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14. ABSTRACT

Many ovarian cancers have specific defects in DNA repair that make them sensitive to a new class of drugs called PARP inhibitors. PARP inhibitors are particularly effective against cancers that have alterations in the *BRCA1* or *BRCA2* genes. BRCA1 and BRCA2 function in DNA repair, and cancers associated with *BRCA1* or *BRCA2* mutations are deficient in homologous recombination directed DNA repair. When homologous recombination does not work right, cancer cells rely on other types of DNA repair that result in more errors in replicating DNA, leading to characteristic patterns of DNA alterations. Whole genome sequencing can detect patterns of alterations in the DNA that are characteristic of homologous recombination deficiency. We will perform whole genome sequencing on cancers from 120 women who participated in ARIEL2, a PARP inhibitor clinical trial for recurrent ovarian cancer. We will use the information that we acquire to develop a new clinical test based on patterns of DNA alterations to better predict which women with ovarian cancer should be treated with a PARP inhibitor. In this manner, we can identify women with ovarian cancer who do not have *BRCA1* or *BRCA2* mutations, but who also have a good chance of responding to PARP inhibitors.

15. SUBJECT TERMS

Ovarian cancer, BRCA1, BRCA2, whole genome sequencing, mutator, DNA repair, signature, homologous recombination deficiency

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. INTRODUCTION

PARP inhibitors (PARPi) have clear therapeutic utility for cancer treatment in individuals with germline mutations in *BRCA1* or *BRCA2 (BRCA)*. These drugs also have activity in a subset of ovarian, peritoneal or fallopian tube carcinoma (OC) without germline mutations. However, the best way to predict which patients with *BRCA*-wildtype OC will respond to PARPi therapy is not defined. Loss of heterozygosity (LOH) as a maker for homologous recombination deficiency (HRD) does not optimally separate responders from non-responders amongst *BRCA*-wildtype OC. ARIEL2 is a monotherapy PARPi trial in platinum-sensitive OC and was powered to identify predictive biomarkers for PARPi response in women without germline *BRCA* mutations. We hypothesize that specific mutator signatures can be identified by whole genomic sequencing that identify HRD and are more predictive of response to PARPis than current diagnostic tests using LOH profiling. Our objective is to develop an HRD test that that will predict benefit from PARPi therapy and provide insight into which genetic alterations lead to HRD and PARPi response.

2. KEY WORDS

Ovarian cancer, BRCA1, BRCA2, whole genome sequencing, mutator, DNA repair, signature, homologous recombination deficiency

3. ACCOMPLISHMENTS

Our first major task was to submit IRB exemption request to our IRB and then submit to the DoD HRPO. This task was successfully accomplished with receipt of human subjects expemption based on non-identifiability of the samples to be studied.

Our second major task is to develop and optimize the bioinformatics pipeline. We have done that successfully collaborating with Serena Nik-Zainal PhD, a well-known expert and developer of mutational signatures at the University of Cambridge.

Our third major task is to complete whole genome sequencing (WGS) on ARIEL2 samples. We are currently in the middle of that process. During this reporting period we identified the company providing the best price and quality sequencing (MedGenome). We wanted to test the quality of our WGS from formalin fixed paraffin embedded (FFPE) tumor sections from ARIEL2 samples using a small set of cases before we run the entire batch. We chose 8 cases and sent matched germline and tumor DNA to Macrogen. We got back very high quality data from 7 of 8 samples. Using Dr. Nik-Zainal's pipeline, we analyzed the samples according to the following algorithm:

- Caveman for substitutions plus additional post-hoc filtering to deal with FFPE-related artefacts and any new sequencing artefacts
- Pindel for insertions/deletions under 100bp
- Brass for structural variation
- ASCAT for copy number, ploidy and aberrant cell fraction

Per Dr .Nik-Zainal, these were the highest quality FFPE samples she has seen using her bioinformatics pipeline and were essentially indistinguishable from fresh frozen samples. Furthermore, the fail rate is usually been about 1/3 with FFPE samples, so we are very encouraged by our first test set. Whilw, this test set is too small to make any definitive conclusions about the relationship between signatures and response, it is encouraging that 2 of 3 cases with high HRDetect scores had measurable response to the PARP inhibitor rucaparib.

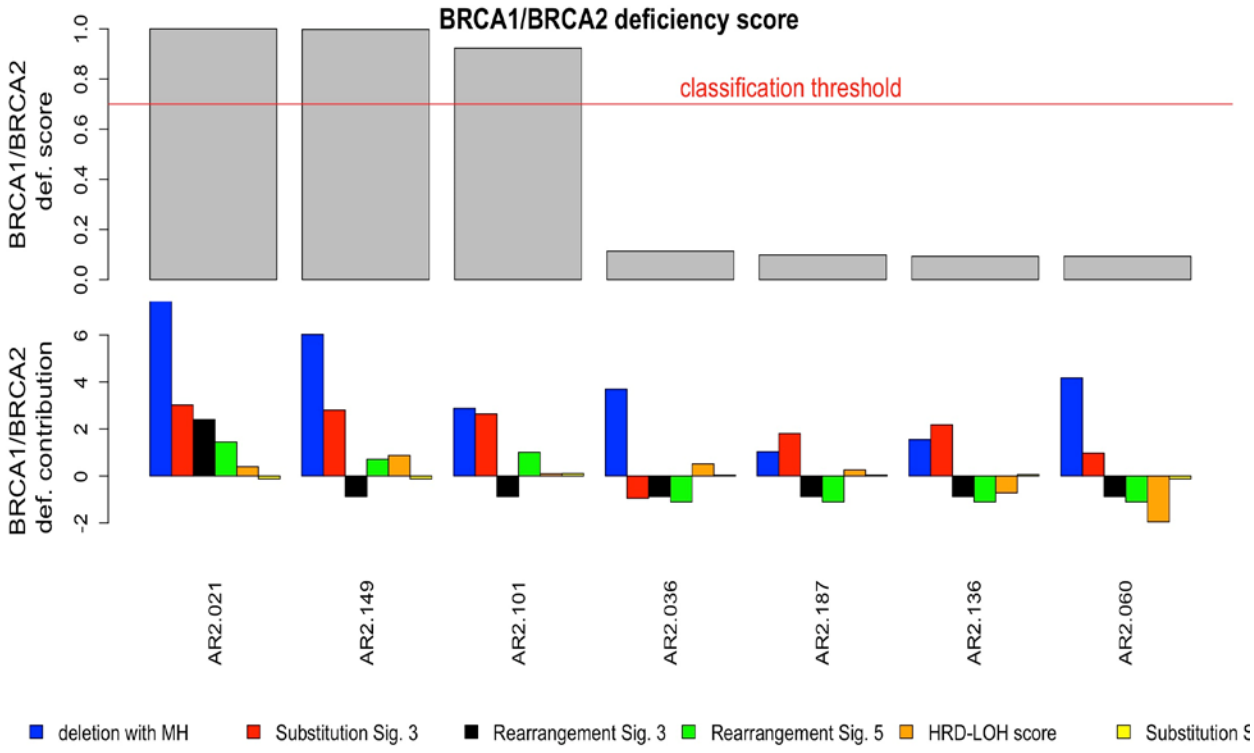

Figure 1. Preliminary mutational signature analysis of first 7 ARIEL2 FFPE cases. Of the first three cases with high HRDetect scores two had a partial response to rucaparib (AR2.021 and AR2.149. In contrast, of the next 4 cases with low HRDetect scores, only one had a partial response (AR2.060) and that tumor had too low neoplastic purity to provide a reliable score. Furthermore, that case has a high microhomology-mediated deletion score, which is one of our signatures of interest in detecting HRD. With a larger sample set, we will compare performance of HRDetect with the microhomology mediated deletions scores as well as with the other mutational signatures to identify the best predictor of PARPi response.

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.
Now that we have confidence in the quality of the WGS data from ARIEL2 FFPE specimens, we will extract DNA and send to Macrogen for the remaining 110 cases. We already know mutational and methylation status. We will then correlate mutational signatures with PARPi response in collaboration with statisticians at Clovis Oncologycore known HR genes. .

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, recruitment, 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 sequencing including quality control. She left the Swisher laboratory in March 2017 and has been replaced by Christopher Pennil MSc
Name:	Marc Radke
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Mr. Radke performs all DNA preparations and quality control prior to whole genome sequencing and monitors data transfer with Macrogen
Funding Support:	
Name:	Chris Pennil., MSc.
Project Role:	He now oversee all sequencing including quality control and took over Dr. Harrell's role on the project
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2

Name:	Nithisha Khasnavis MSc
Project Role:	Ms. Khasnavis is leading the bioinformatics on the project including developing and refining the WGS pipeline.
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6

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