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TITLE: DNA Copy Number Signature to Predict Recurrence in Early-Stage Ovarian Cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT :
Through this project we have determined the status of copy number variation in recurrent and non-recurrent early stage high grade ovarian cancer using a large, fully annotated, consortium cohort of 384 samples from clinical trials (Aim 1). Integrated analysis of copy number variation and RNAseq results from same samples (generated from an ongoing DOD #OC110628) has been performed with clinical data to generate a predictor of the recurrence of early stage ovarian cancer (Aim 2).

15. SUBJECT TERMS
Early Stage Ovarian Cancer, genomic predictive signature, recurrence

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1. INTRODUCTION

The survival of women with high-grade epithelial ovarian cancer is directly related to the spread of the tumor. Women with disease limited to the pelvis do well with many being cured, while those patients whose tumor has spread outside of the pelvis suffer recurrences and the majority will die from the disease. Nevertheless, the standard of care for patients with high-grade ovarian cancer is surgery followed by 6 cycles of chemotherapy (carboplatin/taxol) regardless of the spread of the tumor. Although some early stage patients are benefiting from this strategy, approximately 50-60% of patients with high-grade early stage cancer will not develop recurrent disease even in the absence of chemotherapy. These patients thereby suffer unnecessary short and long-term toxicities of chemotherapy with no benefit. Thus, the development of accurate biomarkers predictive of tumor recurrence becomes essential to identify women with early-stage disease who will benefit from chemotherapy while sparing the rest the unnecessary treatment with quality-of-life and cost-effectiveness ramifications. This approach parallels efforts in breast cancer where tests like "oncotype dx" provide valuable information on disease recurrence to women with early stage breast cancer. To the best of our knowledge, there is no available large-scale molecular characterization of early-stage ovarian tumors. Here, we propose to develop genomic signatures correlated with clinical outcomes and in particular tumor recurrence for early stage ovarian cancer. The status of DNA copy number variation (CNV) in recurrent and non-recurrent early stage high grade ovarian cancer will be investigated using a large, fully annotated, consortium cohort of 1628 samples from clinical trials. Integrated analysis will be performed by combining the gene expression profiles obtained from a recently terminated ongoing DOD project (W81XWH-12-1-0521) using the same 1628 samples with the gene copy results from this proposal. Through the integrated analysis of deleted, amplified and aberrantly expressed genes in early stage ovarian cancer, we expect to develop predictive biomarkers for future prospective stratification of women with early stage ovarian cancer to adjuvant carboplatin/taxol chemotherapy versus careful follow-up. This study will also contribute the identification of therapeutic biomarkers and stratification of early stage ovarian cancer patients most likely to benefit from targeted interventions.

2. **KEYWORDS:** Early Stage Ovarian Cancer, genomic predictive signature, recurrence, DNA copy number variation

3. ACCOMPLISHMENTS

Major Task 1: Obtain DNA samples from consortium specimens. Months 3-8

The tasks for the first project period included: 1) Obtain DNA FFPE specimens collected through the consortium of early stage high grade ovarian cancer, 2) Analyze about 50% of all required samples through IlluminaHumanOmniExpress-FFPE BeadChip system.

Through a previous DOD award (W81XWH-12-1-0521) we have: 1) Established an international consortium through which we have collected 1628 FFPE samples of serous ovarian cancer, 2) identified 592 early-stage high-grade ovarian cancers with 5-year follow-up, clinical annotation and accurate pathological review (228 recurrent and 364 non-recurrent), 3) established a specimen repository and clinical data inventory at MGH, 4) Sequenced RNA from these tumor samples, 5) Obtained preliminary RNAseq data indicating the need to

analyze 384 samples at a ratio of 2 non recurrent tumors versus 1 recurrent tumor to obtain a statistically significant genomic signature.

In addition, in the last budget period of this award we have optimized an SOP for double DNA and RNA extraction from our FFPE tissues and a procedure to randomize these samples to avoid any batch effect.

Major Task 2: To determine the copy number variation (CNV) for early stage high grade ovarian cancers through IlluminaHumanOmniExpress-FFPE BeadChip system. Months 9-18

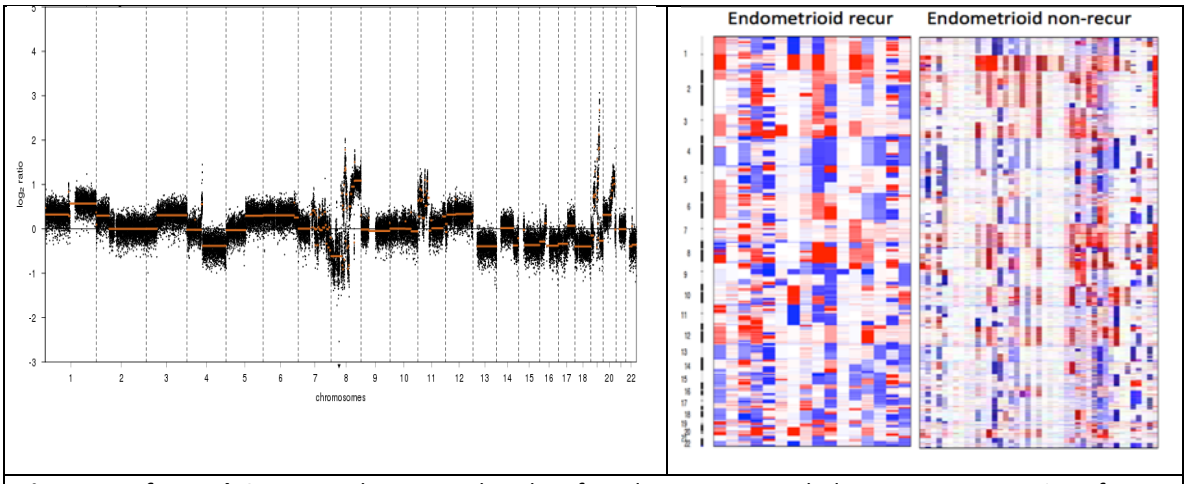


Figure 1. Left panel. Segmented copy number data from low-coverage whole genome sequencing of a recurrent endometrioid ovarian cancer sample. Libraries were prepared with the Swift Biosciences 2S PCR-free kit and sequenced on the Illumina NextSeq500 at 5x coverage. A total of 17 million reads were generated for this sample. Adaptor sequences were removed using cutadapt. Duplicates were removed using Picard tools. Data was normalized using R package QDNASeq followed by circular binary segmentation. **Right panel**. Heatmap of segmented copy number data for Endometrioid samples.

Unfortunately, the Illumina Hybridization Chips utilized for this analysis have revealed to be inefficient as we had less than 30% hybridization in more than 50% of samples of the initial batch of 48 samples that was analyzed. We have thus decided to perform DNA CNV analysis procedure based on shallow (5X) whole genome sequencing (WGS) technology using the Illumina NextGen platform. Shallow WGS has recently emerged as an alternative to microarray for copy-number detection, including the utilization of FFPE DNA as input. Through working with the DFCI core, we have overcome two major obstacles to ensure that copy-number can be reliably estimated through standardized workflow of segmentation. This include: 1) elimination of the amplification bias during the library preparation by using a PCR-free protocol, and 2) elimination of the necessity of using a reference 'normal' specimen by using observed-sequencing-depth across the genome to infer copy-number variation. A bioinformatic pipeline requiring the correction of sequencing bias has been executed using *QDNAseq* R/Bioconductor package, which implements the correction of low mappability, extreme GC content, as well as difficult regions known to be associated with Illumina platform as previously disclosed by large

studies like ENCODE or 1000 Genome Project. The results of this shallow WGS sequencing procedure is illustrated in Figure 1.

Additional Task 2 subtasks: To perform miRNAseq and IHC analysis to be integrated to DNA-CNV and RNAseq and identify a signature for recurrence of early stage ovarian cancer.

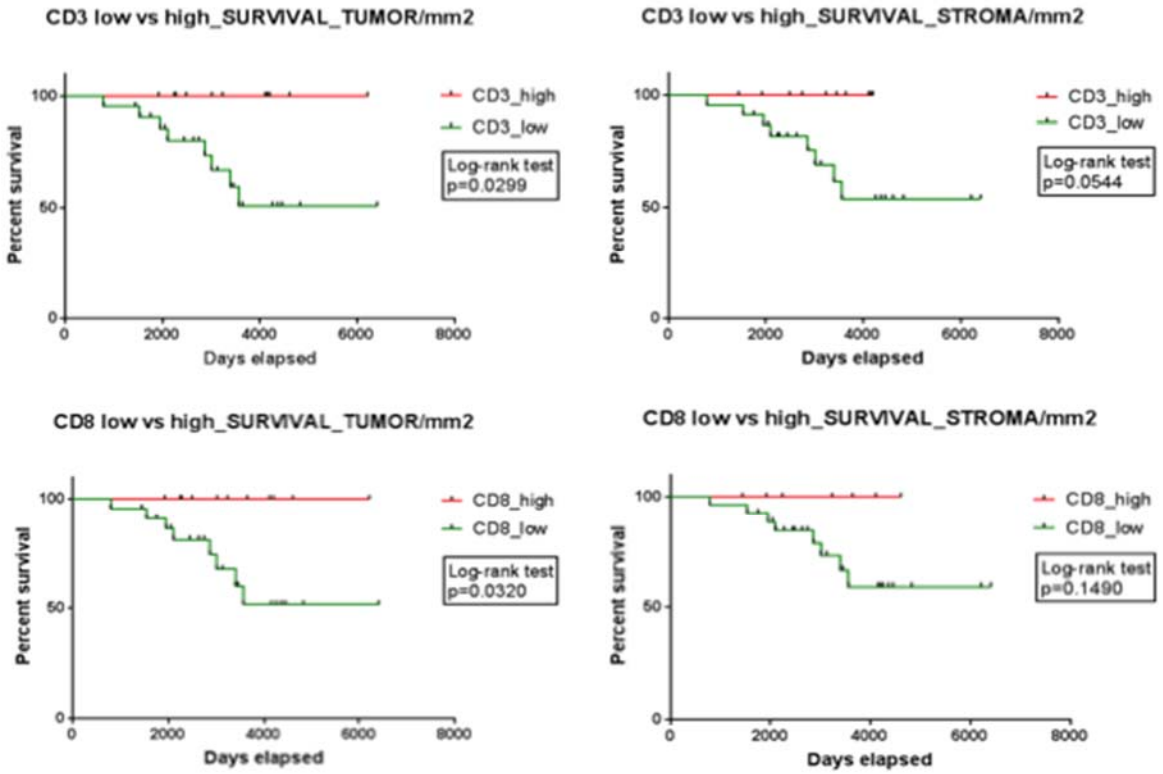
We have received an additional funding from the Ovarian Cancer Research Fund (OCRF) complementing these studies that includes analysis of micro-RNA expression in these samples. Thus, we performed a parallel analysis of miRNAseq on 384 early stage-high grade ovarian tumors. This analysis was performed using the miRNAseq HTG molecular diagnostic platform which is capable to automatically extract and sequence miRNA from 5-micron FFPE tumor tissues.

Finally, we have established collaboration with George Coukos at the Ludwig Institute @ CHUV Lausanne to perform high-throughput IHC analysis and determine if there is different infiltration of immune cells in recurrent versus non-recurrent early stage tumors. For this analysis each sample will be analyzed through the Ventana IHC platform for the following 2 markers panels:

1) T - cells: Granzyme B, PD1, CD8, CD38, CD3, Keratin, KI - 67, DAPI
2) B cells: CD68, CD86, CD163, PDL1, iNOS, Keratin, KI – 67, DAPI

This effort has just started and the initial results can be seen in Figure 2. The presence of high levels of CD8 or CD3 cells dramatically predicts for non-recurrence. The full panels listed above are ongoing and we anticipate them being finished by the first quarter of 2019. We will continue the work and analysis based upon additional funds available at UAB and from OCRF.

Figure 2: IHC analysis of immune cells in early stage tumors.
CD3 and CD8 immune markers predict for non-recurrence in early stage tumors



Major Task 3: Integrated analysis of the DNA-CNV results and the RNAseq results obtained from a paralleled DOD study of the PI W81XWH-12-1-0521. Months 18-24

384 early stage/high grade ovarian cancer FFPE samples have now been sequenced and divided in training and validation sets and data analysis is ongoing.

We have accomplished the analysis of 340 tumor samples from patients diagnosed with ovarian cancer at early stages (less than the 384 due to insufficent quality of some samples). The scope of this analysis is to identify a signature for recurrence of early stage tumors (Figure 2). The genomics and IHC analysis of these samples can then be integrated with the data obtained from advanced tumors to identify the real differences existing between tumor diagnosed at early stage versus those diagnosed at advanced stage.

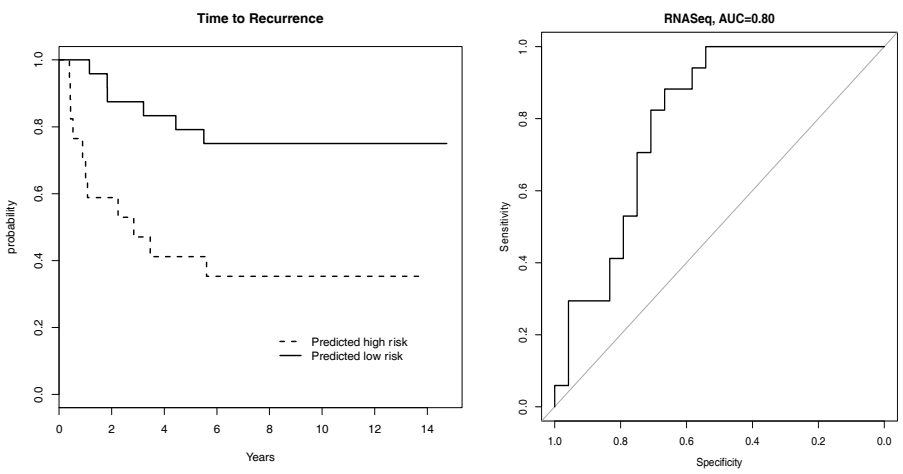


Figure 3. Signatures for recurrence in early stage tumors obtained through RNAseq. Tumors predicted to have high risk for recurrence recur significantly earlier than tumors predicted to have low risk. Cross-validated AUC=0.80.

130 of 340 samples have been set aside as a validation set and a cross-validation scheme has been used for model building using the remaining 210 samples to predict recurrence. Data have been normalized and corrected for possible technical differences among batches using methods implemented in Bioconductor pakcages edgeR and sva. We have developed and refined models to predict recurrence using techniques suitable for high-dimentsional data such as LASSO and randomForest. We have obtained an mRNA expression signature consisting of about 20 features for the endometrioid tumors with median cross-validated AUC of 0.80. The median cross-validated AUC for our best miRNA model is 0.67.

Results disseminated to communities of interest: The results of this project will be published in broadly read high impact scientific journals. The results will be disseminated rapidly to all professional organizations focused on ovarian cancer

Actual or anticipated problems or delays and actions or plans to resolve them: None

4. IMPACT

Impact on the development of the principal discipline(s) of the project: Creation of a well annotated biorepository of early-stage tumors allows performing correlative clinical and genomic studies on these tumors that are so poorly characterized and yet significantly affect the life of so many women.

Impact on other disciplines: Nothing to report

Impact on technology transfer: We anticipate that genomic discoveries in this project will have commercial application.

Impact on society beyond science and technology: This study will be used also in a different DOD award to the PI (W81XWH-16-2-0038) which is being developed with strong engagement of patient advocates. Advocate participation will help spreading the results to the

society in part by educating patients and their family members about new discoveries and therapeutic opportunities in ovarian cancer.

5. CHANGES/PROBLEMS

Not applicable

6. PRODUCTS

Nothing to Report

7. PARTICIPANTS & Other COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Michael Birrer, M.D., Ph.D.
Project Role	Principal Investigator
Researcher Identifer (ORCID ID)	mbirrer
Nearest Person Month Worked	1
Contribution to Project	Dr. Birrer served as the Principal Investigator of this project.
Funding Support	Not Applicable

Name	Wei Wei, Ph.D.
Project Role	Postdoc
Researcher Identifer (ORCID ID)	
Nearest Person Month Worked	2
Contribution to Project	Dr. Wei was responsible for experimental work in both Aims of the study including coordinating the Nanostring assay and performing the immunohistochemical staining.
Funding Support	Not Applicable

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

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report

9. APPENDICES

Not applicable