Single-cell trajectories of metastatic urothelial cancer and individual patterns of resistance to immune checkpoint inhibitors

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BACKGROUND

Immune checkpoint inhibitors (ICI) improved survival in patients with metastatic urothelial cancers (mUC) but only ~20% derive long-term responses, and most ultimately experience disease progression. Primary and acquired mechanisms of resistance are unknown.

OBJECTIVE

Identify primary and acquired mechanisms of resistance to single-agent immune checkpoint inhibitors in patients with mUC, using single sequential single-nuclei RNAseq of metastatic biopsy samples.

PATIENTS AND METHODS

The MATCH-R trial (NCT02517892) included patients with mUC treated with single-agent PD-(L)1 inhibitors.

We performed longitudinal single-nuclei RNA-seq analyses of metastatic samples to explore tumor and immune features predicting ICI response at baseline or associated with acquired resistance at relapse.

After stringent quality control and cell type annotation, we compared the proportions and transcriptomic signatures of tumor and immune cell subsets between responders and non-responders and explored their evolution during treatment.

A total of 32 mUC patients were included.
- 7/32 (22%) achieved objective response.
- 55 biopsies were performed: all underwent biopsies at baseline, 6 (19%) on therapy and 17 (53%) at progression.

RESULTS

Differentiation states drive tumor heterogeneity and ICI response

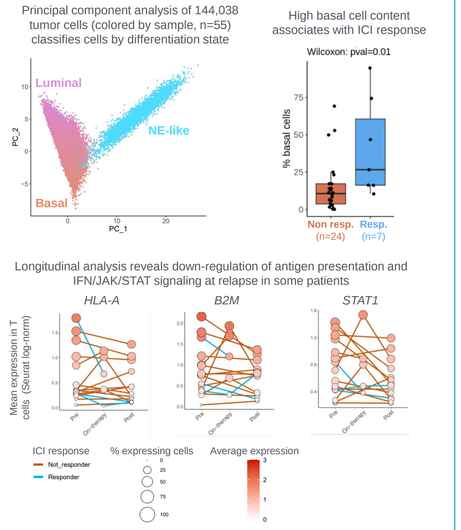

***Fig. 1**. Tumor cell heterogeneity and evolution upon ICI treatment*

CD8 T cell exhaustion is associated with primary and secondary resistance to ICI

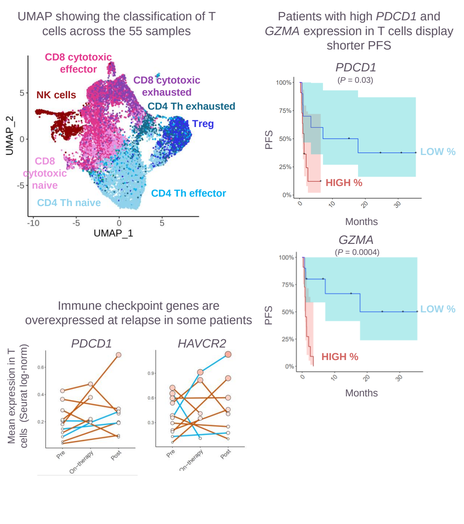

***Fig. 2**. Exhausted CD8 T cells predicts poor ICI response and increase post-treatment*

Pro-tumoral macrophage shift is a hallmark of ICI resistance in mUC

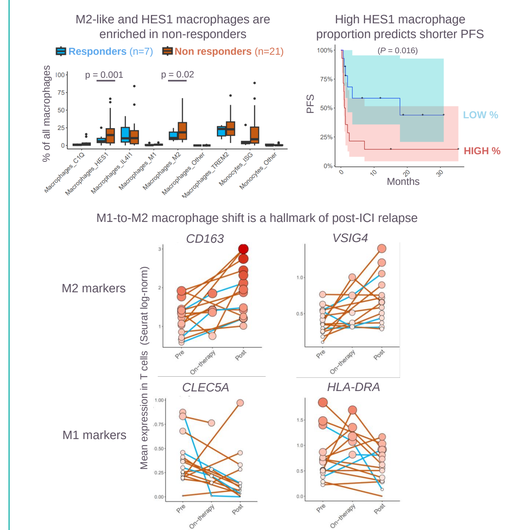

***Fig. 3**. Pro-tumoral macrophages predict poor ICI response and increase upon treatment.*

Single-nucleus RNA sequencing pinpoints individual trajectories towards ICI resistance

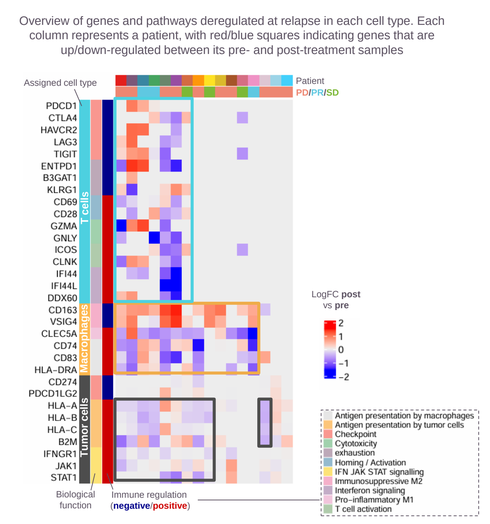

***Fig 4**. Overview of tumor and immune cell reprogramming after ICI.*

CONCLUSION

Single cell states may inform outcomes on ICI for patients with mUC and identify individual patterns of resistance

Basal tumor cell proportion at baseline predict mUC response to ICI. Conversely, adverse immune features include abundant **pro-tumoral HES1 macrophages** (poor response) and a higher proportion of **exhausted CD8+ lymphocytes** expressing immune checkpoint genes (poor response).

Longitudinal analyses uncovered **molecular shifts linked to ICI progression**, involving both tumor and immune compartments: **downregulation of HLA genes and IFN signaling in tumor cells**; a shift from **M1 to M2 macrophage polarization**; increased **expression of immune checkpoints and downregulation of type-I interferon induced genes in T cells**.

Implementation of single-cell transcriptomics in a clinical setting may help predict ICI response and to enable dynamic, personalized therapeutic strategies.

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Evolution of transcriptomic and epigenomic intra-tumor heterogeneity in high-grade serous ovarian cancer with chemotherapy

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SCAN ME

SUMMARY

Keywords. High-grade serous ovarian cancer; single-nuclei RNA-sequencing; single-nuclei epigenomics sequencing; sequential biopsies

Background. Intra-tumor heterogeneity (ITH) refers to the presence of diverse cell populations within a single tumor, each with distinct genetic, epigenetic, and phenotypic characteristics. This complexity presents significant challenges in treatment, as different subpopulations may respond variably to therapies, yet the non-genetic component of ITH and its contribution to treatment response remains poorly understood. We mapped transcriptomic and epigenomic ITH to explore evolution of non-genetic ITH in response to therapy in high-grade serous ovarian cancer (HGSOC).

Methods. The SCANDARE study (NCT03017573) included patients with HGSOC that are treated with neo-adjuvant chemotherapy (NAC). Biopsies were performed at baseline, after NAC surgery and at recurrence for single-cell RNA sequencing, single-nuclei RNA sequencing and single-cell epigenomics (snCUT&Tag). Our snCUT&Tag dataset is one of the first single-cell epigenomic map of cancer patients under treatment, focusing on the histone modification H3K4me1, which accumulates at primed and active enhancers and promoters. We integrated these clinically annotated single-cell/nuclei datasets with publicly available scRNA-seq data of HGSOC.

Results. We constructed a consensus map of functional tumor states in HGSOC, based on scRNA-seq analysis of over 200,000 tumor cells. This analysis revealed **13 recurrent tumor cell phenotypes**, ranging from stressed to cycling or inflammatory states. We identified the transcription factors and cell-cell communications specific to each tumor cell state. These tumor states are encoded at the epigenomic level, we could identify in each tumor epigenetic clones – clusters of cells with the same epigenome - each displaying a H3K4me1 landscape characteristic of tumor states. Longitudinal sampling by snRNA-seq further showed that tumor state composition consistently evolves upon chemotherapy exposure in patients: tumors lose their cycling cells while gaining cells with partial mesenchymal and TNFa characteristics.

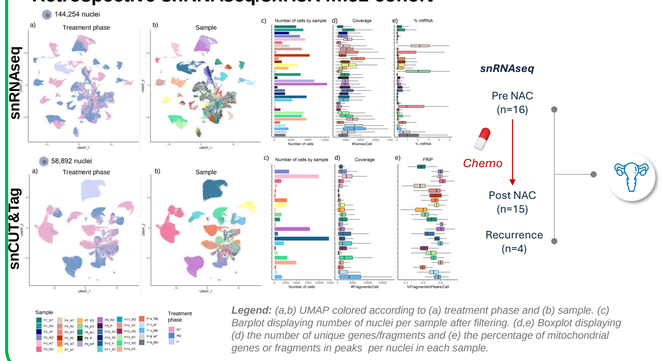
Conclusions. We propose a consensus map of transcriptomic and epigenomic tumor states in HGSOC. Our work is a proof of concept that we can monitor functional ITH with high resolution snRNA-seq from frozen biopsies of the standard of care. We show that these consensus tumor states consistently evolve upon treatment in all patients, pinpointing tumor cell states that are not successfully targeted by NAC, that would need to be targeted by adjuvant therapies.

Transcriptomic and epigenomic data sets

Large scRNAseq public &Curie SCANDARE cohort

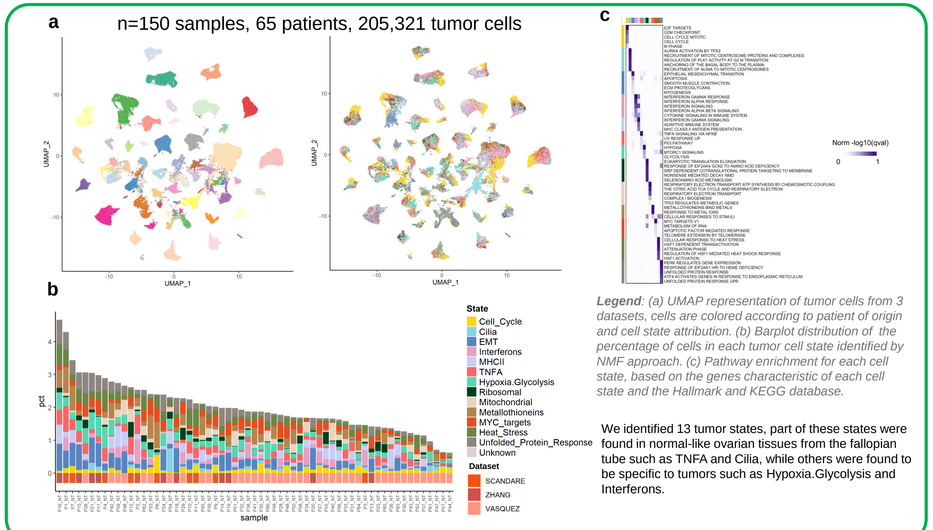
	Dataset	SCANDARE	ZHANG	VASQUEZ	TOTAL CANCER
	Number of cells	27760	8758	174663	226414
	Number of samples	13	20	115	150
Treatment phase	Treatment-naive	5	10	110	125
	Treated	8	10	5	13
HRD status	HRD	7	13		20
	HRP	6	35		43
	Undetermined				20
Anatomical location	Adnexa	5	1	47	53
	Omentum	2	12	30	44
	Peritoneum	2	6	11	19
	Upper quadrant			9	9
	Bowel			14	14
	Other		1	4	5
	Undetermined	6			6

Retrospective snRNAseq/snH3K4me1 cohort



Legend: (a,b) UMAP colored according to (a) treatment phase and (b) sample. (c) Barplot displaying number of nuclei per sample after filtering. (d,e) Boxplot displaying (d) the number of unique genes/fragments and (e) the percentage of mitochondrial genes or fragments in peaks per nuclei in each sample.

ID of consensus cell states in ovarian cancer across scRNAseq datasets



Legend: (a) UMAP representation of tumor cells from 3 datasets, cells are colored according to patient of origin and cell state attribution. (b) Barplot distribution of the percentage of cells in each tumor cell state identified by NMF approach. (c) Pathway enrichment for each cell state, based on the genes characteristic of each cell state and the Hallmark and KEGG database.

We identified 13 tumor states, part of these states were found in normal-like ovarian tissues from the fallopian tube such as TNFA and Cilia, while others were found to be specific to tumors such as Hypoxia.Glycolysis and Interferons.

High quality snRNAseq profiling for samples of the standard of care

Today, single-cell omics remain firmly in Research space

- **Low data quality** from **bio banked samples (FFPE, frozen)**
- **Data analysis is a bottleneck** 20Go/patient
- **2-6 months** from sample to cohort analysis

With wet-lab & AI innovations, we have unlocked use of single-cell

- **Unmatched quality of data** from bio banked samples (**new standards for frozen, FFPE**)
- **Integrative AI analyses** Unravel cell & gene functions in disease ecosystem, linked with therapy strategies
- **Actionable insights, fast & reliable delivery:** 2-3 weeks to deliver patient molecular profiling **'as of' patient #1**

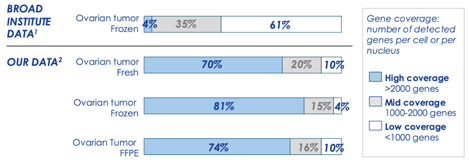
A combination of wet-lab and AI developments to produce snRNAseq from frozen and FFPE samples with high signal/noise ration and a majority of nuclei > 1,000 genes

We have set a new standard

More than 60%[2] nuclei with coverage above 2k genes for frozen samples - validated across multiple tissues & biocollections

Initial FFPE results are consistent and matching this quality

Distribution of nuclei from one biopsy, based on their gene coverage (low, mid or high coverage), reflecting **quality of single-cell / nucleus data**

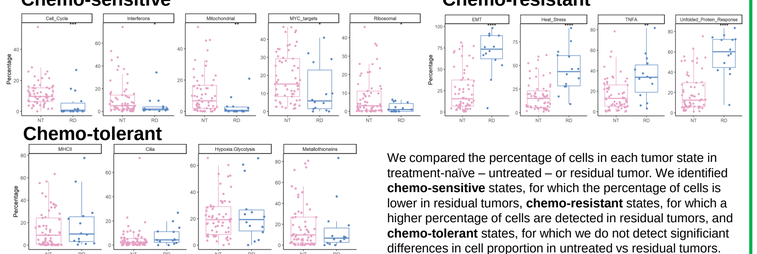


Performances were validated across tumor types (ovarian, glioblastoma, colon, bladder, pancreas). snRNAseq can be performed from 10mg frozen biopsies or 4 FFPE slides (5µm).

We obtained more than 70%[2] nuclei with coverage above 2k genes from an ovarian tumor FFPE sample - matching our high-quality standards from fresh and frozen samples

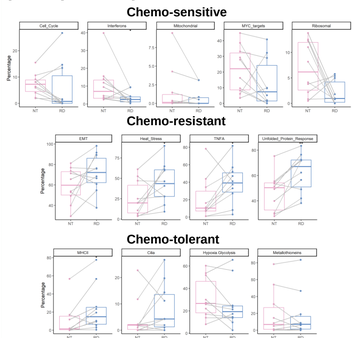
Sc/snRNAseq for longitudinal follow-up of tumor states

All public scRNA-seq samples (n=65 patients, n= 205,321 cells)



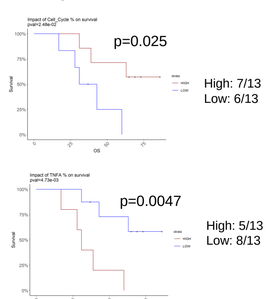
We compared the percentage of cells in each tumor state in treatment-naïve – untreated – or residual tumor. We identified **chemo-sensitive** states, for which the percentage of cells is lower in residual tumors, **chemo-resistant** states, for which a higher percentage of cells are detected in residual tumors, and **chemo-tolerant** states, for which we do not detect significiant differences in cell proportion in untreated vs residual tumors.

Paired pre/post samples SCANDARE & Zhang cohorts (n=10 patients)



Paired comparison of pre and post treatment percentages of tumor states shows state evolution in individual patients upon NAC.

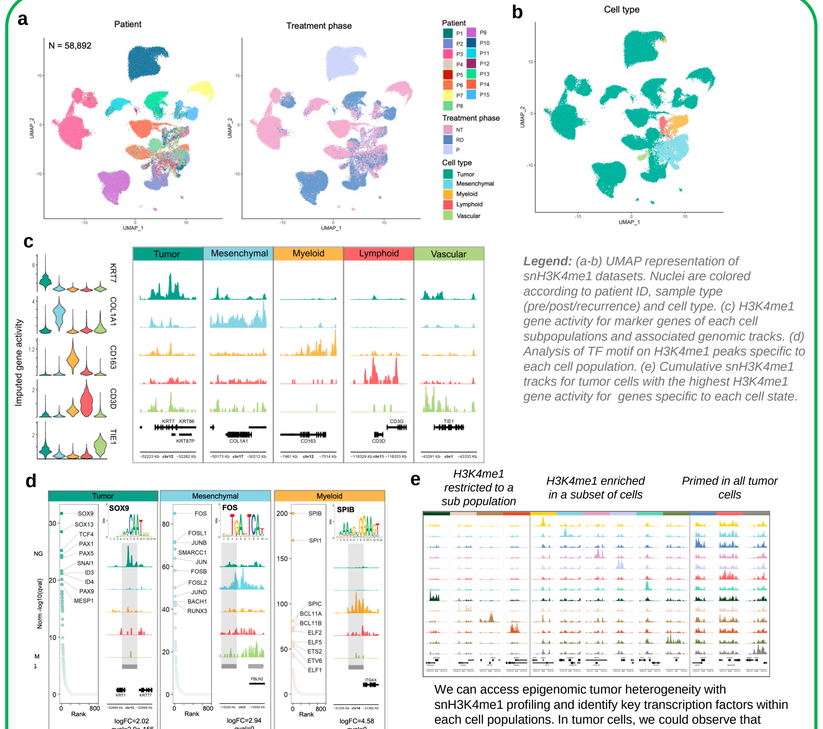
Treatment-naïve samples SCANDARE snRNAseq cohort (n=13 patients)



→ High content in chemo-sensitive cell cycling state ~ good OS
→ High content in chemo-resistant TNFA state ~ poor OS

Evaluation of tumor states content at baseline could be a tool to predict outcome/response to chemotherapy

Epigenomic encoding of tumor heterogeneity: snH3K4me1



Legend: (a-b) UMAP representation of snH3K4me1 datasets. Nuclei are colored according to patient ID, sample type (pre/post/recurrence) and cell type. (c) H3K4me1 gene activity for marker genes of each cell subpopulations and associated genomic tracks. (d) Analysis of TF motif on H3K4me1 peaks specific to each cell population. (e) Cumulative snH3K4me1 tracks for tumor cells with the highest H3K4me1 gene activity for genes specific to each cell state.

We can access epigenomic tumor heterogeneity with snH3K4me1 profiling and identify key transcription factors within each cell populations. In tumor cells, we could observe that some cell state identified above are primed with H3K4me1 in a subset of cells only, while others are primed in all tumor cells.

Future directions

Collecting and processing fresh patient samples is logistically challenging and the interface between the clinics and research is sometimes not reliable enough to ensure the processing of very precious samples – e.g. paired samples in a longitudinal study. The development of protocols to generate high quality single-nuclei RNA-Seq data from frozen biopsies gives more control over processing timing and gives the opportunity to access samples from large biobanks containing precious samples that have not been exploited yet. One Biosciences has led snRNAseq retrospective studies in bladder, glioblastoma and ovarian cancer and will open prospective pilot studies in mid 2025 with Institut Curie, Institut Gustave Roussy and AP-HP to characitrize patient samples @ single-cell resolution in less than 2 weeks.
The combination of our initial study with the longitudinal study of Zhang et al. enabled us to detect significant increase and decrease of relevant states after exposure to chemotherapy. Notably, using this landscape of ITH on longitudinal datasets with treatment-naïve and treated samples, we detected the expected effect of chemotherapy on highly proliferative cells, with a decrease in proportions of cells associated with this state. However, we also noticed that most states remain after NAC. It shows (i) the importance to reassess the functional decomposition of a tumor during treatment as it can evolve, (ii) the efficiency of chemotherapy on very specific tumor states and (iii) the need to identify new therapeutic options to tackle the remaining states.