

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

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## 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.

## 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

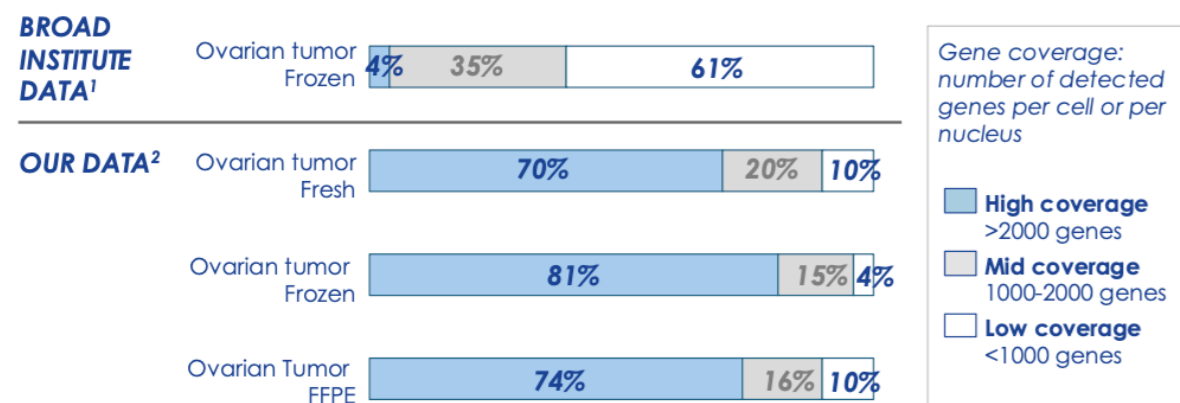
A combination of wet-lab and AI developments to produce snRNAseq from frozen and FFPE samples with high signal/noise ratio and a majority of nuclei > 1,000 genes

We have set a new standard

More than 60%<sup>2</sup> 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



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

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

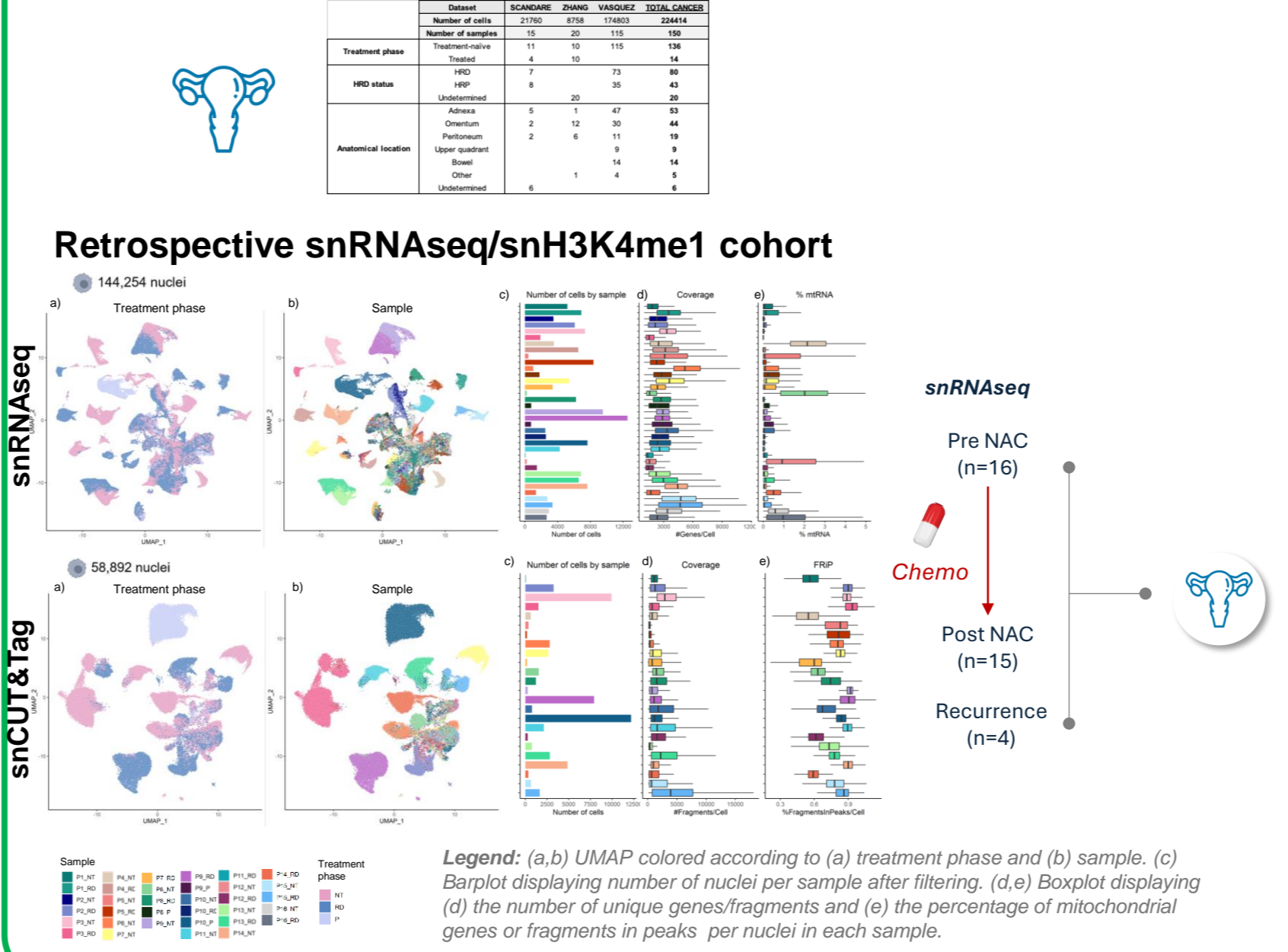
## Future directions

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 characterize patient samples @ single-cell resolution in less than 2 weeks.

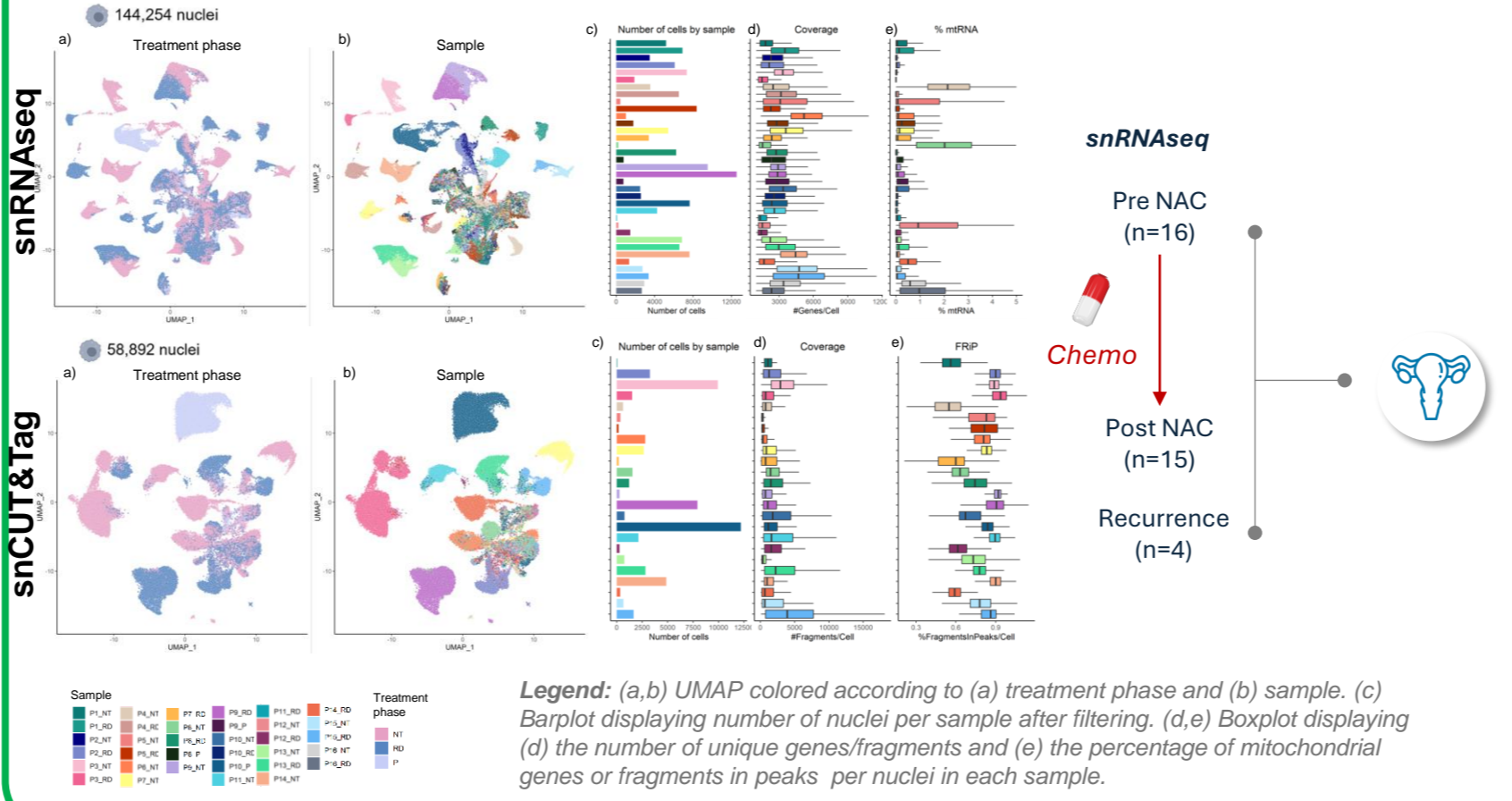
The combination of our initial study with the longitudinal study of Zhang et al. enabled us to detect significant increase and decrease of relevant states after exposure to chemotherapy. Notably, using this landscape of ITH on longitudinal datasets with treatment-naïve and treated samples, we detected 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.

## Transcriptomic and epigenomic data sets

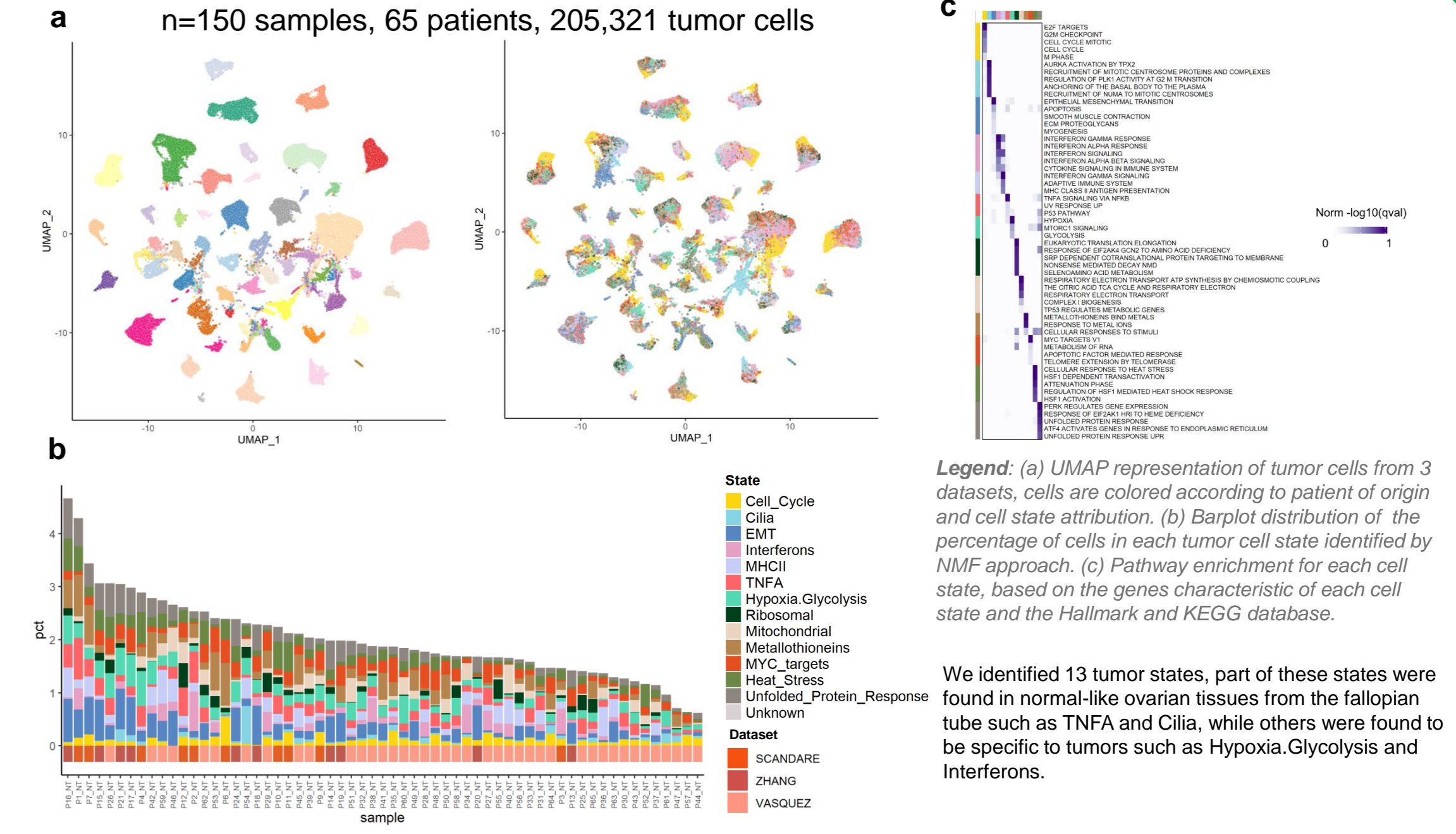
Large scRNAseq public & Curie SCANDARE cohort



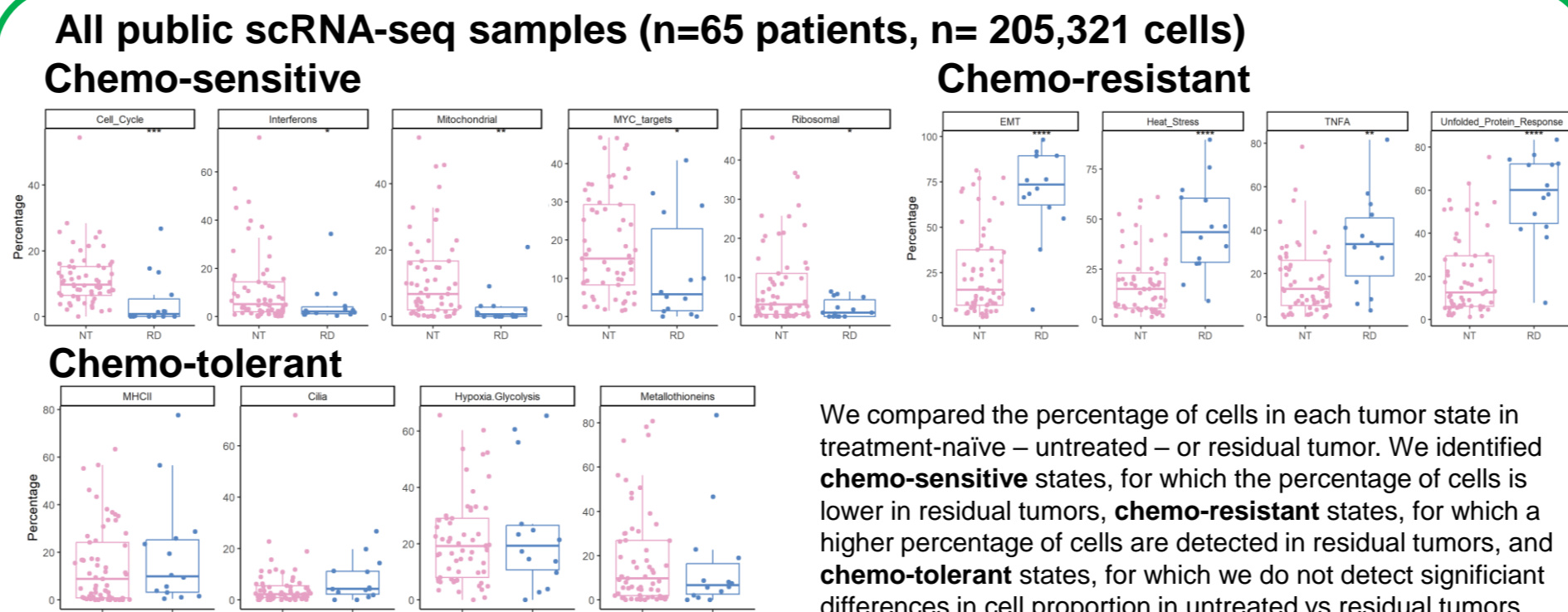
Retrospective snRNAseq/snH3K4me1 cohort



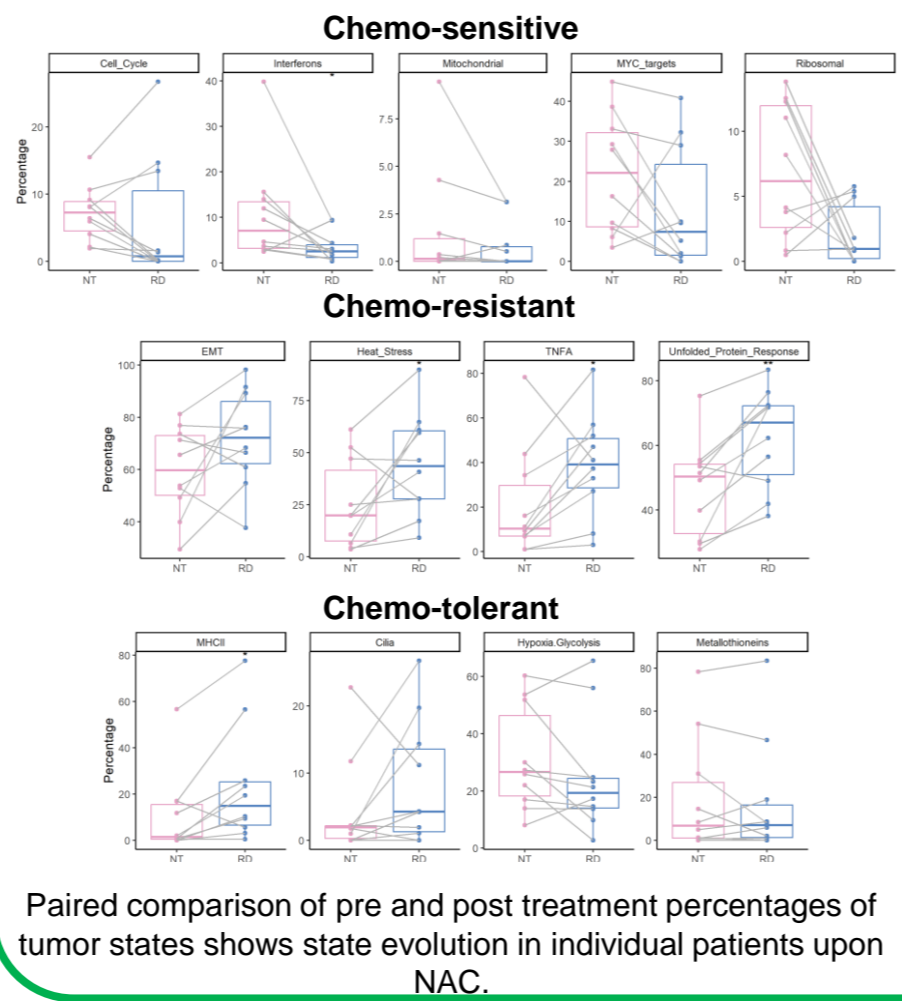
## ID of consensus cell states in ovarian cancer across scRNAseq datasets



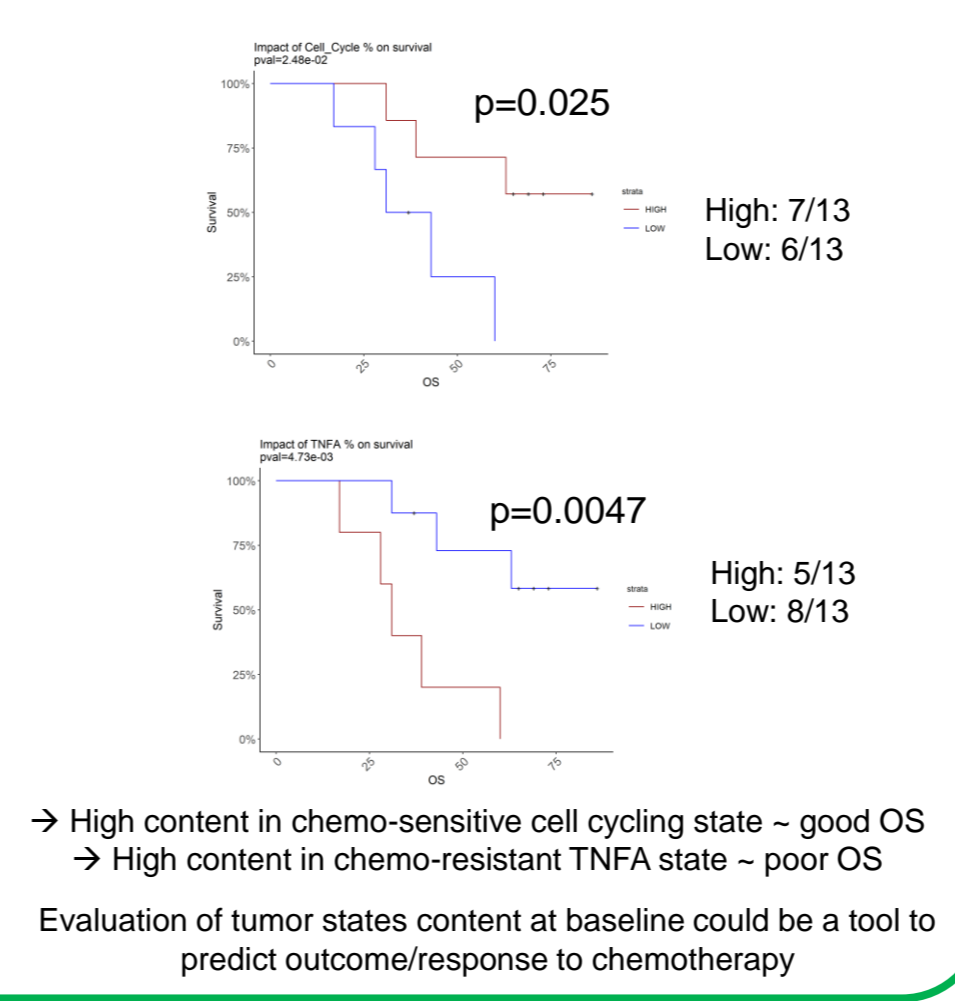
## Sc/snRNAseq for longitudinal follow-up of tumor states



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



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



## Epigenomic encoding of tumor heterogeneity: snH3K4me1

