

Patients with ovarian cancer generally have a good response to this therapy, but most cancers eventually reappear and require additional rounds of chemotherapy. "Precision medicine" uses individual characteristics of a specific cancer to help choose the best therapy for that patient. Many ovarian cancers have defects in DNA repair that make them vulnerable to specific types of chemotherapy as well as a new class of drugs called PARP inhibitors. Over time the cancer changes and develops resistance to these therapies. Choosing the best treatment could require repeated painful and expensive biopsies in order to identify changes in the cancer that impact response to therapy. We propose to develop a blood test to monitor tumor characteristics in real-time by evaluating free-floating tumor DNA (called cell-free DNA) in the bloodstream. This novel assay will use cell free DNA to evaluate DNA repair alterations in ovarian cancers and how those change during the course of treatment. We hope these studies will lead to new, less invasive ways of providing ongoing tumor information that will facilitate monitoring women with ovarian cancer and choosing the therapies most likely to be effective.

15. SUBJECT TERMS

Ovarian cancer, cell free plasma DNA, circulating tumor DNA, BRCA1, BRCA2, DNA repair, homologous recombination deficiency, next generation sequencing, liquid biopsy

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	9	

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1. INTRODUCTION

BRCA1 and *BRCA2 (BRCA)* are critical genes in the *BRCA*-Fanconi anemia pathway which repairs double strand DNA breaks via homologous recombination. Defective homologous recombination repair (HRR) confers increased sensitivity to platinum based chemotherapy and is synthetically lethal with PARP inhibitors. We and others have demonstrated that secondary somatic reversion mutations can restore *BRCA* function in *BRCA*-mutated ovarian carcinoma (OC). These reversion mutations correlate with platinum and PARP inhibitor resistance but are difficult to detect in clinical samples with current methods. In addition to the difficulty in identifying reversion to wildtype mutations in patients with HRR mutations, another barrier to predicting responsiveness to PARP inhibitors in the clinical setting is the need for pre-treatment biopsies to assess HRR status in real-time. We propose that assays that reveal the dynamic HRR status in tumors will lead to improved prediction of which women will benefit from PARP inhibitor therapy and provide essential information that will lead to better personalized therapies. To overcome the challenge of needing repeat biopsies, we propose to develop an HRR assay in cell-free DNA (cfDNA); this "liquid biopsy" will provide real-time assessment of HRR during therapy

2. KEY WORDS

Ovarian cancer, cell free plasma DNA, circulating tumor DNA, BRCA1, BRCA2, DNA repair, homologous recombination deficiency, next generation sequencing, liquid biopsy

3. ACCOMPLISHMENTS

Our first major task was to obtain DoD HRPO approval, which was accomplished on schedule.

Our second major task was to optimize a next generation sequencing assay to detect reversion mutations in tumor tissues using next generation sequencing. We performed high depth targeted NGS in cases with known reversion mutations identified by laser capture microdissection purification of neoplastic cells followed by Sanger sequencing. The identification of novel indel or single nucleotide substitutions that restore the open reading frame is relatively easy and was possible to do with our usual bioinformatics pipeline. However, the detection of secondary reversion to wildtype sequencing occurring on the mutant allele is challenging. We used tumor tissues with a variety of neoplastic purities to determine the lower limit of reliable detection of reversion to wildtype somatic mutations. We spent some time using different bioinformatics strategies to correct for neoplastic purity using allelic ratios both intragenic and across the genome. Despite these efforts, we were not able to get the threshold for reliable detection of reversion to wildtype below a minimum of 70% neoplastic purity. That means that for less pure tumor samples tested by NGS, one cannot rule out the presence of a sub-clonal reversion to wildtype somatic mutation.

Our third major task was to develop, optimize and test a cfDNA assay to identify HRR status. We designed a cfDNA NGS assay using IDT probes for target identification. We are closely collaborating with investigators in the University of Washington on development of this assay, so that it will be CLIA ready for clinical application at the conclusion of the project. First we tested the performance of the assay on high quality tumor DNA to compare with our standard tumor targeted NGS assay. In this quality control step, no mutations were missed with the new design. We've included unique molecular identifiers (UMIs) in our pipeline, which are small molecular tags that are added to each molecule prior to amplification that facilitate identification of amplification errors from true low-level variants. When combined with bioinformatic digital error correction, our sensitivity is significantly increased to the <0.2% variant range.

We are evaluating the assay both for standard single nucleotide subsitutions and indels, but also for larger copy number variations (CNV) and again, we have not missed any known mutations as evidence by the table below.

miniOnco_Dataset	BROCA Sample	Gene	Expected Mutation(s) in overlapping genes based on BROv10
169R01_A01_MONCv1_NA0250	56_H05_BROv7_HA0198	PMS2	partial deletion exon 2
169R02_B01_MONCv1_NA0250	6624_H03_BROv8_HA0215	MSH2	exon 7 duplication
169R03_C01_MONCv1_NA0250	9659_C08_BROv8_HA0255	MSH2	exons 3-16 deletion
169R04_D01_MONCv1_NA0250	LMG2140	MLH1	exon 10 deletion
169R05_E01_MONCv1_NA0250	18224_H03_BROv10_HA0323	BRCA1	exon 17 deletion
169R06_F01_MONCv1_NA0250	23438_F05_BROv10_HA0410	BRCA1	exon 13 duplication
169R07_G01_MONCv1_NA0250	LMG1847	PALB2	exons 12 and 13 deletion
169R08_H01_MONCv1_NA0250	LMG2145	BRCA1	exons 21-24 deletion

Now that we are confident in the performance of our design using high purity tumor DNA, we have begun testing on cfDNA samples from patients with OC. We have optimized our cfDNA collection and DNA extraction process. We have just tested our first batch of cfDNA samples

Opportunities for training and professional development has the project provided?
Nothing to report

Dissemination of Results
Nothing to report

Plans during the next reporting period.
We will test a number of cfDNA samples and compare results in our cfDNA NGS assay to direct tumor sequencing to identify sensitivity and specificity of cfDNA for tumor DNA alterations.

4. IMPACT

Impact on the principal discipline
Nothing to report

Impact on other disciplines
Nothing to report

Impact on technology transfer
Nothing to report

Impact on society
Nothing to report

5. CHANGES/PROBLEMS

Changes in approach
Nothing to report

Problems or delays and plans to resolve them:
Nothing to report

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

6. PRODUCTS

Publications, conference papers, and presentations
Nothing to report

Website(s) or other Internet site(s)
Nothing to report

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
Nothing to report

Other Products
Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Elizabeth Swisher MD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-2331-0434
Nearest person month worked:	1
Contribution to Project:	Dr. Swisher is directing all aspects of the project including IRB oversight, sequencing analyses, and data interpretation
Name:	Maria Harrell, PhD
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Harrell was overseeing all experiment and coordinating efforts between the Swisher Laboratory and Lab Medicine Department. She left the Swisher laboratory in March 2017 and has been recently replaced by Christopher Pennil MSc
Name:	Marc Radke
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Mr. Radke identify specimens, purify DNA including from plasma and perform all library preparations.
Name:	Chris Pennil., MSc.
Project Role:	He is now overseeing all experiment and coordinating efforts between the Swisher Laboratory and Lab Medicine Department and took over Dr. Harrell's role on the project.

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Name:	Mallory Beightol
Project Role:	Ms. Beightol is a senior technician in the Clinical Molecular Genetics Laboratory. She perform cfDNA library preps and sequencing.
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Name:	Colin Pritchard MD, PhD
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Together with Dr. Swisher, Dr. Pritchard oversees assay development and validate the assay in a CLIA environment.
Name:	Stephen Salipante MD, PhD
Project Role:	Computational biologist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Salipante has developed the pipeline to assess somatic reversion mutations and to identify homologous recombination mutations in cell-free plasma DNA. He will perform the bioinformatics analyses for all samples.

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

No, Nothing to report

What other organizations were involved as partners?

Nothing to report

Appendices
None