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14. ABSTRACT

High grade serous ovarian cancer (HGSOC) is the most lethal gynecological malignancy and while treatment with PARP inhibitors has shown some promise for patients with BRCA1/2 mutations these mutations remain an imperfect predictor for response. Our team has established structural variant-associated mutational processes for patient risk stratification: homologous recombination deficient (HRD) tumors, either associated with BRCA1 mutation-linked duplications (HRD-Dup) or BRCA2 mutation-linked interstitial deletions (HRD-Del), have a better prognosis than homologous recombination proficient tumors, including CDK-12 associated tandem duplications (TD) and foldback inversion (FBI) bearing tumors. Our hypothesis is that these **distinct mutational processes confer differential evolutionary capacity** on the malignant cells and impact treatment response.

Aim 1 *defined the contemporary vs vestigial DNA defects resulting from specific structural mutational processes in HGSOC*. Analyzing mutational patterns in >22,000 single cell whole genomes (scDNA) from HRD and FBI tumors we observed widespread aneuploidy and continuous whole genome duplication in HR deficient cancer cells, whereas FBI tumors showed early ploidy fixation and large clone-specific variation in local high-level amplifications with substantial breakpoint variability, often impacting oncogenes and increasing genome plasticity.

Aim 2 *defined the functional impact of mutational processes on the transcriptome*. To probe the effect of mutational processes on gene expression and cellular phenotype we performed single cell RNA sequencing (scRNA-seq) on 42 HGSOC tumors with different mutational signatures. We observed increased inflammatory signaling and ongoing immunoediting in HRD tumors, while FBI tumors exhibited elevated TGFβ signaling and immune exclusion. We also developed a workflow that includes the inference of clone-specific copy number alterations from scRNA data which made it possible to dissect the sub-clonal structure of tumors from transcription data. TreeAlign, a new tool to map scRNA data onto a scDNA-derived phylogenetic trees allowed us to define allele-specific gene expression in each clone for accurate phenotype to genotype mapping. We determined pathways driven by gene dosage effects and those regulated by epigenetic effects.

Aim 3 *tested structural mutational processes as a determinant of response to genotoxic therapy.* We carried out *in vitro* testing on cells derived from HGSOC PDXs with different classes of genotoxic drug. We focused on PDXs from a homologous recombination competent FBI tumor and identified two classes of drugs, a G quadruplex stabilizer and a CDK12 inhibitor, that reduced viability. Our work of detailed genomic and transcriptomic profiling of HGSOC clones provides a high resolution sub-clonal map and points to clone-specific molecular mechanisms that likely underly drug resistance.

15. SUBJECT TERMS					
High grade serous ovarian cancer, mutational signatures, copy number variants, single cell genome, single cell transcriptome, drug resistance, tumor evolution					
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1. INTRODUCTION:

In this work we tested the hypotheses that the different mutational processes, which stratify HGSOC tumors into distinct subtypes with different prognostic outcomes, confer specific evolutionary capacity on malignant cells, including transcriptional pathway activation unique to each subtype. We tested the response to different classes of genotoxic drugs in PDX models derived from tumor subtypes to understand their specific mechanistic underpinning of drug resistance. The goal of our research is to discover how mutational processes shape tumor evolution and to gain mechanistic insight into the development of drug resistance that will guide future targeted treatment options for patients.

2. KEYWORDS:

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aims	Timeline	Milestones achieved
Aim 1 To define the contemporary vs vestigial DNA defects resulting from specific structural mutational processes in HGSOC	Months	
Aim 1.1 Patient accrual and tumor tissue collection (n=50)	5-24	Protocol approved by MSK-IRB; collection from 42 patients
Aim 1.2 Single cell DNA Sequencing	5-24	Data from 42 patients
Aim 1.3 Identify active mutational processes through phylogenetic analysis	7-24	Defined for 36 patients from WGS data
Aim 1.4 Identify clone-specific variation in mutational processes	7-24	Established computational pipeline for allele-specific analysis of clones
Aim 2 To define the functional impact of mutational processes on the transcriptome	Months	
Aim 2.1 Single cell RNA-seq data generation and analytical pre-processing	5-24	scRNA-seq data generated from 41 patients
Aim 2.2 Measure intrinsic cellular variation and stability of activated DNA damage response (DDR) pathways linked to structural mutational processes	7-30	Decomposition of cell-specific DRR activation from scRNA-seq and integration with scDNA-seq
Aim 2.3 Estimate phenotypic diversity within and between genomic clones as a function of mutational signature	10-33	Tools and pipeline to analyze HGSOC samples
Aim 3 - Establish structural mutational processes as a determinant of response to genotoxic therapy		
Aim 3.1 Identify the working ranges for index PDX tumors	5-12	8 PDXs were tested with 4 classes of drugs
Aim 3.2 Establish the clonal vs transcriptomic relationships of drug sensitivity/resistance	13-36	Due to slow growing PDXs this goal will be accomplished in future work
Aim 3.3 Determine resistance phenotypes and identifying cross resistance	19-30	Due to slow growing PDXs this goal will be accomplished in future work
Aim 3.4. Identifying the effects of pairwise drug combinations	21-30	Due to slow growing PDXs this goal will be accomplished in future work

What was accomplished under these goals?

Aim 1 **To define the contemporary vs vestigial DNA defects resulting from specific structural mutational processes in HGSOC**

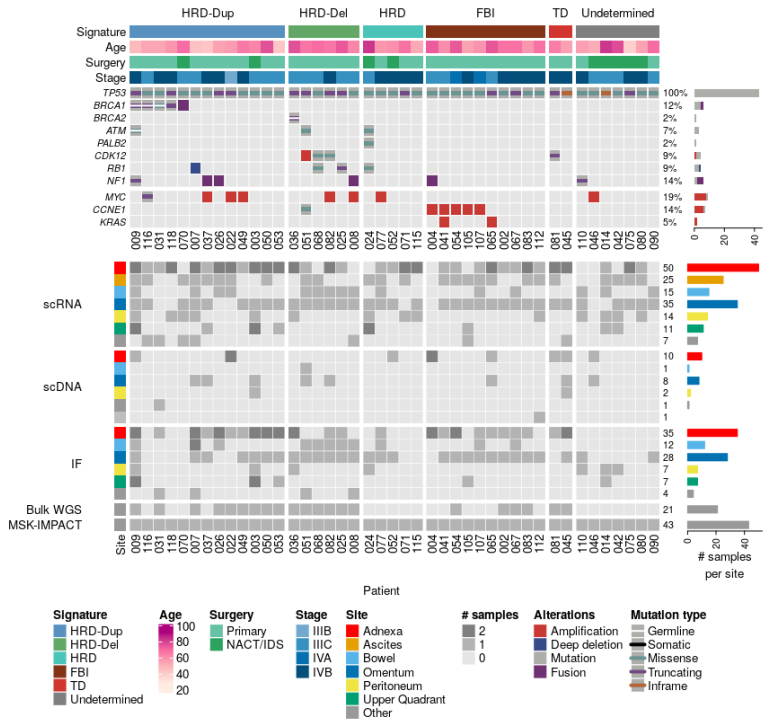

Fig. 1 Cohort overview Top panel: Oncoprint of selected somatic and germline mutations per patient. Bottom panel: sample and data inventory indicating number of co-registered datasets: scDNA-seq, scRNA-seq, bulk WGS and targeted panel sequencing (MSK-IMPACT).

We have collected multi-site tumor biopsies from **42 treatment-naïve HGSOC patients** working closely with the Disease Management Team (DMT) at MSK (see **Fig. 1** for an overview of the data collected for the cohort). For 36 samples we obtained whole genome sequencing (WGS) and employed a method established in the Shah group (*Funnell et al PLoS Comp. Bio. 2019*) to stratify patients by their mutational signatures into prognostically relevant groups: 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 are characterized by foldback inversion (FBI) and tandem duplications (TD) and they show worse outcome.

We have acquired single cell (sc) DNA-seq from 42 patients in the present cohort and used our newly developed tools and computational pipeline to reconstruct phylogenetic trees and separate ancestral from clade-specific and cell-specific events, corresponding to the earliest, later and most recent genomic damage. In addition, we designed SIGNALS, a method to quantify allele-specific copy number alterations (CNAs) at 0.5Mb resolution. We demonstrated the robustness of our pipeline on a study of 22,057 single cell genomes of triple negative breast tumors (TNBC) and HGSOCs (*Funnell et al. Nature 2022*). All subtypes displayed extensive subclonal heterogeneity, but FBI tumors showed higher rates of polyploidy and chromosomal missegregation, and they accrued gains at higher rates than HRD-Dup tumors (**Fig. 2**). These data demonstrate that mutational signatures determine the accrual patterns of CNAs with striking differences between HRD and FBI subtypes. One particularly noteworthy type of copy number variation were high-level amplifications (HLAMPs), with 10 or more copies of a segment, often accompanied by extensive variation in breakpoints between cells. This gave rise to a staircase-like pattern which we termed 'serrate structural variation' (SSV). FBI tumors had an almost 2fold higher HLAMP variance than other subtypes and, importantly, these HLAMPs often impacted the expression of known oncogenes (**Fig. 3**). In one example we showed that an HLAMP in a minor clone over the KRAS locus, corresponded to a higher KRAS expression, assessed via scRNA-seq and validated by immunofluorescence imaging on the primary tissue. These observations would not have been possible with bulk sequencing. Notably,

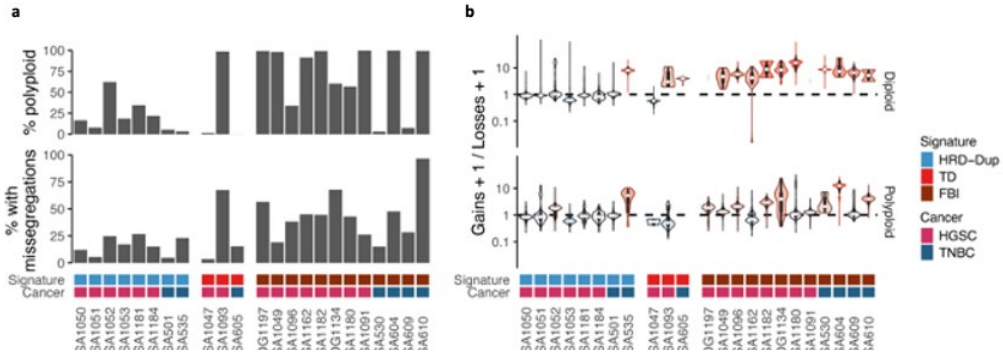

Fig. 2 Single tumor cell genome properties a) % of polyploid cells and missegration events b) Ratio of gains vs losses

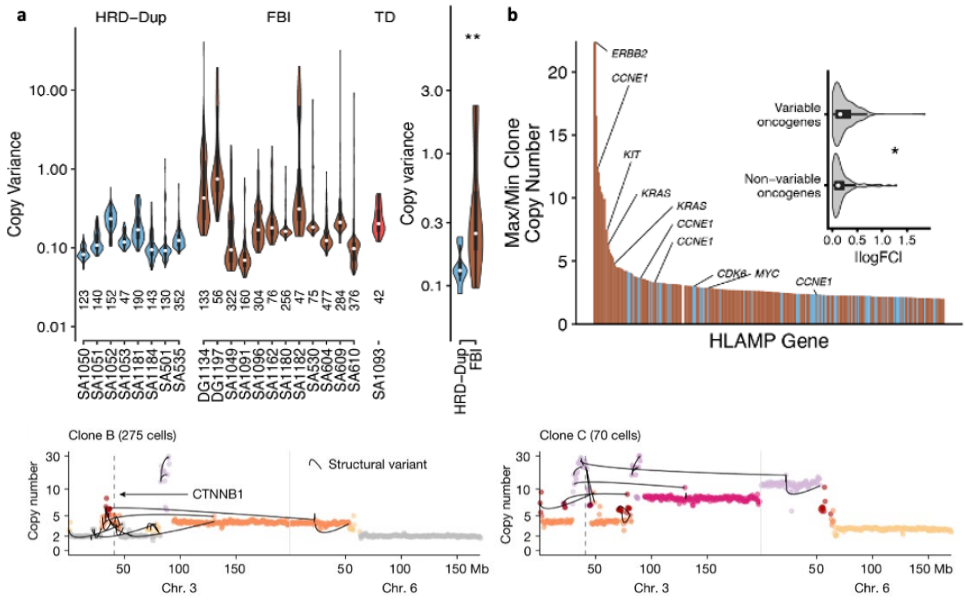

Fig. 3 HLAMP variance a) Copy number variance for HLAMPs **b)** Clone max/min copy number ration of oncogenes overlapping HLAMP regions. Inset shows distribution of gene expression in matched scRNA-seq data for the same genes. Bottom panel shows consensus copy number profiles in two clones from FBI tumor SA1096 overlaid with lines indicating structural variants.

the structural processes leading to the HLAMPs differed between the subtypes: while foldback inversions, as a result of breakage fusion bridge cycles, drove HLAMPs in FBI tumors, in HRD-Dup tumors they were associated with simple tandem duplications. In general, many clone-specific HLAMPs involved complex genomic structures, often involving multiple chromosomes.

We employed long-read single molecule nanopore sequencing to validate the presence of some of these complex rearrangements. For example, the variable amplitude around the CCNE1 locus in the FBI sample SA1096 (**Fig. 3b**) coincided with a translocation between chromosomes 3 and 6 (**Fig. 3**, bottom panel).

Aim 2 **To define the functional impact of mutational processes on the transcriptome**

We obtained scRNA-data from 41 treatment-naïve patients, on flow-sorted cells enriched for either the CD45 negative tumor fraction or CD45 positive immune cells across 156 sites. In addition, we performed bulk whole genome sequencing (WGS) on a single site and FDA-approved targeted sequencing of 505 cancer-related genes (MSK-IMPACT) (**Fig 1**). Single cell RNA (scRNA-seq) data defined epithelial, lymphoid, myeloid, stromal and tumor cells. Immune cells showed significant differences between anatomic sites, with adnexal samples depleted for T, B and dendritic cells, whereas distal sites were more infiltrated with immune cells (**Fig. 4**).

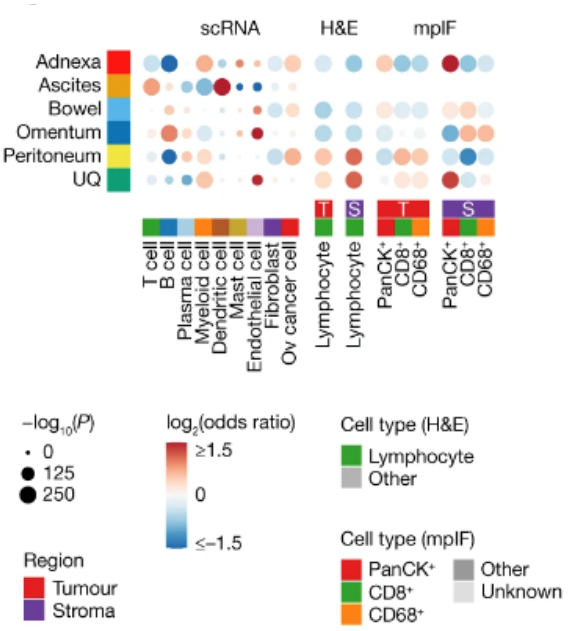

Fig. 4 Tumor microenvironment Site-specific enrichment of cell type composition in scRNA-seq, H&E and mpIF data.

Analysis of H&E-stained slides and multiplexed immunofluorescence (mpIF) with cell-type specific antibodies confirmed this finding of site-dependent immune cell composition (**Fig. 4**). A deeper analysis of the phenotypic states of immune cells, based on known marker genes, identified 10 major T cell and natural killer (NK) cell clusters made up of 10 CD4+ T cell clusters, 9 CD8+ T cell clusters, 4 innate-like T cell clusters, 9 NK cell clusters and 7

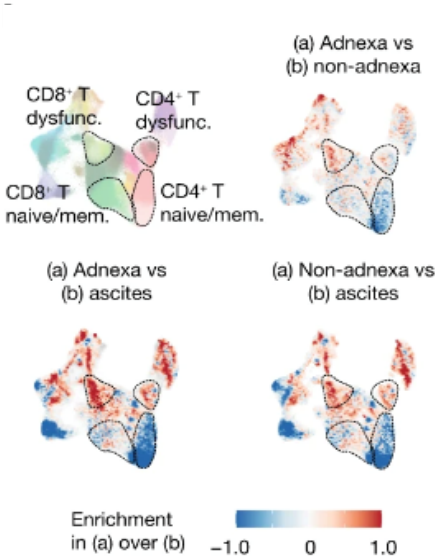

Fig. 5 Site-specific immunophenotypes Pairwise comparison of kernel density estimates in UMAP space.

cycling cell clusters. By fitting a generalized linear model (GLM) to cluster composition we were able to quantify site-specific phenotypic differences. Of particular interest was the depletion of naïve/stem-like and central memory CD4+ T cell cluster in adnexa and its enrichment in ascites, whereas dysfunctional CD4+ and CD8+ T cells were depleted in ascites and enriched in the adnexa and metastatic sites (**Fig. 6**). Our data agree with the hypothesis that chronic antigen exposure in solid tumors drives the development of dysfunctional T cells. An enrichment in regulatory T cells and NK cells in the adnexa also indicates increased immune modulation at this site.

A more granular view of the phenotypic states of immune cell types showed dysfunctional CD4+ and CD8+ T cells enriched in adnexal samples, together with more regulatory T cells, regulatory NK cells and immunosuppressive macrophages, consistent with chronic antigen exposure at the primary tumor site paired with higher immunomodulatory feedback. Focusing on tumor cells we observed increased JAK-STAT signaling in the adnexa of HRD tumors, but not in distal lesions. FBI tumors displayed more prominent TGFβ signaling in non-adnexal sites, compared to the primary tumor, implying that this pathway is activated during metastasis (**Fig. 6A**). Tumor subtypes also differed in their expression of major histocompatibility complex (MHC) genes. MHC class I and II genes were upregulated in HRD relative to FBI adnexal tumors, indicating a possible increase in antigen presentation (**Fig. 6B**).
We observed a compositional difference in the tumor microenvironment (TME) of the different tumor subtypes. FBIs were enriched for naïve/stem like and memory T cells, while depleted for dysfunctional T cells, HRD tumors on the other hand were enriched for dysfunctional T cells (**Fig. 6C**). To investigate whether the variation in immunophenotypes originated in genomic differences at the HLA locus, we compared the loss of heterozygosity (LOH) at the 6p22.1 locus which encodes HLA class I and II genes, using SIGNALs. We observed frequent clonal LOH of 6p in HRD-Dup tumors, while in FBI patients subclonal LOH events were more common (*Vasquez-Garcia et al. Nature 2022*). Taken together our results provide a phenotypic map of the HGSOC tumor microenvironment that is driven by anatomic site as well as by mutational processes. We outline how HRD and FBI tumors have different mechanisms to evade immune recognition. While HRD tumors are immune infiltrated, they show a high rate of dysfunctional T cells, whereas FBI tumors restrict T cell infiltration and are immune excluded. These observations could prove critically important for treatment since different approaches will need to be taken to either re-activate or engage the immune system.

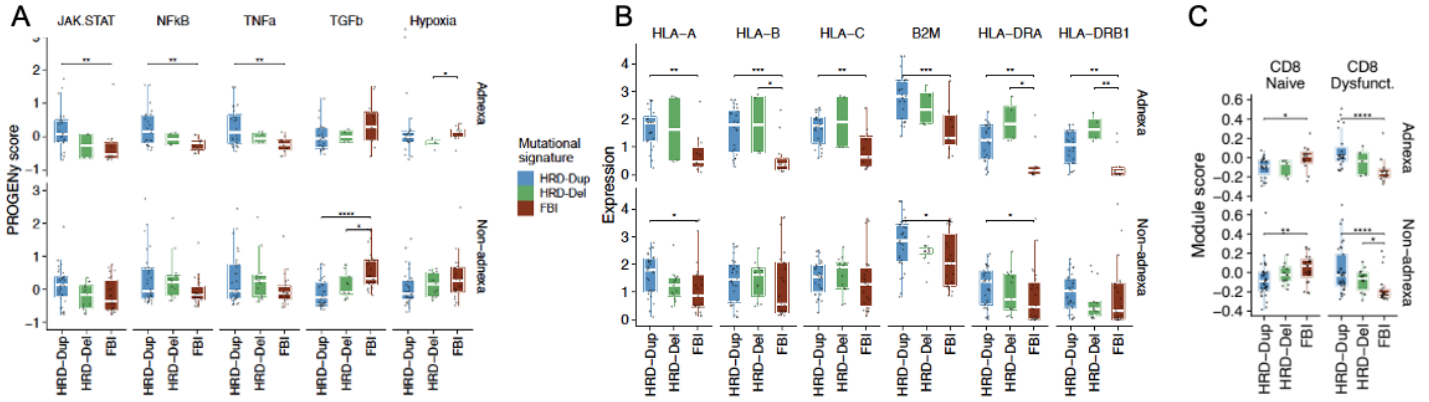

Fig. 6 Immune cell phenotypes as a function of mutational signature A) Signaling pathway activity scores **B)** HLA gene expression **C**) CD8 T cell state module scores.

In our efforts to investigate individual tumors more in depth and link transcriptional responses to clone-specific copy number alterations (CNA) we developed `TreeAlign`, (*Shi et al. BioRxiv 2023*) a modification of `CloneAlign` (*Campbell et al Genome Biology 2019*). `TreeAlign` combines a single cell expression count matrix with a scDNA-derived phylogenetic tree and its underlying CN matrix to obtain the clone assignment for each cell and derives the probability of CN-dependent gene expression (**Fig.7**).

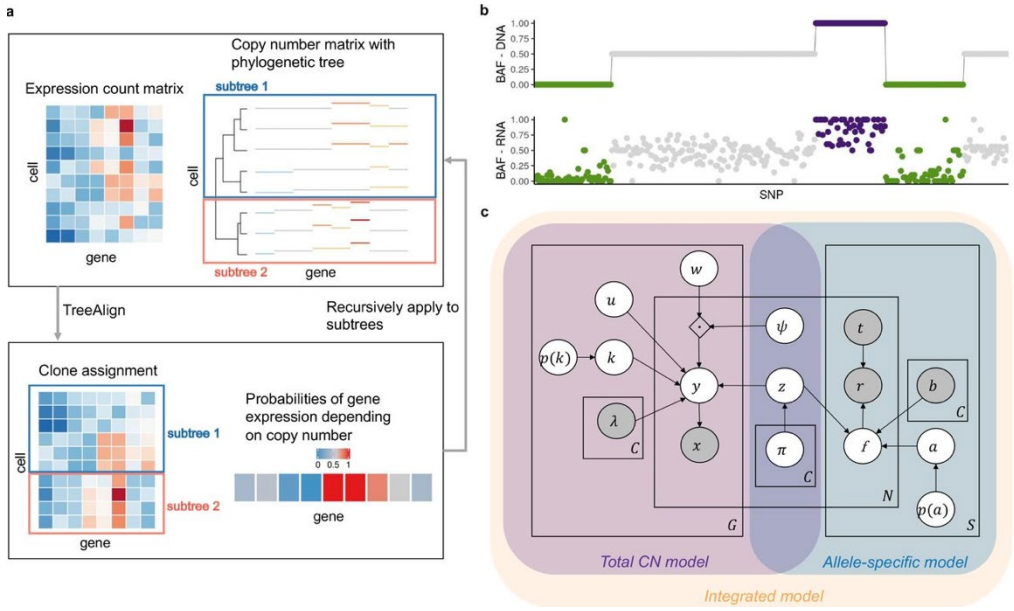

Fig. 7 Overview of `TreeAlign` a) `TreeAlign` infers the clone-of origin of cells identified in scRNA-seq and the dosage effects of clone-specific copy number alterations b) Allelic imbalance, based on B allele frequency (BAF) inferred from DNA or RNA. c) Graphical model of `TreeAlign`.

We found accurate predictions of dosage effects in highly expressed genes and saw that only 5-30% of differential gene expression between clones can be attributed to CN dosage effects. Applied to one HGSOC tumor we assign cancer cells individual expression profiles to 4 subclones identified by scDNA-seq. (**Fig. 8**) While the expression profiles of clones C and D overlapped in the expression-based UMAP, they were clearly separated by `TreeAlign`. This separation indicates that genetic subclones feature distinct transcriptional phenotypes. While clone-specific CNAs were often observed in both the scDNA and scRNA data, it is important to note that inferring copy number from scRNA alone is not always accurate. For example, a focal amplification on chromosome 18 was missed in the RNA data (**Fig. 8A**). To further improve the resolution of clone-assignment, we incorporated allele-specific copy numbers, inferred from scDNA with SIGNALS (*Funnell et al. Nature 2023*). We were able to resolve allele-specific expression in subclones (**Fig. 8B**). Clone B was subdivided into two subclones, with a deletion at 16q, in clone B.1, that resulted in loss of heterozygosity and an allelic imbalance due to gain of 10q. Clone B.2 showed increased B allele frequency after an amplification in 11q. Clone D was split into 4 subclones, with a deletion on chromosome 5 occurring in different alleles and with different breakpoints in clones D.1 and D.2. Clone B also showed a 5q deletion, but with breakpoints distinct from those in clones D.1 and D.2. `TreeAlign` allows an unprecedented granularity of phenotype to genotype mapping.

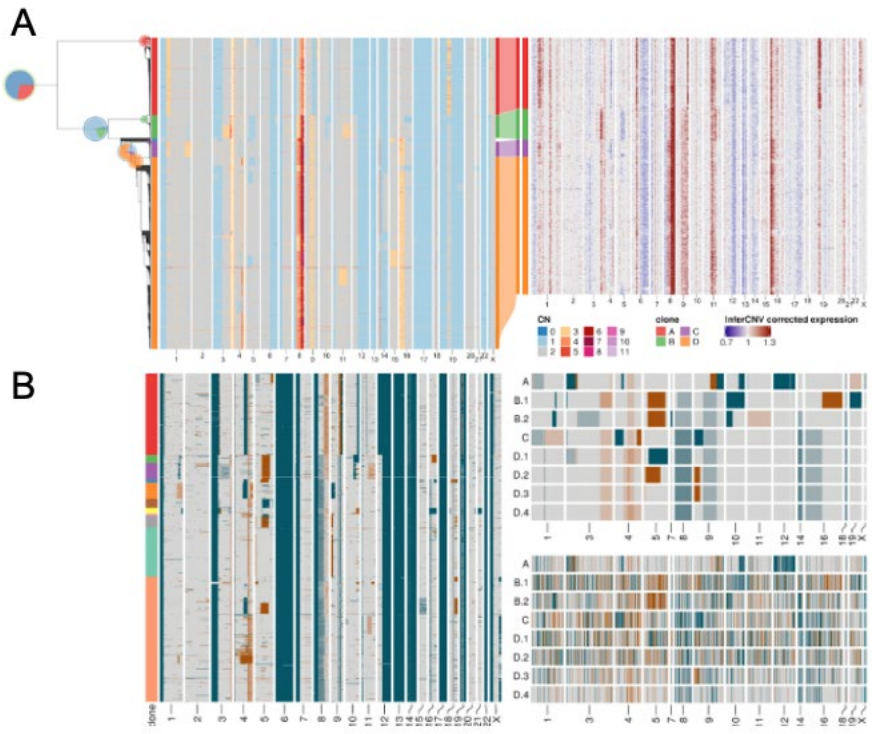

Fig. 8 Inferring clone assignment with `TreeAlign` A) Phylogenetic tree constructed with scDNA-data along with pie charts showing how `TreeAlign` assigns cell expression profiles to subtrees recursively. The pie charts are colored by the proportions of cell expression profiles assigned to downstream 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`. B) Heatmap of B allele frequencies in (left) HGSOC tumor estimated from scDNA data (upper right) subclone scDNA data, (lower right) subclone scRNA data.

We then inferred the copy number dosage effects on data from 2 triple negative breast tumors and 7 HGSOC samples. For highly expressed genes, defined as the top 40% after normalization, located in clone-specific copy number regions, we found their expression to be dependent on copy number. Furthermore, we identified subclonal amplifications of oncogenes, such as MYC, that correlated with changes in gene expression but also pinpointed clone-specific MYC pathway activation that was not driven by CNAs. This suggests that pathways at the subclonal level are regulated both by copy number dosage effects as well as other, possible epigenetic, effects. `TreeAlign` provides the subclonal resolution to interpret the mechanism behind gene dysregulation.

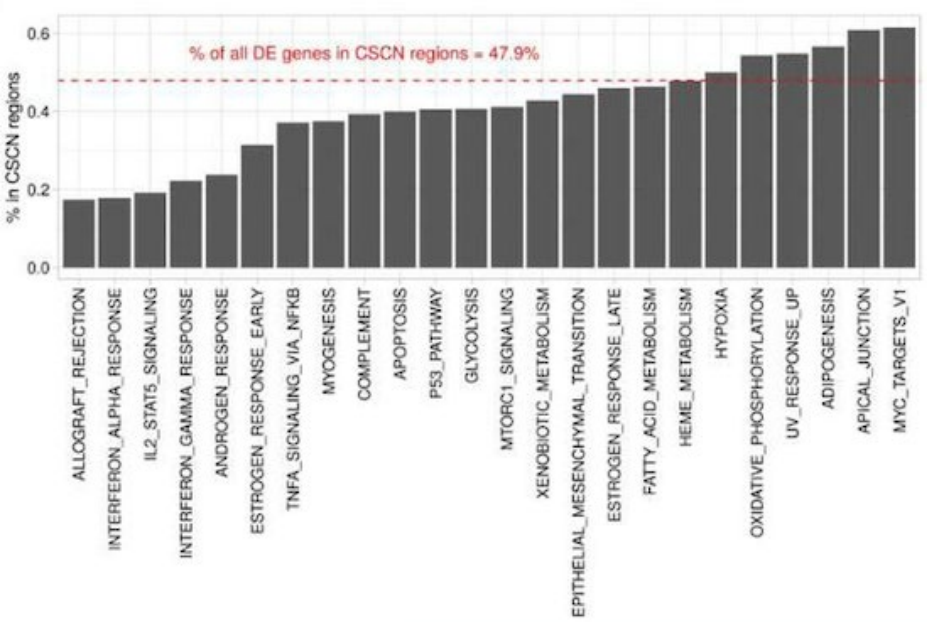

Fig. 9 Differentially expressed genes between subclones in a HGSOC patient Frequencies of DE genes in clone-specific copy number regions summarized by pathways .

Focusing on one HGSOC patient we were able to outline clone-specific transcriptional phenotypes with 1346 genes upregulated in at least one subclone and 701 of these genes not falling into a CNA region. Of the genes that did fall into CNA loci, 90% were regulated by gene dosage effects. Particularly immune related pathways, such as IFN-α and IFN-γ, were differentially expressed between subclones: Clone B.1 was enriched in TNFα and IFN-γ signaling pathways, whereas some MYC target genes were downregulated, compared to clone B.2. Clone D.4 downregulated TNFα signaling, compared to the other subclones within clone D. We note that this differential expression between subclones likely drives clonal divergence. To quantify how much CNAs contribute to differentially expressed pathways, we determined the proportion of differentially expressed genes in subclonal CNAs. We observed clear differences in the impact of subclonal CNAs in different pathways. For example, in the allograft rejection gene set, only 17% of differentially expressed genes fall into clone-specific CNA regions, while for MYC target genes 61% of DE genes are found in CNAs (**Fig. 9**).

Aim 3 **Establish structural mutational processes as a determinant of response to genotoxic therapy**

We used our HGSOC-derived PDX collection, representing multi-site sampling from 15 patients, to begin evaluating the impact of mutational processes on the response to four classes of genotoxic drugs. Xenografts were transplanted subcutaneously into NRG mice (n=3 or 4 per group).

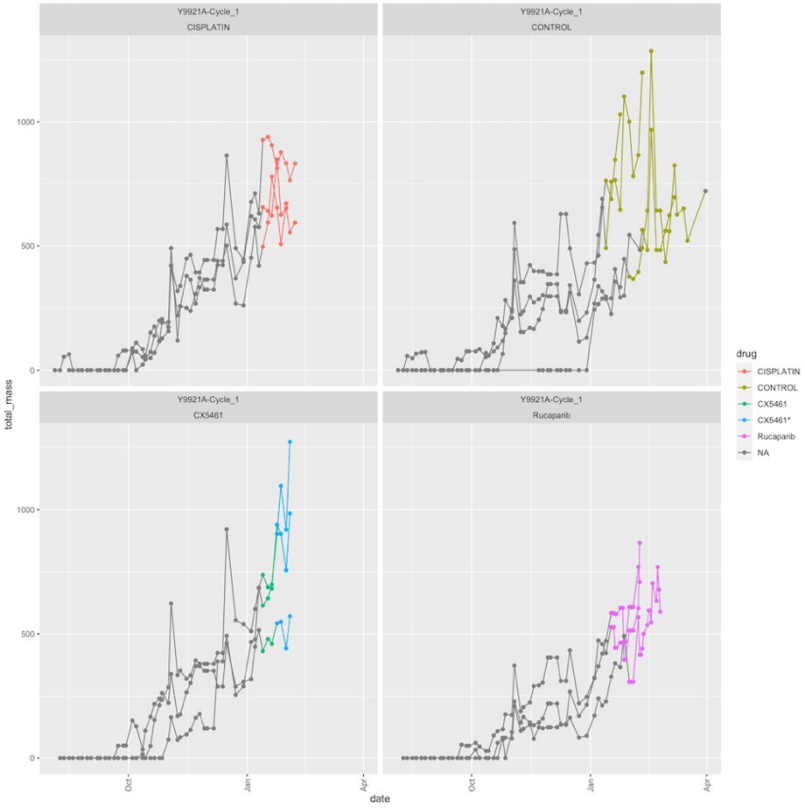

Fig. 10 FBI-derived PDX treated with the indicated drugs

We measured the tumors twice per week and once they reached ~500mm^3, mice were randomized to receive cisplatin (2mg/kg, intraperitoneal), CX5461 (62.5mg/kg, gavage), Rucaparib (10mg/kg, intraperitoneal) or control. Cisplatin and CX5461 doses were administered once every three days up to 8 doses, Rucaparib was administered daily for 5 days for up to 20 doses. Tumors were measured at each time of dosing. Mice were euthanized if tumors reached 1000mm^3 or following completion of dosing. In agreement with our original hypothesis that FBI tumors will show higher intrinsic resistance to treatment we see that the FBI-PDX tested showed no response to the three drugs (**Fig. 10**). In a more extensive dose-response drug screen, testing 11 drugs in doses ranging from 10μM to 1nM, we observed that the FBI tumor are sensitive to the G4 stabilizer CX5461 and the CDK12 inhibitor THZ531 (**Fig. 11**). This work built a solid foundation to further elucidate the genomic and transcriptomic underpinnings of drug resistance in HGSOC clones.

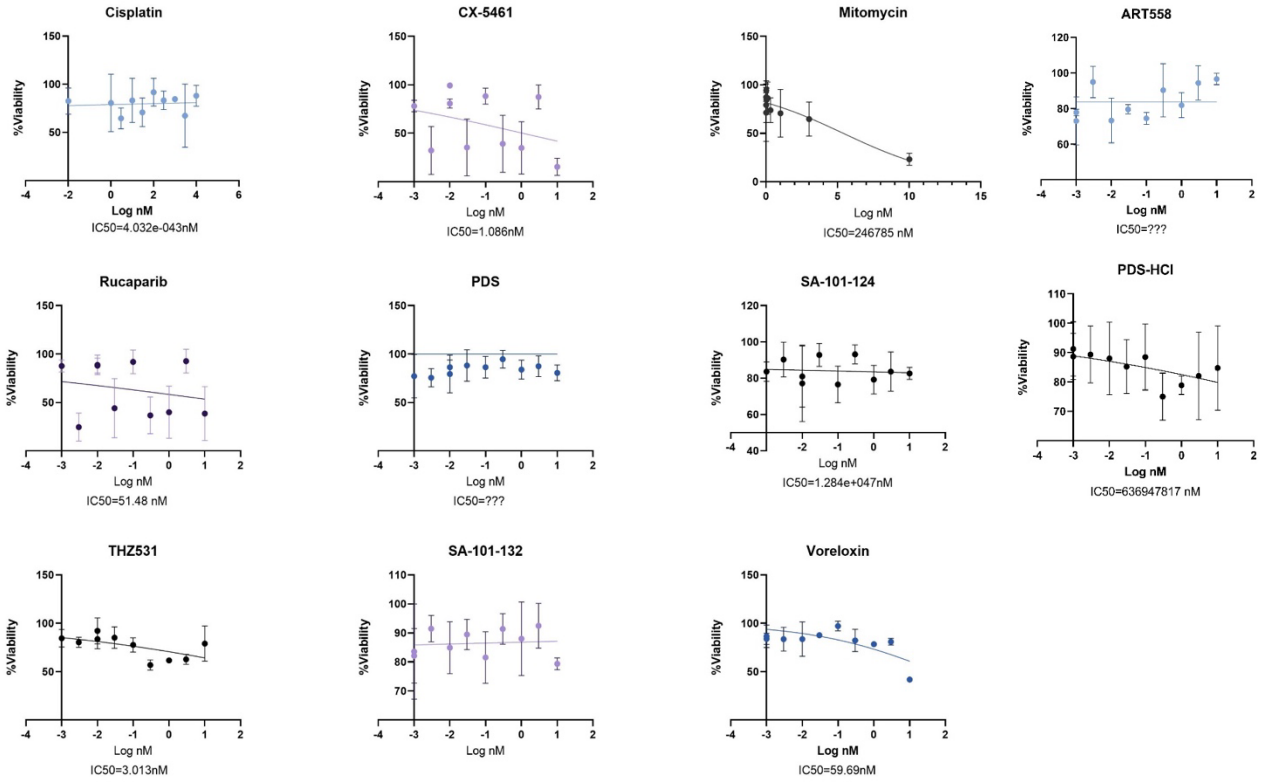

Fig. 11 FBI-derived PDX treated with different doses of the indicated drugs

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

Dr. Shah holds weekly meetings with all the participants in this grant including technicians, students and the post doctoral fellow. Hongyu Shi, the PhD student who developed `TreeAlign`, graduated in November 2023. Part of this work was used in the Mammalian Genetics Course at Rockefeller University, where Dr. Shah gave a lecture on 'Mutational signatures in cancer' in June 2023.
The post doctoral fellow whose work contributed to the goals in this grant presented at the AACR Annual Meeting in April 2023 in Orlando, Florida, at the UPenn Ovarian Cancer Research Syndicate Transient in September 2023 and most recently at the AACR Special Conference Translating Cancer Evolution and Data Science, in Boston.

How were the results disseminated to communities of interest?

Dr. Shah is an active participant in and organizer of scientific meetings (see below for details). In addition, results were shared via BioRxiv preprints prior to publication and software is made available on GitHub.

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
In the course of this work, we established computational pipelines that will be valuable to basic clinical research beyond ovarian cancer, extending to different diseases. Our tools allow the interrogation of single cells, the basic unit of disease, and will bring to light how changes at the single cell level drive the development of disease and resistance to treatment. Our platforms allow the characterization of cells at the genome level, elucidating how mutations reshape genomic content and the effect this has on the ability of clones to grow. Knowing that genomic changes only account for part of cellular evolution we also profile gene expression at the single cell level and thus provide a framework for the comprehensive analysis of clones. Applied to ovarian cancer our tools and approaches will lead to an in depth understanding of the particular drivers of cancer subtypes and highlight biomarkers that can stratify patients by subtype, each associated with a different risk profile. It will also provide candidates for subtype-specific intervention and a treatment plan that is geared towards the individual molecular makeup of a tumor, exploiting its weaknesses for an improved outlook for patients with high grade serous ovarian cancer.

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?
Upon completion of the project, we will have laid the groundwork for better risk-stratification of high grade serous ovarian cancer patients using genomic changes in tumors as biomarkers. This concept of 'genome as biomarker' will likely impact other diseases that are driven by distinct mutational processes and lead to the development of targeted therapies and more personalized treatment. Work like ours will raise public awareness of the importance of genomic profiling and make a case for incorporating clinical sequencing into standard of care to ensure a more targeted disease management.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Progress in Aim 3 was limited by the slow and sometimes unsuccessful growth of PDX samples which prevented us from establishing cell lines from the different HGSOC subtypes and fully testing their change in clonal structure in response to drug treatment. We were successful in deriving a PDX from an FBI tumor and successfully treated it with a panel of drugs. We were able to identify two drugs that successfully reduced tumor viability and will characterize the genotypic and phenotypic changes in drug resistant clones in future work.

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 vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications**

  Journal publications

  Vázquez-García I, Uhlitz F, Ceglia N, Lim JLP, Wu M, Mohibullah N, Niyazov J, Ruiz AEB, Boehm KM, Bojilova V, Fong CJ, Funnell T, Grewal D, Havasov E, Leung S, Pasha A, Patel DM, Pourmaleki M, Rusk N, Shi H, Vanguri R, Williams MJ, Zhang AW, Broach V, Chi DS, Da Cruz Paula A, Gardner GJ, Kim SH, Lennon M, Long Roche K, Sonoda Y, Zivanovic O, Kundra R, Viale A, Derakhshan FN, Geneslaw L, Issa Bhaloo S, Maroldi A, Nunez R, Pareja F, Stylianou A, Vahdatinia M, Bykov Y, Grisham RN, Liu YL, Lakhman Y, Nikolovski I, Kelly D, Gao J, Schietinger A, Hollmann TJ, Bakhoum SF, Soslow RA, Ellenson LH, Abu-Rustum NR, Aghajanian C, Friedman CF, McPherson A, Weigelt B, Zamarin D, **Shah SP**. Ovarian cancer mutational processes drive site-specific immune evasion. Nature. 2022 Dec;612(7941):778-786. doi: 10.1038/s41586-022-05496-1. Epub 2022 Dec 14. PMID: 36517593; PMCID: PMC9771812.

  Funnell T, O'Flanagan CH, Williams MJ, McPherson A, McKinney S, Kabeer F, Lee H, Salehi S, Vázquez-García I, Shi H, Leventhal E, Masud T, Eirew P, Yap D, Zhang AW, Lim JLP, Wang B, Brimhall J, Biele J, Ting J, Au V, Van Vliet M, Liu YF, Beatty S, Lai D, Pham J, Grewal D, Abrams D, Havasov E, Leung S, Bojilova V, Moore RA, Rusk N, Uhlitz F, Ceglia N, Weiner AC, Zaikova E, Douglas JM, Zamarin D, Weigelt B, Kim SH, Da Cruz Paula A, Reis-Filho JS, Martin SD, Li Y, Xu H, de Algara TR, Lee SR, Llanos VC, Huntsman DG, McAlpine JN; IMAXT Consortium; Shah SP, Aparicio S. Single-cell genomic variation induced by mutational processes in cancer. Nature. 2022 Dec;612(7938):106-115. doi: 10.1038/s41586-022-05249-0. Epub 2022 Oct 26. PMID: 36289342; PMCID: PMC9712114.

  Shi H, Williams MJ, Satas G, Weiner AC, McPherson A, Shah SP. Exploiting allele-specific transcriptional effects of subclonal copy number alterations for genotype-phenotype mapping in cancer cell populations. bioRxiv [Preprint]. 2023 Jan 17:2023.01.10.523464. doi: 10.1101/2023.01.10.523464. PMID: 36711951; PMCID: PMC9882029. [*Note that this paper is accepted at Nature Communications and will be published in 2024*]

Other publications, conference papers and presentations.

Conferences Dr. Shah chaired or co-chaired
1) Keystone Symposium Single Cell Biology: From Development to Cancer, Keystone CO, June 19, 2023

Dr. Shah's invited presentation
1) "Towards quantifying evolvability in cancer with single cell approaches", Weill Cornell Medicine 2023 ICB Seminar Series, New York, NY, January 30, 2023
2) "Measuring evolvability in cancer through single cell approaches", Human Technopole, Milan, Italy, May 2, 2023
3) "Tumor evolution and immune evasion in ovarian cancer", Ohio State University (Virtual), May 12, 2023
4) "Genotype-phenotype evolution in cancer studied with single cell approaches", Banff Workshop - Mathematical methods in cancer biology, evolution and therapy, Banff, Canada. May 15, 2023
5) Tumor evolution and immune response in ovarian cancer", Distinguished Ludwig Lecturer in Cancer Research, University of Lausanne, Switzerland, September 14, 2023
6) "Tumor evolution and immune system dynamics in ovarian cancer", Sarma Lectureship on Oncologic Pathology at the University of Toronto, Toronto CA, September 25, 2023
7) "Mutational processes as determinants of immune evasion in ovarian cancer", AACR Special Conference on Ovarian Cancer, Boston, MA, October 7, 2023
8) "Co-evolution of ovarian cancers and their tumor microenvironments", Dana-Farber Cancer Institute Center for BRCA & Related Genes Scientific Symposium, Boston, MA, November 15, 2023

- **Website(s) or other Internet site(s)**

  Data will be publicly disseminated upon publication of the results

- **Technologies or techniques**

  To map scRNA-seq data to scDNA-derived clones we modified the tool `CloneAlign` (Campbell et al. Genome Biology 2019) to improve accuracy and the ability to infer gene dosage. The `TreeAlign` tool can be found at Github: ttps://github.com/AlexHelloWorld/clonealign_pyro

- **Inventions, patent applications, and/or licenses**

  Nothing to report

- **Other Products**
  Software: `TreeAlign`

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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 2.
Funding Support:	

Name:	Gryte Satas
Project Role:	Post doctoral fellow
Researcher Identifier (ORCID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Satas advised Hongyu Shi on the development of TreeAlign and contributed to clonal tracking in Aim 2.
Funding Support:	N/A

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 2, she will be supported in the clinical interpretation of the data by Dr. Aghajanian. Ms Shi is fully supported by her PhD program and based her PhD dissertation on the work described in this proposal.
Funding Support:	N/A

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?

Dr. Samuel Aparicio (ORCID: 0000-0002-0487-9599), Head of Molecular Oncology at BC Cancer, Vancouver, Canada, oversees the work in Aim 3 which involves the analysis of previously established patient-derived xenografts (PDXs) from HGSOC patients.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*