Epigenomic priming shapes chemoresistant tumor states in high-grade serous ovarian cancer

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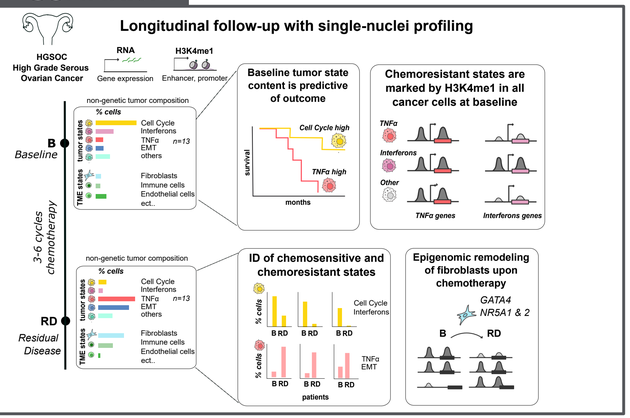
BACKGROUND

Resistance to platinum-based chemotherapy is the main cause of mortality in high-grade serous ovarian cancer (HGSOC). While **intratumor heterogeneity** (ITH) drives treatment failure, it remains unclear whether resistant states arise through therapy-induced reprogramming or selection of pre-existing populations.
Using a clinically compatible multimodal single-nucleus approach, we investigated the epigenomic and transcriptomics basis of tumor and immune cell state dynamics.

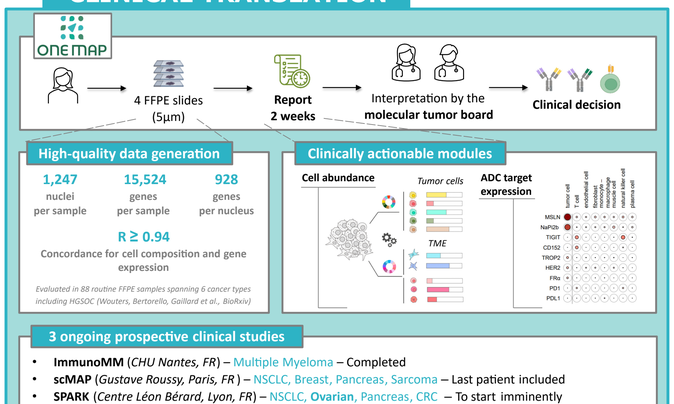
PATIENTS AND METHODS

Within the **SCANDARE** study (NCT03017573), longitudinal biopsies were collected from **16 HGSOC patients** at diagnosis, post–neoadjuvant chemotherapy (NAC), and relapse. We performed multimodal single-nucleus profiling combining **snRNA-seq** and **H3K4me1 CUT&Tag** from **frozen biopsies**. Data from 203,146 nuclei were integrated with public cohorts to define consensus tumor and immune states and their epigenomic encoding.

SUMMARY



CLINICAL TRANSLATION

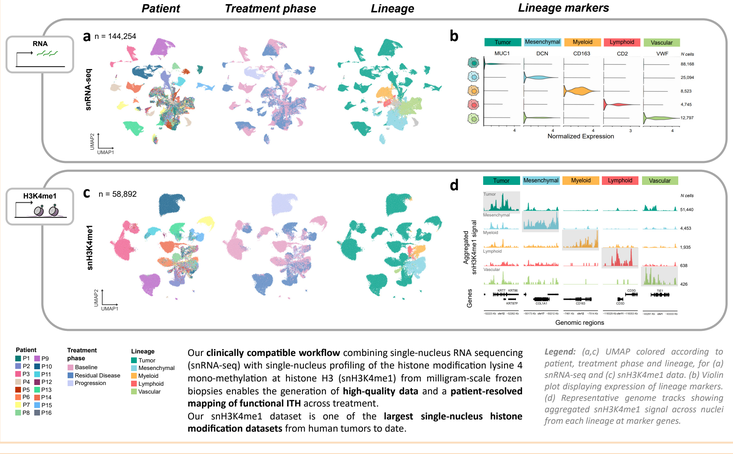


3 ongoing prospective clinical studies

- **ImmunoMM** (*CHU Nantes, FR*) – Multiple Myeloma – Completed
- **scMAP** (*Gustave Roussy, Paris, FR*) – NSCLC, Breast, Pancreas, Sarcoma – Last patient included
- **SPARK** (*Centre Léon Bérard, Lyon, FR*) – NSCLC, **Ovarian**, Pancreas, CRC – To start imminently

RESULTS

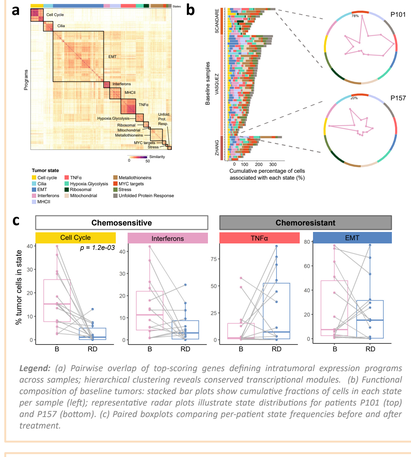
Generation of high-quality transcriptomic and epigenomic datasets



Our **clinically compatible workflow** combining single-nucleus RNA sequencing (snRNA-seq) with single-nucleus profiling of the histone modification lysine 4 mono-methylation at histone H3 (snH3K4me1) from milligram-scale frozen biopsies enables the generation of **high-quality data** and a **patient-resolved mapping of functional ITH** across treatment.
Our snH3K4me1 dataset is one of the **largest single-nucleus histone modification datasets** from human tumors to date.

Legend: *(a,c) UMAP colored according to patient, treatment phase and lineage, for (a) snRNA-seq and (c) snH3K4me1 data. (b) Violin plot displaying expression of lineage markers. (d) Representative genome tracks showing aggregated snH3K4me1 signal across nuclei from each lineage at marker genes.*

Identification of chemoresistant tumor states

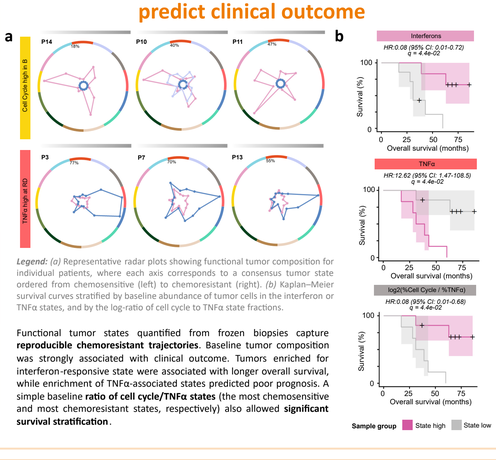


Legend: *(a) Pairwise overlap of top-scoring genes defining intratumoral expression programs across samples; hierarchical clustering reveals conserved transcriptional modules. (b) Functional composition of baseline tumors: stacked bar plots show cumulative fractions of cells in each state per sample (left); representative radar plots illustrate state distributions for patients P101 (top) and P157 (bottom). (c) Paired boxplots comparing per-patient state frequencies before and after treatment.*

We developed a framework to identify **HGSOC tumor states** from a large discovery cohort comprising two public datasets and one generated from fresh SCANDARE samples (205,321 tumor cells).
We identified **13 tumor states.** The proportion of cells in each state can be computed to establish a **sample tumor state profile**.

We compared the percentage of cells in each tumor state in baseline 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 significant differences in cell proportion in untreated vs residual tumors.

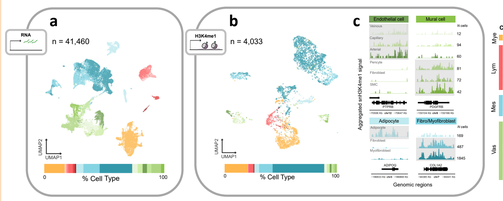
Monitoring baseline tumor composition to predict clinical outcome



Legend: *(a)* Representative radar plots showing functional tumor composition for individual patients, where each axis corresponds to a consensus tumor state ordered from chemosensitive (left) to chemoresistant (right). *(b)* Kaplan–Meier survival curves stratified by baseline abundance of tumor cells in the interferon or TNFα states, and by the log-ratio of cell cycle to TNFα state fractions.

Functional tumor states quantified from frozen biopsies capture **reproducible chemoresistant trajectories**. Baseline tumor composition was strongly associated with clinical outcome. Tumors enriched for interferon-responsive state were associated with longer overall survival, while enrichment of TNFα-associated states predicted poor prognosis. A simple baseline **ratio of cell cycle/TNFα states** (the most chemosensitive and most chemoresistant states, respectively) also allowed **significant survival stratification**.

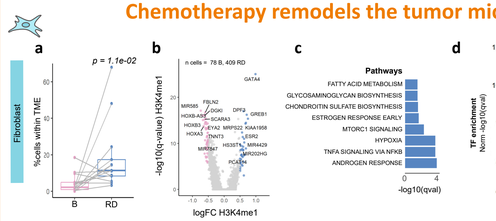
Resolving high cell type granularity from both datasets



Legend: *(a,b) UMAP colored according to cell type, for (a) snRNA-seq and (c) snH3K4me1 data. (c) Representative genome tracks showing aggregated snH3K4me1 signal across nuclei from vascular (top) and mesenchymal (bottom) cell types at marker genes.*

Single-nucleus profiling resolves coordinated transcriptional and epigenomic diversity and provides an **integrated framework** for **dissecting non-genetic ITH** across **immune** and **stromal** compartments with **unprecedented resolution**.

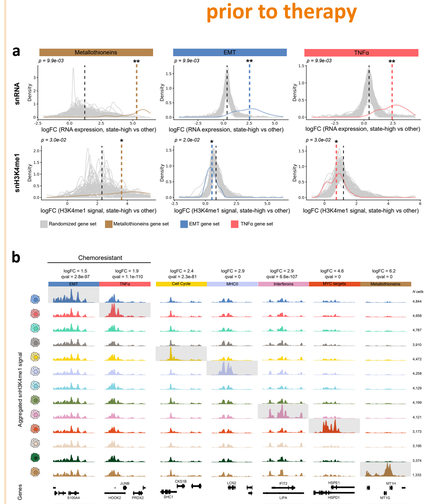
Chemotherapy remodels the tumor microenvironment



Legend: *(a) Paired boxplot showing the proportion of fibroblasts before (B) and after treatment (RD). (b) Volcano plot of differential H3K4me1 activity across fibroblasts before and after treatment. (c) Pathway enrichment for genes up-regulated in fibroblasts after chemotherapy based on pseudo-bulk snRNA-seq analysis. (d) Scatter plot showing TF-motif enrichment in top-specific peaks from RD fibroblasts, highlighting candidate regulators of fibroblast reprogramming.*

We show that chemotherapy induces **coordinated remodeling of the tumor microenvironment** at both the cellular and epigenomic levels, with fibroblasts undergoing pronounced enhancer reprogramming.
Tumor and stromal compartments evolve in parallel under therapeutic pressure, collectively establishing a **chemoresistant tumor ecosystem**.

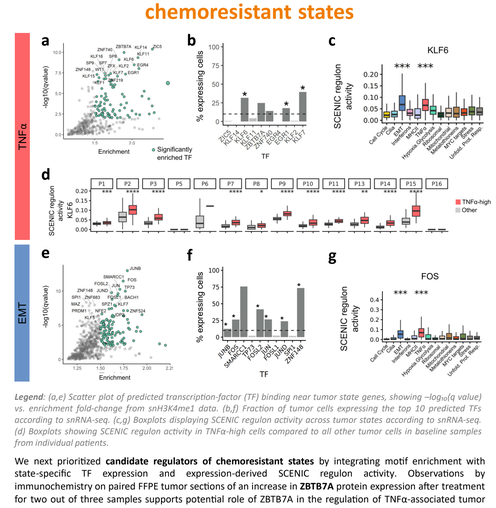
Enhancer landscapes encode chemoresistant states prior to therapy



Legend: *(a) Density plot representing the log fold change at state genes of snRNA expression (top) and snH3K4me1 signal (bottom) for 3 states, along with similar measurements from randomized gene sets of similar size. Two-sided empirical p-values were computed based on the deviation of the observed statistic from the mean of the null distribution. (b) Representative genome tracks showing aggregated snH3K4me1 signal across nuclei from individual states, with corresponding $\log_2$ fold-change and q values for differential activity indicated above each locus.*

While most programs are specifically enriched for H3K4me1 in a subset of tumor cells - mirroring their restricted expression patterns - the TNFα and EMT programs exhibited **significantly broader H3K4me1 distributions** across tumor cells than expected from matched random gene sets, suggesting that a large fraction of tumor cells retains the **regulatory potential to activate TNFα- and EMT-associated programs under therapeutic stress**.

Candidate transcription factor regulators of chemoresistant states



Legend: *(a,e) Scatter plot of predicted transcription-factor (TF) binding near tumor state genes, showing $-\log_{10}$(q value) vs. enrichment fold-change from snH3K4me1 data. (b,f) Fraction of tumor cells expressing the top 10 predicted TFs according to snRNA-seq. (c,g) Boxplots displaying SCENIC regulon activity across tumor states according to snRNA-seq. (d) Boxplots showing SCENIC regulon activity in TNFα-high cells compared to all other tumor cells in baseline samples from individual patients.*

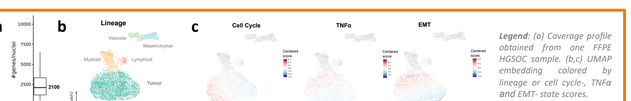
We next prioritized **candidate regulators of chemoresistant states** by integrating motif enrichment with state-specific TF expression and expression-derived SCENIC regulon activity. Observations by immunochemistry on paired FFPE tumor sections of an increase in **ZBTB7A** protein expression after treatment for two out of three samples supports potential role of ZBTB7A in the regulation of TNFα-associated tumor state.

HIGHLIGHTS

- **Epigenomic priming** emerges as a potential determinant of therapeutic resistance.
- **Baseline tumor profiling** could identify patients at risk of relapse and guide earlier interventions.
- **Multimodal single-nucleus profiling** provides a scalable framework to anticipate and potentially intercept non-genetic resistance mechanisms.

PERSPECTIVES

We further show proof of concept that our workflow is **compatible with FFPE tumor sections**, which is instrumental to support the deployment of ITH quantification to **routine clinical practice**.



Legend: *(a) Coverage profile obtained from one FFPE HGSOC sample. (b,c) UMAP embedding colored by lineage or cell cycle-, TNFα and EMT- state scores.*

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