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14. ABSTRACT

High grade serous ovarian cancer (HGSOC) accounts for >75% of ovarian cancers and is the most lethal gynecological malignancy. Unlike other cancer types, HGSOC has not benefited from advances in cancer immunotherapy despite high levels of tumor-infiltrating lymphocytes (TIL) in some cases. This necessitates a deeper investigation into the recognition of tumor cells by host immunity and causal factors of immune cell avoidance, suppression and evasion. HGSOC presents with widespread disease at diagnosis and within-patient variation in immune microenvironments.

HGSOC is an archetype tumor of genomic instability, with profound copy number alterations and genomic rearrangements on a genetic background of near ubiquitous mutation in *TP53* rendered bi-allelic through loss of heterozygosity of 17p. Somatic and germline alterations in the homologous recombination repair pathway such as *BRCA1* and *BRCA2* mutations, lead to homologous recombination deficiency (HRD) in approximately half of HGSOCs. Distinct patient strata are associated with endogenous mutational processes inferred from structural variation patterns in whole genome sequencing: HRD subtypes (*BRCA1*-associated tandem duplications: HRD-Dup; *BRCA2*-associated interstitial deletions: HRD-Del), foldback-inversion (FBI) bearing tumors and *CDK12*-associated tandem duplicators (TD), amongst related mutational processes defined by copy number alteration and other recently described structural variation patterns. Notably, the mutational processes are associated with different clinical outcomes, with FBI and TD tumors exhibiting the worst prognoses. The malignant-immune interface is also influenced by these endogenous mutational processes. Specifically, FBI tumors display reduced immune infiltration, in contrast to HRD-Dup and HRD-Del groups which harbor increased levels of cytotoxic T cells. Our key hypotheses indicate that both anatomic site and mutational process have influence over the constituent immune cell phenotypes comprising the tumor microenvironment (TME). Our goal is therefore to comprehensively map the 3D spatial architectures of HGSOC TMEs, and identify the tissue architecture determinants of immune evasion and avoidance.

15. SUBJECT TERMS

High grade serous ovarian cancer, mutational signatures, copy number variants, single cell genome, single cell transcriptome, drug resistance, tumor evolution, tumor microenvironment, spatial modeling

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1. INTRODUCTION:

Based on preliminary observations, advances in technology and newly established mouse models we will test the following hypotheses: i) Anatomical sites within patients exhibit cellular architectural differences which lead to immunosuppressed adnexal sites relative to intraperitoneal sites such as omental lesions (Aim 1); ii) FBI tumors elicit structural features in cellular architectures resulting in immune avoidance through physical stromal repatterning (Aim 1&2); iii) HRD but not FBI tumors elicit early immune infiltration leading to PD-L1 expression and consequent differentiation of T-cells into a dysfunctional state (Aim 2); iv) therapeutically resistant tumors exhibit a repatterning of TMEs, leading to increase in immunosuppressive phenotypes (Aim 3). We will use the unique patient tissue repository, novel model systems and advances in 3D in situ spatial molecular profiling technology to address these hypotheses.

2. KEYWORDS:

High grade serous ovarian cancer, mutational signatures, copy number variants, single cell genome, single cell transcriptome, drug resistance, tumor evolution, tumor microenvironment, spatial modeling

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1: Contrasting 3D topological maps of HGSOC patient tissue immune microenvironments in primary and intraperitoneal sites

Major Task 1 Tissue collection and sample selection	**Timeline**	**Site**	**Milestones achieved**
Subtask 1 – We will collect adnexal and omental masses from 10 HRD-Dup and 10 FBI patients	1-24	MSKCC	Protocol approved by MSK-IRB, we collected multi-site tumor samples from 4 patients
Milestone(s) Achieved: Given our experience with prior collection protocols of >90 patients in two years we expect to have the samples collected in Q2 of year two.	By 24 months		
Major Task 2 Spatial imaging of matched adnexal and omental lesions			
Subtask 1 - For each sample we will collect matched FFPE and frozen samples	1-24	MSKCC	We collected matched FFPE and frozen samples from 4 patients
Subtask 2 - We will perform STPT on 40 tumor samples (10 patients HRD-Dup and 10 samples FBI, 2 sites each)	3-27	CAM	Optimization of STPT embedding protocol for frozen tissue still ongoing – expected on track by end of Y2
Subtasks 3 - We will obtain scRNA-seq and (where it exists) whole-genome sequencing data on tumor samples from 20 patients	6-24	MSKCC	We have obtained scRNAseq and scWGS of 4 samples from 2 patients
Subtask 4 - We will perform CITE-seq on 6 samples to register merFISH and IMC data	12-24	MSKCC	
Subtasks 5 - We will develop merFISH and IMC panels on the basis of scRNAseq and whole genome sequencing data	6-9	CAM	~150 antibodies validated on multiple human tissues incl. ovarian tumors. Several panels developed including one focused on immune markers. Initial selection of clone specific and cell-type specific genes for merFISH. MerFISH

			protocol improved for human tumor tissue.
Subtask 6 - We will perform merFISH with a 250-gene panel and IMC with a 35-40 antibody panel on 5 sections (each) for each sample	9-30	CAM	4 samples from 2 patients received, 2 processed for IMC
Milestone(s) Achieved: spatial model of tumor histology augmented with gene and protein expression	By 30 months		
Major Task 3 Data analysis			
Subtask 1 - We will perform Image alignment, co-registration and segmentation	6-30	CAM	Processing performed for all analyzed samples. Segmentation pipeline optimized comparing two different algorithms
Subtask 2 - Cell phenotype identification to identify cell types and phenotypic states		MSKCC	
Subtask 3 - Geospatial statistical 3D models		MSKCC	
Milestone(s) Achieved: Quantitative analysis of 3D tumor models, including phenotypic characterization of constituent cell types	By 30 months		

Aim 2: Temporal repatterning of tissue architectures as a function of mutational process			
Major Task 1 Generate EPO-GEMMs representing each of the four mutational subtypes			
Subtask 1: We will generate HDR-Del tumor models by BRCA2 KO	14/24	MSKCC	
Subtask 2: we will generate FBI tumor models by overexpressing cyclin E	14/24	MSKCC	
Subtask 3: we will generate TD tumor by CDK12 KO	14/24	MSKCC	
Subtask 4: we will generate scRNAseq and CITE-seq data for the three models (3 replicate animals/model)	14/24	MSKCC	
Subtask 5 - We will perform scRNA-seq analysis with CellAssign to identify cell types and phenotypic states	14/24	MSKCC	
Milestone(s) Achieved: Mouse models for the three HGSOC subtypes and single-cell data describing them (including the HRD-Dup model we already created)			ongoing

Major Task 2: Spatial analysis of TME structure on time series of EPO-GEMM tumors			
Subtask 1: We will generate a time course dataset for the HRD-DUP, FDI and TD models (60 animals in total, 4 time points, 3 models, 5 replicate animals/time point)	12-16	MSKCC	
Subtask 2: We will perform STPT analysis on the samples above and quantify stromal abundance / profile through our pipeline	16-26	CAM	
Subtask 3: We will develop merFISH and IMC panels for EPO-GEMM models based on single-cell data	16-18	CAM	
Subtask 4: We will perform merFISH with a 250-gene panel and IMC with a 35-40 antibody panel on 1-2 sections for each sample (or possibly more if time/budget allows)	18-30	CAM	
Subtask 5 - We will perform Image alignment, co-registration and segmentation	18-30	CAM	
Subtask 3 - We will compare the spatial/molecular features of tumours at different time points and of different mutational profiles using geospatial models	20-30	MSKCC	
Milestone(s) Achieved: Understanding of the relationship between tumour progression, tumour mutational origin, and TME remodeling/structure in EPO-GEMM HGSOC tumors.			
Aim 3: Establish changes in tissue architecture after drug perturbation in mouse and patient tumors			
Major Task 1 Pre and post- treatment comparisons from patient analyses.			
Subtask 1 We will collect pre-treatment biopsies from multisite laparoscopic procedures in 10 HRD and 10 FBI patients	10-12	MSKCC	
Subtask 2 We will collect samples from the same patients undergoing interval debulking surgery after neoadjuvant chemotherapy	10-24	MSKCC	
Subtask 3 We will perform STPT, IMC and merFISH to delineate the impact of chemotherapy on tumor architecture on all (n=40) specimens	24-36	CAM	
Milestone(s) Achieved: Matched pre- and post treatment samples from 20 patients representing the four HGSOC subtypes			

Major Task 2 EPO-GEMM comparisons of BRCA1 and CCNE1 models			
Subtask 1 We will treat the mice representing HRD-Dup (BRCA1 mutant) and FBI (CCNE1 overexpressing) with vehicle, cisplatin or cisplatin + immunotherapy	10-24	MSKCC	
Subtask 2 We will analyze 5 replicates per condition (2 models under 3 treatments) with STPT/IMC	20-36	CAM	
Milestone(s) Achieved: 3D tumor models that recapitulate subtype specific drug resistance mechanisms			

Aim 1: Contrasting 3D topological maps of HGSOC patient tissue immune microenvironments in primary and intraperitoneal sites

MSKCC Site

We successfully obtained approval for the protocol that describes this work with human and animal samples by the MSK internal review board (IRB) and Institutional animal care and use committee (IACUC) respectively. In the past year we have collected 13 frozen tissue samples from 8 patients as well as 77 single cell suspensions from 33 total sites across 7 patients.

Our previous work has demonstrated that mutational signatures, determined from genomic sequences, stratify patients into four clinically relevant categories: homologous recombination deficient (HRD) subtypes, characterized by BRCA1 mutation linked duplications (HRD-Dup) and BRCA2- variant linked deletions (HRD-Del), both of which are associated with a better prognosis. In contrast, homologous recombination competent groups, characterized by foldback inversion (FBI) and tandem duplications (TD), show worse outcome (*Funnell et al Nature 2022*). In further studies on pre-treatment HGSOC samples we saw that different mutational signatures had different mechanisms of immune escape. HRD-Dup cases adnexal tumors had a high prevalence of clonal loss of heterozygosity (LOH) on chromosome 6p, harboring HLA class I and II genes, pointing to early immune selection in the primary site. The functional consequences of the 6pLOH in HRD-Dup included upregulation of JAK-STAT signaling, which was most pronounced in bowel metastases (*Vasquez Garcia et al. Nature 2022*). Bowel lesions are of particular clinical interest, since malignant bowel obstructions are the most severe complication in HGSOC. Additionally, the presence of bowel metastases in HGSOC can prevent surgical complete gross resection, leading to poor outcomes. We therefore decided to prioritize the collection of bowel samples with our clinical collaborators. Our technician processed the surgical samples straight from the OR for single cell RNA (scRNA) and DNA in a same-day workflow.

During the past year, we have focused on the multi-modal analysis of 4 samples from 2 patients, constituting primary tumor and one metastatic lesion each, either from the omentum (patient OV-36, HRD-Del) or the small bowel (patient OV-82, HRD-Del). H&E staining identified the tumor regions in the histological sections (**Fig. 1**), with clearly delineated primary tumors (**Fig. 1a and c**) and metastases in the small bowel (**Fig. 1b**) and omentum (**Fig. 1d**). We performed scRNA sequencing on dissociated and flow sorted CD45+ and CD45– cells. The CD45– fraction consisted mainly of tumor cells, with a small fraction of fibroblasts and endothelial cells in one HRD-Del patient (OV-36), and a larger fraction of fibroblasts in the other HRD-Del patient (OV-82), particularly in the primary tumor site. The CD45+ immune fraction displayed an intriguing difference in cell type composition between the two patients: in one case the vast majority of cells were T cells, with only very few myeloid and plasma cells, whereas the other was mainly made up of myeloid cells, with only a small lymphoid fraction (**Fig. 2**). Multiplexed immuno-fluorescence (mpIF) with markers for tumor cells (panCK), cytotoxic T cells (CD8+)

and macrophages (CD68+) showed the cell type distributions between tumor and the tumor microenvironment (TME) in the tumor and adjacent stroma regions (**Fig. 3**). This spatial profiling of the TME showed a greater immune infiltration in the tumor and adjacent stroma in the bowel metastatic region of patient 082 compared to the adnexa (Fig. 3, right panel). Comparing the two patients we observed a higher % of immune cells in the metastatic samples from patient 082 as compared to the adnexa. Similarly, the stroma of patient 082 patient showed a higher prevalence of immune cells compared to the stroma of patient 036, in both the primary and secondary sites. We are in the process of collecting samples from HRD-Dup and FBI patients to repeat this analysis.

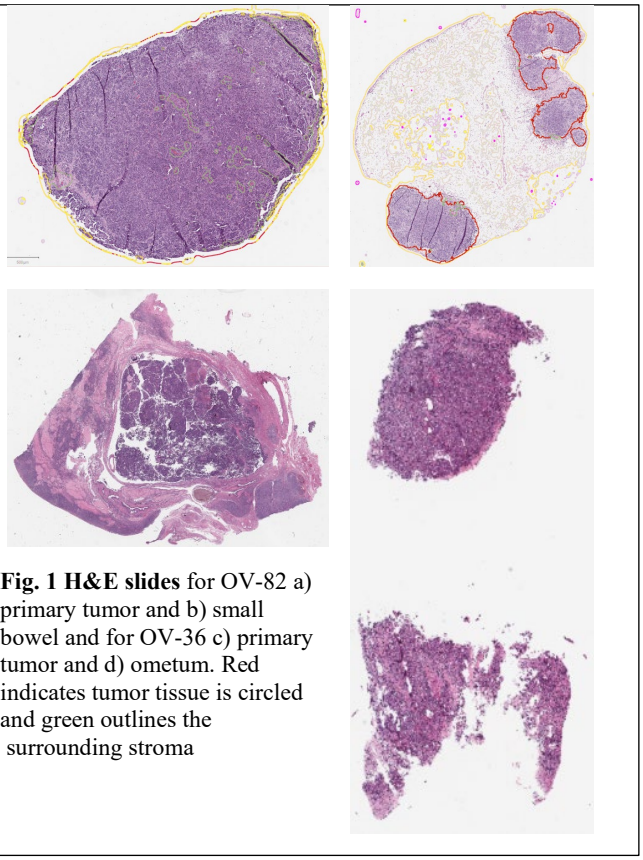

Fig. 1 H&E slides for OV-82 a) primary tumor and b) small bowel and for OV-36 c) primary tumor and d) ometum. Red indicates tumor tissue is circled and green outlines the surrounding stroma

We performed single cell whole genome sequencing (scWGS) using our Direct Library Preparation (DLP+) platform on the metastatic tumor from the omentum in OV-082 and on the tumor from the left ovary and right adnexa in OV-036. Our analysis pipeline determined copy number alterations (CNA) across the entire genome in each cell and reconstructed phylogenetic trees from this CNA matrix. In an effort to link transcriptional responses to clone-specific copy number alterations we had developed `TreeAlign` (*Shi et al. Nat. Communications 2023 in press*). It combines the single cell expression count matrix with the scWGS-derived phylogenetic tree and copy number count matrix to obtain the clone assignment for each cell. It then derives the probability of CN-dependent gene expression, which allows for the accurate prediction of dosage effects in highly expressed genes. Applying `TreeAlign` to the scWGS and scRNA samples from our two patients we observed that clade-specific copy number events are reflected in the site-matched scRNA data (**Fig. 4**). Based on these results we are confident that merFISH probes designed for genes in these amplified regions, will allow us to identify clade-specific CNAs from merFISH data.

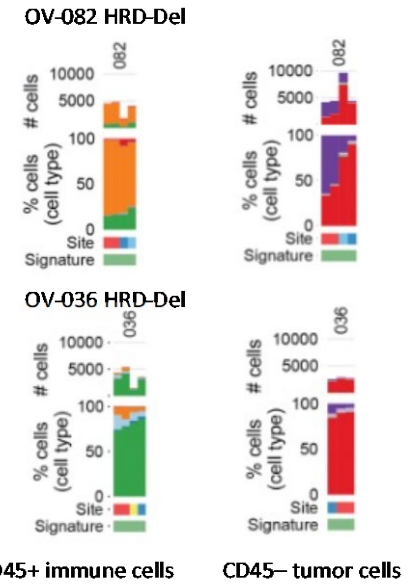

Fig. 2 scRNA analysis CD45+ cells: T cells (green), plasma cells (blue), myeloid cells (orange); CD45– cells: tumor cells (red), fibroblasts (purple). Tumor sites are adnexa (red), bowel (light blue), omentum (dark blue) and peritoneum (yellow). Signatures are HRD-Del (light green).

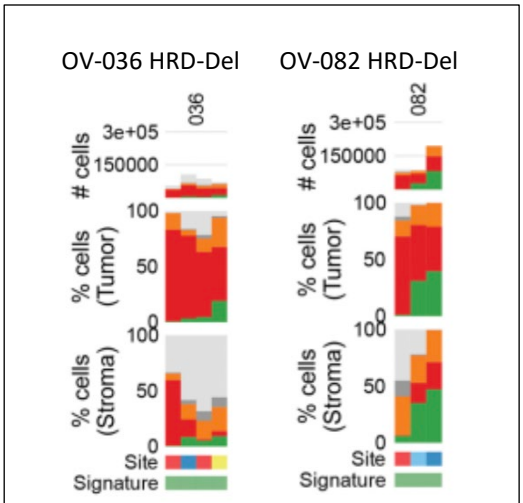

Fig. 3 mpIF analysis Fraction of panCK tumor cells (red), CD8+ T cells (green) and macrophages (orange) were determined from mpIF for tumor and adjacent stroma from different sites in the two patients: adnexa (red), ascites (orange), omentum (dark blue) peritoneum (yellow), bowel (light blue)

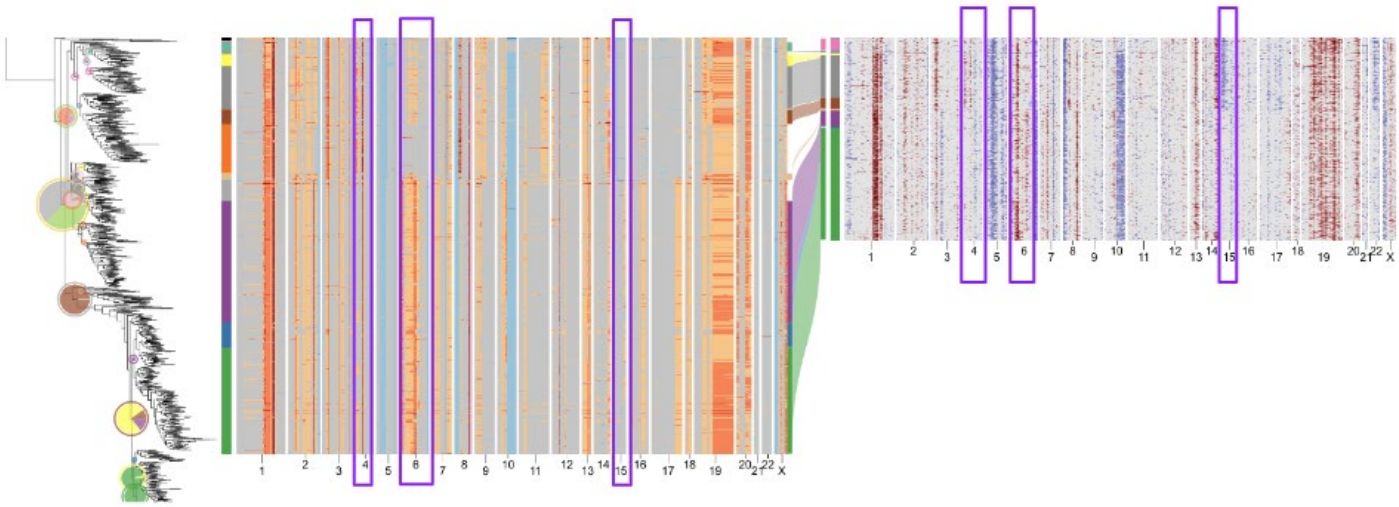

Fig. 4 Inferring clone assignment with `TreeAlign` Phylogenetic tree constructed with scDNA-data along with pie charts showing how TreeAlign assigns cell expression profiles to subtrees recursively. The pie charts are colored by the proportions of cell expression profiles assigned to downstream subtrees. The outer ring color of the pie charts indicates the current subtree. Heat maps of copy number profiles from scDNA (left) and InferCNV normalized expression profiles from scRNA (right). The sankey chart in the middle shows clone assignment from expression profiles to copy number based clones by `TreeAlign`. The chromosomes highlighted in purple show that the same CN events are also recovered in the scRNA data.

CAMBRIDGE SITE

In our proposal, we described the use of two parallel spatial profiling methods (Imaging Mass Cytometry and merFISH) to characterize the tumor micro-environment of different types of HGSOC lesions (primary and/or metastatic). Over the last year, we have taken significant steps to optimize and develop panels for both these technologies, and begun to apply them to patient samples.

Imaging Mass Cytometry is a multiplexed immunohistochemistry technique, and as such is critically dependent on the quality, efficiency and specificity of antibody staining. As a first step in our project, therefore, we established an antibody validation pipeline based on a set of best practices derived from both the literature and the advice of a leading antibody supplier with whom we are collaborating (Abcam). In particular, our pipeline is composed off the following steps (**Fig. 5**): 1) Target antigens are chosen based on curated lists and/or analysis of existing data, 2) Several commercially available antibodies are identified and purchased for each antigen, and they are conjugated to metal polymers 3) Antibodies are combined in sub-pools of 10 antibodies each, and tested in 5 different dilutions on a tissue micro-array (TMA) composed by 43 different normal and tumour human tissue samples. 4) The stained TMAs are acquired by IMC, and the staining pattern of each core is evaluated and matched against known positive and negative control tissues. Importantly, the tissues used in the validation TMA are the same used for validation by the original supplier, which enables a comparison between the staining patterns of the native vs. IMC-conjugated antibodies, 5) the antibodies that passed validation are combined in larger panels at their final dilution, and re-tested to ensure that they work well in combination.

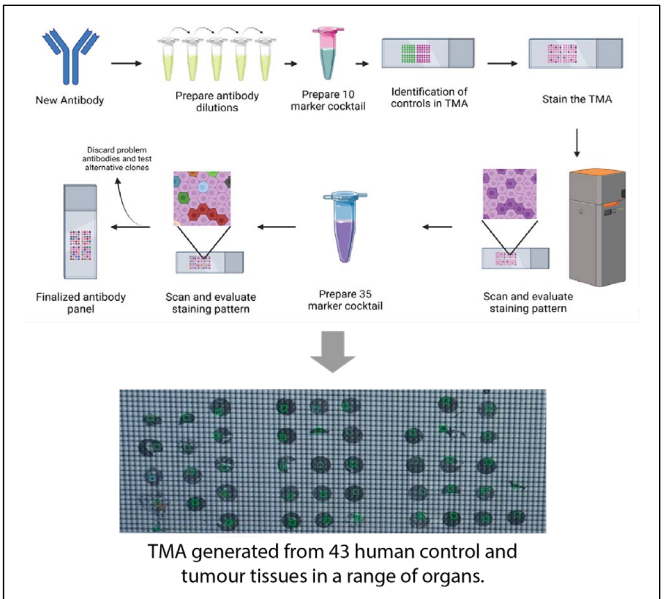

Fig 5: Outline of the antibody validation pipeline

Using this pipeline, we have developed and validated 4 panels of ~35 antibodies each as a shared effort between this project and another project ongoing in the Hannon/IMAXT laboratory focused on triple-negative breast

Metal	Antibody	Supplier	Clone	Concentration (ug/mL)	Dilution (1:X)
142Nd	CD19	Abcam	SP291	2.5	200
176Yb	CD3e	Abcam	EP449E	2.5	200
156Gd	CD4	Abcam	EPR6855	2.5	200
162Dy	CD8a	Abcam	CAL66	2.5	200
141Pr	CD11c	Abcam	EP1347Y	5	100
144Nd	CD14	Abcam	EPR3653	2.5	200
171Yb	CD68	Abcam	EPR20545	2.5	200
163Dy	CD56	Abcam	EP2567Y	2.5	200
146Nd	CD16	Abcam	EPR16784	2.5	200
145Nd	LYVE1	Abcam	RM1008	2.5	200
170Er	CD138	Abcam	SP152	1.25	400
151Eu	CD31	Abcam	EPR3094	2.5	200
147Sm	C1q or APO-E	Abcam	EP1374Y	2.5	200
148Nd	PDPN	Abcam	EPR22182	5	100
169Tm	PD-1	CST	D4W2J	5	100
150Nd	PD-L1	Abcam	E13N	10	50
149Sm	TIM3	Abcam	EPR22241	2.5	200
152Sm	TIM1	R&D systems	219211	2.5	200
165Ho	LAG3	Abcam	EPR20261	10	50
153Eu	CD28	Abcam	EPR22076	5	100
155Gd	FoxP3	Abcam	236A/E7	2.5	200
154Sm	Pan-CK	Abcam	C-11	5	100
158Gd	CCR7	Abcam	EPR23192-57	2.5	200
174Yb	MHCII/HLA-DR	Abcam	EPR11227	2.5	200
168Er	Ki67	SBT	B56	1.25	400
159Tb	Granzyme B	Abcam	EPR8260	2.5	200
160Gd	IFNg	Novus	IFNG/466	5	100
161Dy	CXCL10	Abcam	EPR24674-84	10	50
167Er	IgG	Abcam	EPR4421	1	500
164Dy	IgA	Abcam	EPR5367-76	1.25	400
166Er	IgM	Abcam	EPR5539-65-4	1.25	400
172Yb	Tyrosine hydroxylase	Abcam	EP1532Y	2.5	200
175Lu	B-catenin	Fluidigm	D13A1	2.5	200

Fig.6: One of our IMC antibody panels (Panel 3 – immune focused)

cancer. The panels are focused on general cell type identification (panels 1 and 2), identification of immune cell subtypes, activation and exhaustion, immune structures and immune interactions (panel 3), and cell intrinsic drug response and resistance mechanisms (panel 4). Antibodies from each panel can be mixed and matched to produce new "focused" panels if necessary. Given the importance of immune interactions to differentiate HGSOC of different mutational origin, we decided to focus on Panel 3 for the initial sample acquisitions. The panel composition, as well as representative validation images, are show in **Fig. 6-7**.

We received four samples from MSKCC, corresponding to the two HRD-Del patients described above (OV-82 and OV-36) and to their primary and metastatic sites. So far, we have performed staining on both samples from OV-36 using IMC Panel 3. While analysis is still in progress, preliminary results (**Fig. 8**) indicate a strong infiltration of CD8+ lymphocytes, in agreement with what was detected by single-cell RNA sequencing and mpIF analysis (**Fig 2** and **3**). In addition to this, we could map the position of macrophages (CD68+) and proliferating cells (Ki67+).

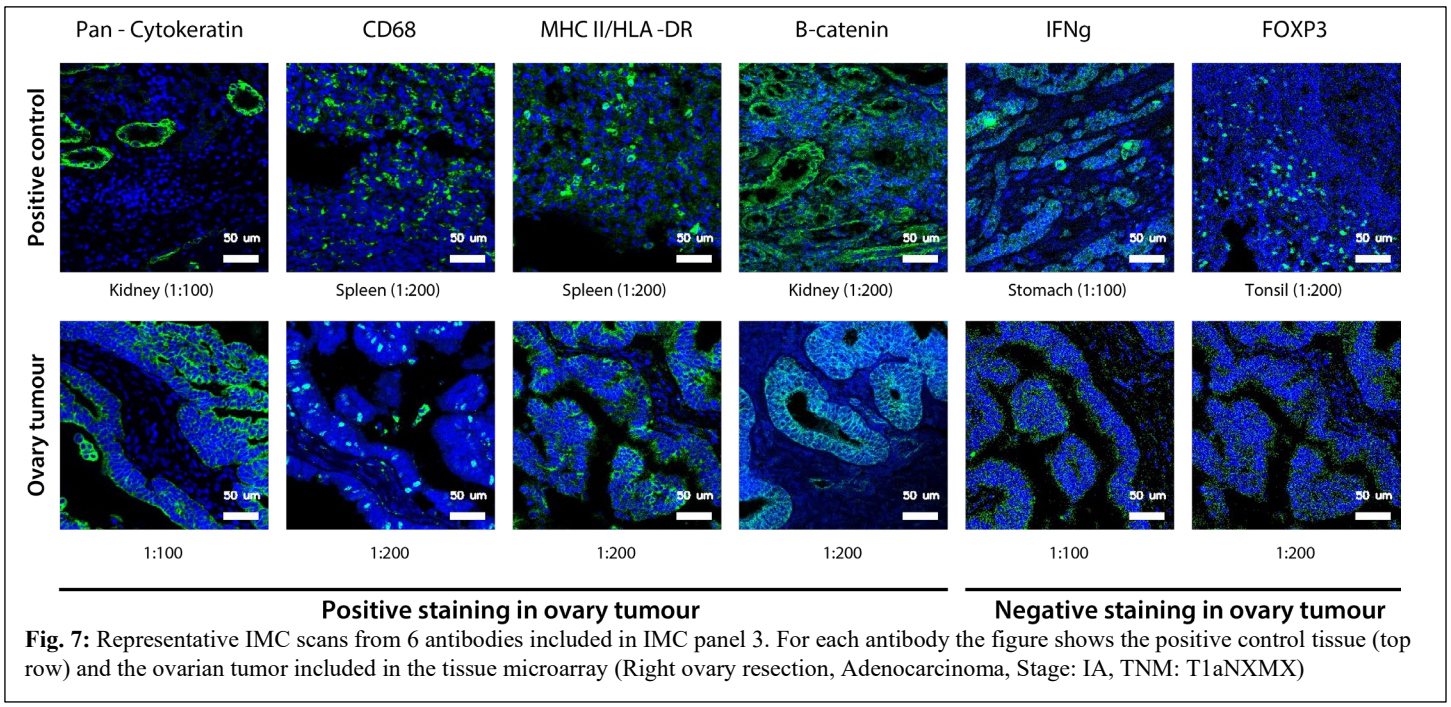

Fig. 7: Representative IMC scans from 6 antibodies included in IMC panel 3. For each antibody the figure shows the positive control tissue (top row) and the ovarian tumor included in the tissue microarray (Right ovary resection, Adenocarcinoma, Stage: IA, TNM: T1aNXMX)

For merFISH, our work this year focused on improving the protocol for the processing of dense tumor tissue as well as metastatic lesions. This type of tissue presented significant challenges in terms of both RNA stability, cell segmentation, and probe penetration. Using mouse models, we optimized several components of the merFISH protocol, including buffer compositions, oxygen scavenger system, readout probe concentration, flow order and volumes and exposure times. While a shorter protocol provided superior RNA detection for some samples, most sections provided higher quality data using an optimized longer protocol. In addition to this, we also developed custom training models for cell segmentation (using the UNET-based *cellpose 2* algorithm), leading to a much improved detection of cells. Finally, we tested a new spot caller (Savannah) developed by the laboratory of Andrew Roth at the British Columbia Cancer Centre in Vancouver in the context of the IMAXT project (personal

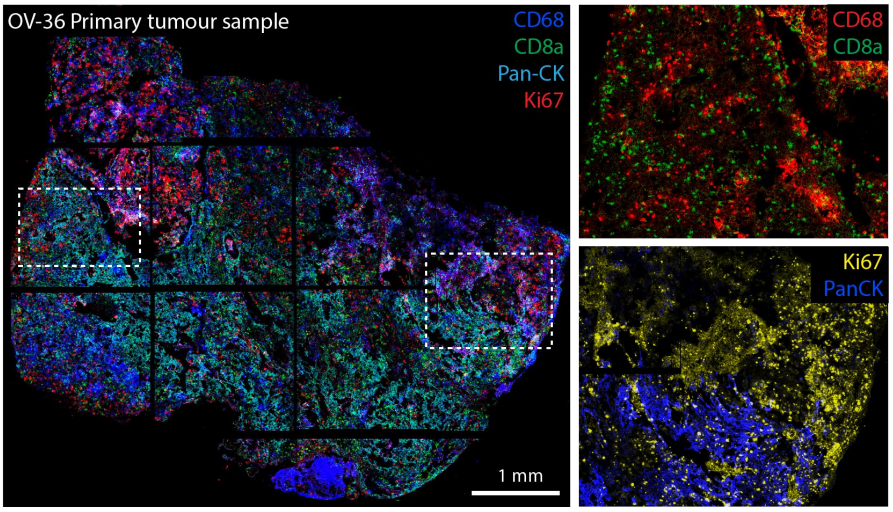

Fig. 8: OV-36 primary tumour sample analysed by IMC using Panel 3. The dotted lines indicate the position of the insets on the right. It is possible to identify a substantial amount of immune infiltrate (predominantly CD8+ T cells), as well as localized areas of high proliferation within the tumour (Ki67 signal)

communication, manuscript in preparation). Savannah enabled a significantly RNA profiling depth, in particular in terms of the average number of genes detected per cell, compared to the traditional merFISH processing algorithm (*merlin*). The results of these optimization are reported in **Fig. 9**.

In addition to spatial molecular profiling by IMC and merFISH, we proposed to perform whole-organ imaging by Serial Two Photon tomography. We encountered a challenge here as this technology is normally performed on tissue that have been fixed in formalin overnight, while the samples we received from MSKCC are predominantly frozen in OCT resin. For this reason, we initially focused on optimizing a protocol to thaw and fix OCT samples without damaging their histology. We did this using animal samples. In parallel, we worked on developing methods to stain human STPT samples using an analogue of the H&E stain. Since there are no endogenous fluorophores in human tissue, this was required to produce sufficient contrast to discriminate anatomy. Currently, our preferred method for this is performing the staining on the histological sections produced by STPT, re-imaging them on a slide scanner and using our co-registration pipeline to produce a coherent 3D dataset. However, we are in the process of testing a method for whole-mount DAPI&Eosin stain which will allow direct 3D imaging.

Finally, we are about to test a new method for tissue clearing enabling whole-mount immunostaining with native antibodies called wildDISCO, which will allow us to use a small number of markers directly in STPT to better elucidate tissue morphology.

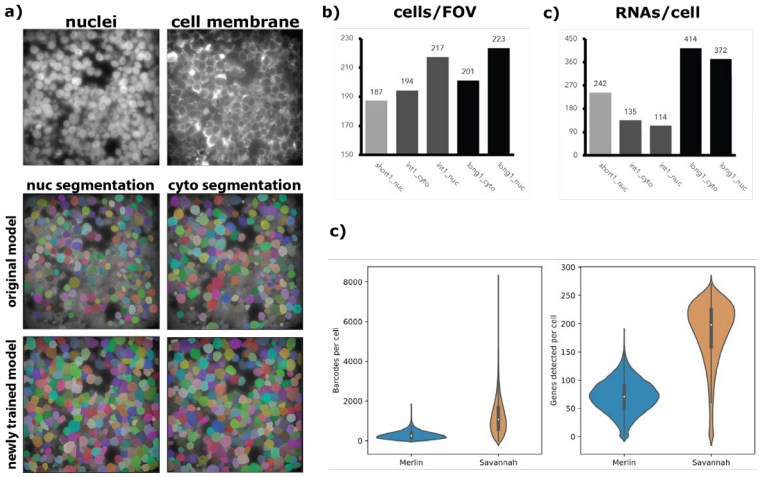

Fig. 9: Improvements to merFISH protocol. A) Comparison of the conventional cellpose training model for nuclei and cytoplasm segmentation (top row) vs. the newly developed training models (bottom row). B) Cell segmentation and RNA detection/assignment metrics for different variations of the merFISH staining protocol. C) An improved spot caller based on probabilistic models, developed by the laboratory of A. Roth at the British Columbia Cancer Centre in the context of the IMAXT projext, provides increased RNA and gene detection compared to traditional merFISH processing (merlin software)

What opportunities for training and professional development has the project provided?

Dr. Shah holds weekly meetings with all the participants in this grant including the technician and the trainees. The graduate student, Hongyu Shi, who developed `TreeAlign` and carried out the experiments mapping scRNA to scWGS-derived clones, successfully graduated in October 2023. She will be replaced in this project by post doctoral fellow Dr. Marc Williams, who already participated in the analysis.

The Hannon laboratory recruited a full-time technician (Mi Chween Chan) to work on IMC panel design, staining and analysis. Ms Chan received extensive training and is now proficient with these techniques, which she plans to further apply in the future as she is interested in pursuing a PhD in cancer biology. The technician

tasked with merFISH optimization (Nicole Hemmer) has participated in several meetings presenting the results of the optimization, and will progress to graduate school next year.

How were the results disseminated to communities of interest?

Dr. Shah and Dr. Hannon regularly meet with each other and with the respective teams. Dr. Shah is an active participant in and organizer of scientific meetings (see below for details). All software used in this work is made available on GitHub.

What do you plan to do during the next reporting period to accomplish the goals?
To advance Aim 1 we will continue to collect samples, with a particular focus on patients with an FBI signature, and proceed with the multi-modal analysis described above. We will obtain IMC and merFISH data for all our samples and be able to reconstruct topological maps for each patient. These data will enable us to develop a framework to associate topological features across covariates of adnexal vs bowel lesions and different mutational processes.
For Aim 2, **Temporal repatterning of tissue architectures as a function of mutational process**, we will continue to collaborate with the team of Dr. Scott Lowe to generate mouse models that represent the four different mutational subtypes.
To ensure progress for Aim 3 **Establish changes in tissue architecture after drug perturbation in mouse and patient tumors** we will continue to collect samples pre-treatment and post NACT and analyze them with scRNA and scWGS as well as IMC and merFISH to compare tumor and TME architecture before and after therapy.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
We have established a sample processing pipeline together with experimental and analytic tools that will allow us to first profile RNA and DNA from single cells, the basic unit of disease, and then put them in the larger context of tissue architecture. We will be able to determine how tumors at primary and distant sites are shaped by tumor intrinsic factors, such as mutational signatures, and extrinsic factors, such as tumor-immune cell interactions and tumor site. We anticipate that a detailed spatial understanding of primary and metastatic lesions, particularly in the bowel, will provide much needed insight in the main players in disease progression and yield potential candidates for intervention. We will also uncover how tumor architecture is impacted by treatment and how immune evasion mechanisms shed light on the therapeutic response in patients. We will explore whether we can find biomarkers that are induced in tumor cells during invasion of the colon or markers that predispose primary tumors to invade the colon and whether there may be targets for early intervention to prevent this lethal complication. Our findings will be valuable to basic cancer research, beyond ovarian cancer, as they establish a way to elucidate the link between etiologic mutational processes to therapeutically relevant immune escape and suppression.

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
HGSOC is the most lethal gynecologic cancer and patients with bowel metastasis fare especially poorly. Our work will extend the concept of ‘genome as biomarker’ and integrate molecular, cellular and tissue features to better identify high risk patients, understand disease progression and identify markers that can aid in targeted disease management.

5. CHANGES/PROBLEMS:

Changes and reasons for change
Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
The graduate student who created the EPO-GEMM mouse models for Aim 2 has left MSK, leading to slight delays but we are in the process of finding a solution to resume the work.

STPT acquisition of the first batch of samples has been delayed by the need to develop methods to thaw, fix and embed frozen samples in gel block compatible with the technique, as well as methods to produce contrast in the samples allowing the discrimination of cellular features. We have a viable solution for this now, and we expect to get back on track early in year 2.

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

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use or care of vertebrate animals
Nothing to report

Significant changes in use of biohazards and/or select agents
N/A

6. PRODUCTS

Publications, conference papers and presentations
Dr. Shah's invited presentations
1) "Co-evolution of ovarian cancer genomes and the tumor microenvironment", Mayo Clinic's Gynecologic Cancer Seminar Series (virtual), December 2022
2) "Towards quantifying evolvability in cancer with single cell approaches", Weill Cornell Medicine 2023 ICB Seminar Series, New York, NY, January 2023
3) "What are the determinants of evolvability in cancer?", The Anderson Center for Cancer Research Lecture Series, New York, NY, February 2023
4) "Toward Multimodal Data Integration", 2023 National Academies workshop on Incorporating Integrated Diagnostics into Precision Oncology Care, Washington, DC, March 2023
5) "Measuring evolvability in cancer through single cell approaches", Human Technopole, Milan, Italy, May 2023
6) "Tumor evolution and immune evasion in ovarian cancer", Ohio State University (Virtual), May 2023
7) "Genotype-phenotype evolution in cancer studied with single cell approaches", IMAXT Legacy Meeting, Titignano, Italy, May 2023
8) "Genotype-phenotype evolution in cancer studied with single cell approaches", Banff Workshop - Mathematical methods in cancer biology, evolution and therapy, Banff, Canada, May 2023
9) "Biostatistics and Computational Genomics Core" (with Ronglai Shen), MSK Breast Cancer SPORE in Genomic Instability: Annual Review, New York, NY, June 2023
10) Welcome and Keynote Address, Keystone Symposium Single Cell Biology: From Development to Cancer, Keystone, CO, June 2023

Websites or internet sites
Data will be publicly disseminated upon publication of the results.

Technologies or techniques
Our new tool `TreeAlign` can be found at Github https://github.com/AlexHelloWorld/clonealign_pyro and is in press in Nature Communications. (*Shi et al. Nat. Comms. 2023*)

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

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Dr. Sohrab Shah
Project Role:	Principal Investigator
Researcher Identifier (ORCID):	0000-0001-6402-523X
Nearest person month worked:	0.24
Contribution to Project:	Dr. Shah is leading the overall program and is mentoring the trainees focusing on Aims 1 and 3.
Name:	Dr. Britta Weigelt
Project Role:	Co-I
Researcher Identifier (ORCID):	
Nearest person month worked:	0.24
Contribution to Project:	Dr. Weigelt is a member of the Gynecology DMT and assisted with coordinating sample processing.
Name:	Hongyu Shi
Project Role:	Graduate Student
Researcher Identifier (ORCID):	0000-0002-8541-6261
Nearest person month worked:	12
Contribution to Project:	Hongyu Shi led the analysis of scRNA-seq data generated for Aim 1. She developed TreeAlign as part of her PhD thesis.
Funding Support:	
Name:	Dr. Dmitriy Zamarin
Project Role:	Co-I
Researcher Identifier (ORCID):	
Nearest person month worked:	0.24
Contribution to Project:	Oncologist who helped to identify suitable cases for the project and contributed to data interpretation.
Name:	Scott Lowe
Project Role:	Co-I
Researcher Identifier (ORCID):	
Nearest person month worked:	0.24
Contribution to Project:	Dr. Lowe supervises the generation of transgenic HGSOC mouse models for Aim 2.
Name:	Emily Ali
Project Role:	Research Technician
Researcher Identifier (ORCID):	
Nearest person month worked:	2.4
Contribution to Project:	Ms Ali is a research technician receiving samples from the surgeon and processing them under the supervision of Dr. Weigelt.

Name:	Greg Hannon
Project Role:	Principal Investigator
Researcher Identifier (ORCID):	
Nearest person month worked:	0.6
Contribution to Project:	Prof. Hannon will oversee all the research proposed in this application for the Cambridge site.
Name:	Dario Bressan
Project Role:	Co-Investigator
Researcher Identifier (ORCID):	
Nearest person month worked:	4
Contribution to Project:	He will oversee all the spatial mapping components of this project, perform experiments (in particular focusing on IMC and merFISH) and assist in data analysis.
Name:	Mi Chween Chan
Project Role:	Research assistant
Researcher Identifier (ORCID):	
Nearest person month worked:	3.15
Contribution to Project:	Tasked with running STPT, IMC and merFISH runs, and preparing associate reagents.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Dr. Zamarin has left MSK in October 2023 and will be replaced in year 2 of this grant by oncology surgeon Dr. Kara Long-Roche.

What other organizations were involved as partners?
This proposal is a collaborative award with PIs Dr. Sohrab Shah at MSK and Dr. Greg Hannon at the University of Cambridge.

8. SPECIAL REPORTING REQUIREMENTS

9. APPENDICES
N/A