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Poster #1

Comprehensive single cell analysis of a patient's primary, recurrent, and xenograft ovarian cancer

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Objectives: Demonstrate a novel precision medicine approach in the diagnosis and treatment of ovarian cancer through comprehensive genomic and histologic analysis of a *BRCA1*+ primary, recurrent, patient-derived xenograft (PDX), and chemo-resistant PDX tumor.

Methods: The patient was consented as part of a prospective precision medicine study in ovarian cancer utilizing single cell sequencing technology. A tumor specimen was taken at the time of primary debulking for single cell RNA sequencing (scRNA-seq, 10x Genomics) and exome sequencing (C1 Fluidigm). Portions were also sent for bulk RNA & DNA sequencing and used to create mouse xenograft avatars. Avatar mice were treated with carboplatin and paclitaxel to generate chemo-resistant PDX tumors which were also single cell sequenced. Tumor was similarly collected and analyzed at the time of the patient's platinum sensitive recurrence. The patient also underwent genetic counselling with subsequent germline medical exome sequencing (covering ~4000 clinically significant loci). Histologic examination of the primary, recurrent, and xenograft tumors was performed by a board-certified pathologist.

Results: scRNA-seq was able to identify 7 distinct subpopulations of stromal and cancer cells, reproducible via multiple clustering algorithms. The patient's known *BRCA1* founder mutation, 187delAG, was identified by both somatic single cell exome sequencing and germline exome analysis. No additional clinically relevant mutations were identified via the extended germline panel. Histologic analysis of the primary, recurrent, and PDX tumors did demonstrate evidence of treatment effect. Analysis of the tumor subpopulations is ongoing to identify cell lineages shared between primary and recurrent samples, with a focus on chemo-resistant/stem-like populations.

Conclusions: We have created a compilation of single cell data on a *BRCA1*+ patient and demonstrated the increased resolution of this method over traditional approaches. Further analysis of cell subgroups may help identify chemo-resistant/stem-like populations present within the primary tumor which increase the risk for recurrence. Identification of these populations may guide novel treatment approaches after front-line therapy to ultimately improve patient outcomes.

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Poster #2

How does microbiome change with chemotherapy? Using an in vivo model of uterine cancer to assess changes in gut microbiome

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Objectives: Recent studies have suggested interactions between microbiome and cancer therapeutics. In addition to direct cytotoxicity, chemo-induced changes in microbiome may play significant role in disease outcomes. Our objective was to evaluate the efficacy of therapeutic agents in a mouse model of human endometrial cancer (EC) and their effect on tumor growth and gut microbiome.

Methods: An EC cell line, HTB-112 (ATCC), was injected subcutaneously into female, athymic mice. After 1week, mice were randomized and treated with one or a combination of 3 chemotherapies, carboplatin (C), paclitaxel (T), and a new investigational agent, Minnelide (M). Mice were treated for 4weeks with either single agent C, T, M, or combination C/T, C/M, T/M, or C/T/M. Mice were euthanized and tumors weighed. Fecal samples were collected prior to establishing tumors and weekly after. Microbial DNA was isolated from fecal samples and processed (Qiagen) for sequencing 16S rRNA gene, variable region 4 (U. of Minnesota, Genomics Center). Diversity between groups was calculated using Bray-Curtis dissimilarity.

Results: Tumors from mice treated with M, or C/M, or T/M were significantly smaller than tumors treated with single agent C or T (both, $p<0.0005$) or with C/T ($p<0.02$). There was no significant difference between single agent M, C/M, or T/M. Triplet therapy C/T/M treated tumors were significantly smaller than all other treatments ($p<0.05$).

Untreated controls with tumor were compared to treatment groups. Both single agent C and T changed beta diversity of fecal microbial composition significantly ($p=0.02$ for both), as did the combination of C/T ($p=0.04$) and tumors were larger. Monotherapy with M or in doublet, C/M or T/M, did not significantly change the microbial beta diversity ($p>0.05$) and tumors were smaller. In taxonomic analyses, M treated tumors were smaller and retained a greater percent of Firmicutes. C or T alone or C/T lost Firmicutes and increased Bacteroides relative abundance (figure attached).

Conclusions: We used a mouse model of EC, with 3 chemotherapies and compared chemotherapeutic efficacy and changes in microbiome. There was a correlation with increasing Bacteroides species abundance and increased tumor size. Interestingly, C treatment showed no significant change in tumor size and correlated with the

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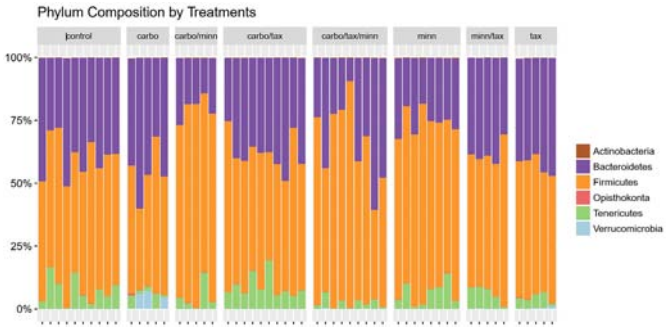
emergence of Verrucomicrobia. Further studies are underway to delineate the role of the microbiome observed.



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Poster #3

Whole genome CRISPR/Cas9 screen identifies established and novel genes required for ovarian cancer dissemination

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Objectives: A majority of high-grade serous ovarian cancers (HGSOC) arise from the exfoliation of transformed cells from the fimbriated end of the fallopian tube, indicating that these cells must escape anoikis to disseminate. Our aim was to identify novel genes and pathways critical for HGSOC dissemination.

Methods: We performed a CRISPR/Cas9 whole genome screen of HGSOC cells grown in adherent and suspension settings. 19,050 genes were targeted with at least 6 guide RNAs (gRNA) per gene totaling 123,411 gRNAs. We conducted principal component and differential gRNA expression analyses, as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and gene ontology (GO) analyses. We completed next generation sequencing of cells grown in adherent and suspension (RNA-seq). Known databases, including Protein Atlas, Oncomine, and The Cancer Genome Atlas, were utilized to refine gene list. We validated a subset of candidate genes, using small hairpin RNA (shRNA) knockdown and cell viability studies. Student t-test was used with threshold of < 0.05 for significance.

Results: CRISPR/Cas9 screen of cells grown in adherent compared to suspension settings identified 15,636 differentially expressed guide RNAs (gRNAs) (adj. $p<0.05$), mapping to 11,571 genes. 3,395 genes had ≥ 2 targeting gRNAs, suggesting greater significance. RNA-seq analysis of the cells grown in adherent compared to suspension setting identified 804 differentially regulated genes ($p<0.0001$).

CRISPR/Cas9 and RNA-Seq overlap analysis found 444 genes, representing a 3.2-fold enrichment. Of these genes, 108 had ≥ 2 gRNAs with similar directionality. KEGG pathway analysis of these 108 genes revealed enriched pathways including dorso ventral axis formation (NOTCH signaling), TGFbeta and calcium signaling. GO for biological processes showed enrichment for regulation of cell differentiation and positive regulation of development. In two independent ovarian cancer datasets, Oncomine analysis of the 108 genes revealed a significant enrichment in metastatic associated genes. Utilizing The Protein Atlas, 13 genes predominantly expressed in ovarian cancer were selected for further validation and elucidation of their role in anoikis. All 13 genes were significantly upregulated in suspension cells.

Cross-referencing the 108 genes with TGCA we found the expression of eight genes were significantly correlated with overall survival (Log Rank, $p<0.05$). Notably, most had not been previously correlated with survival.

Conclusions: CRISPR/Cas9 and RNA-seq uncovered numerous regulators of anoikis escape. Known pathways, including NOTCH and TGFbeta were identified, increasing confidence in our findings. We also found novel regulators of anoikis escape that correlated with overall survival and metastatic disease. This genomic-based endeavor highlights potential new markers and therapeutic targets in HGSOC dissemination.

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Poster #4

Complications and costs of minimally invasive versus open radical hysterectomy for early stage cervical cancer

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Objectives: Recent results suggest that when compared to open (ORH), a minimally invasive (MIS) approach to radical hysterectomy, results in worse overall survival. Herein, we evaluate the effects of surgical approach on perioperative outcomes including complications and costs

Methods: We performed an observational retrospective cohort analysis of 2,830 women cervical cancer recorded in the 2010-2015 Premier database (Premier, Inc.) to evaluate complications and costs. Premier is a comprehensive electronic healthcare database which provides U.S. hospital-based, service-level, all-payer data, in the form of ICD-9 procedure and diagnosis codes and billing data. Premier was used to compare complications, length of stay (LOS), readmission, and costs between surgery types among propensity score matched patients. All p values were 2 sided.

Results: From Premier, 2,830 women had radical hysterectomy: 45.1% ORH and 54.9% MIS. After propensity score matching, 2,433 patients were selected: 51.2% ORH and 48.8% MIS. ORH was associated with longer LOS than RRH or LRH (days, median (IQR): ORH 3 (3-5); MIS 1 (0-2), $P < 0.001$). ORH also had a higher composite complication rate than RRH or LRH (ORH 52.9%; MIS 25.5%, $P < 0.001$), with increased bowel injuries, urinary tract injuries, vascular injuries, respiratory failure, minor infections, electrolyte or fluid disorders, and ileus (all $P < 0.05$) associated with ORH. Thirty-day readmission rates were similar (ORH 2.4%; MIS 1.3%, $P = 0.078$). Total surgical hospitalization costs favored MIS ($P < 0.001$) with median (IQR) values: ORH $11,545 (8,622-15,461); MIS $10,636 (8,023-13,640). Additionally, ORH was associated with additional operations during the surgical admission (ORH: 20.9%; MIS 7.8%, $P<0.001$) with extra bowel and urinary tract surgeries (all $P < 0.001$) associated with ORH.

Conclusions: MIS hysterectomy is associated with decreased morbidity and costs compared to open radical hysterectomy. Though MIS radical hysterectomy is associated with decreased survival, it may still be a reasonable choice for some women who are deemed lower risk. Patients and physicians should consider morbidity when deciding on surgical approach.

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Poster #5

Assessment of the false negative rate of preoperative imaging in cervical cancer patients undergoing primary radical surgery

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