# Unlocking the Tumor Microenvironment: Accelerating Precision Oncology with Single Cell Sequencing

## Introduction

Tumors are complex ecosystems composed of diverse cell types, including cancer cells, immune cells, stromal fibroblasts, and vascular networks. This cellular heterogeneity plays a critical role in tumor progression, therapeutic resistance, and variability in patient outcomes. Although traditional bulk RNA sequencing is widely used in oncology research, it measures average gene expression across all cells in a tumor, often obscuring rare but clinically important cell populations that can drive drug resistance or metastasis.

To develop more effective therapies, it is essential to resolve this intra-tumor heterogeneity and understand the intricate cellular interactions within the tumor microenvironment (TME). Single cell RNA sequencing (scRNA-seq) addresses this challenge by enabling transcriptomic profiling at single cell resolution. This technology allows researchers to identify distinct cell populations, reconstruct cellular trajectories, and uncover molecular mechanisms underlying therapeutic response and resistance.

### From Tissue to Clinical Insight

We offer a full range of single cell multi-omics solutions to address key oncology challenges including tumor heterogeneity, rare cell detection and treatment resistance.

| Solutions | Features |
|---|---|
| **sCelLiVE® Tissue Preservation and Dissociation Kit** | Preservation and dissociation of fresh, clinical samples including biopsies. |
| **GEXSCOPE® Single Cell RNA Library Kit** | High-sensitivity gene expression profiling. |
| **GEXSCOPE® Single Nucleus RNA Library Kit** | Optimized for frozen samples and difficult-to-dissociate tissues (e.g., bone, brain). |
| **FocuSCOPE® Single Cell mRNA Capture Kit** | Simultaneous detection of gene expression, targeted mutations (SNVs, gene fusions), viral and other non-poly(A) RNAs. |
| **MobiuSCOPE® Full Length Single Cell RNA Sequencing Kit** | Combines 3′ and 5′ RNA-seq approaches in one solution, providing full-length transcript insight. |

## Singleron Single Cell Analysis Workflow

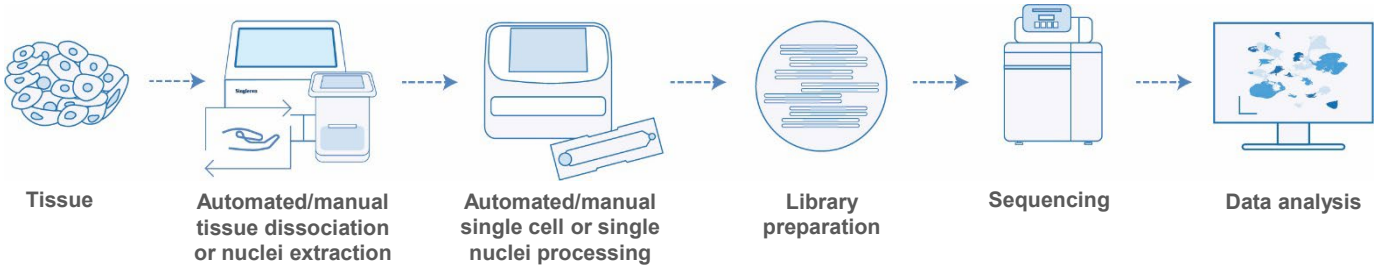


**Figure 1.** Single cell analysis workflow

## Case Study 1. Revitalizing Immunotherapy

In a landmark 2024 study published in ***Nature***, Feng et al. (2024) utilized scRNA-seq to investigate novel strategies for enhancing cancer immunotherapy. While Type 1 immune responses are the standard target for cancer treatment, they often fail to achieve long-term remission. The study explored the potential of Fc–IL-4, a Type 2 cytokine-based therapy, to revitalize exhausted CD8⁺ Terminally Exhausted-like ($T_{TE}$) cells.

The scRNA-seq analysis clustered 15,983 single cells into transcriptionally distinct groups.

Data revealed that Fc–IL-4 treatment selectively expanded a specific population of CD8⁺ $T_{TE}$ cells.

Differential gene expression analysis showed these expanded cells expressed high levels of functional effector genes such as *Ifng* (Interferon-gamma) and *Gzmb* (Granzyme B), confirming that the therapy successfully reprogrammed the T cells to attack the tumor.

**Case study: The type 2 cytokine Fc–IL-4 revitalizes exhausted CD8⁺ T cells against cancer**

Scan to read the full article in *Nature* →

**Data Showcase:**

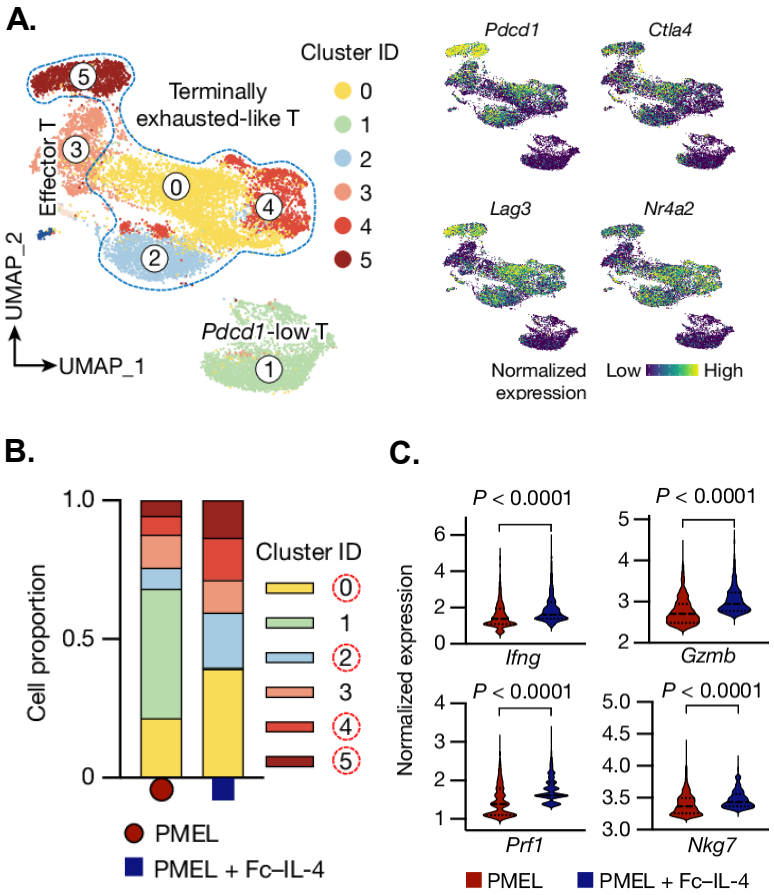


**Figure 2.** Mouse tumor tissues were collected and PMEL CD8⁺ T cells were sorted for scRNA-seq. **A.** UMAP clustering of all the PMEL CD8⁺ T cells with co-inhibitory gene marker expression on the UMAP. **B.** Comparison of cell proportion in each cluster. **C.** Comparison of functional gene expression in the $T_{TE}$ cells. Image from Feng et al. (2024). CC BY 4.0

## Reference

Feng, B., Bai, Z., Zhou, X. et al. (2024). The type 2 cytokine Fc–IL-4 revitalizes exhausted CD8⁺ T cells against cancer. *Nature* **634**, 712–720. https://doi.org/10.1038/s41586-024-07962-4

## Case Study 2. Profiling Tumor Heterogeneity

Advanced non-small cell lung cancer (NSCLC) is characterized by profound cellular and molecular heterogeneity, making it one of the most clinically challenging malignancies to study and treat. Key obstacles include the extremely limited tissue availability from minimally invasive biopsies and the poor viability of tumor cells during sample collection and processing. To address these challenges, Wu et al. (2021) applied sCelLiVe tissue preservation solution to preserve cell integrity and enable high-quality single cell data even from low viability samples, followed by GEXSCOPE single-cell RNA sequencing (scRNA-seq) platform to minimize cell loss during sample loading, enabling efficient capture of scarce cells.

42 tissue biopsy samples from stage III/IV NSCLC patients were analyzed by scRNA-seq. Rare tumor cell populations, including follicular dendritic cells and T helper 17 cells were identified. The study revealed an association between tumor heterogeneity and tumor-associated neutrophils, providing insights into their potential roles in NSCLC progression.

**Case study: Single cell profiling of tumor heterogeneity in advanced NSCLC**

Scan to read the full article in *Nature Communications*→

**Data Showcase:**

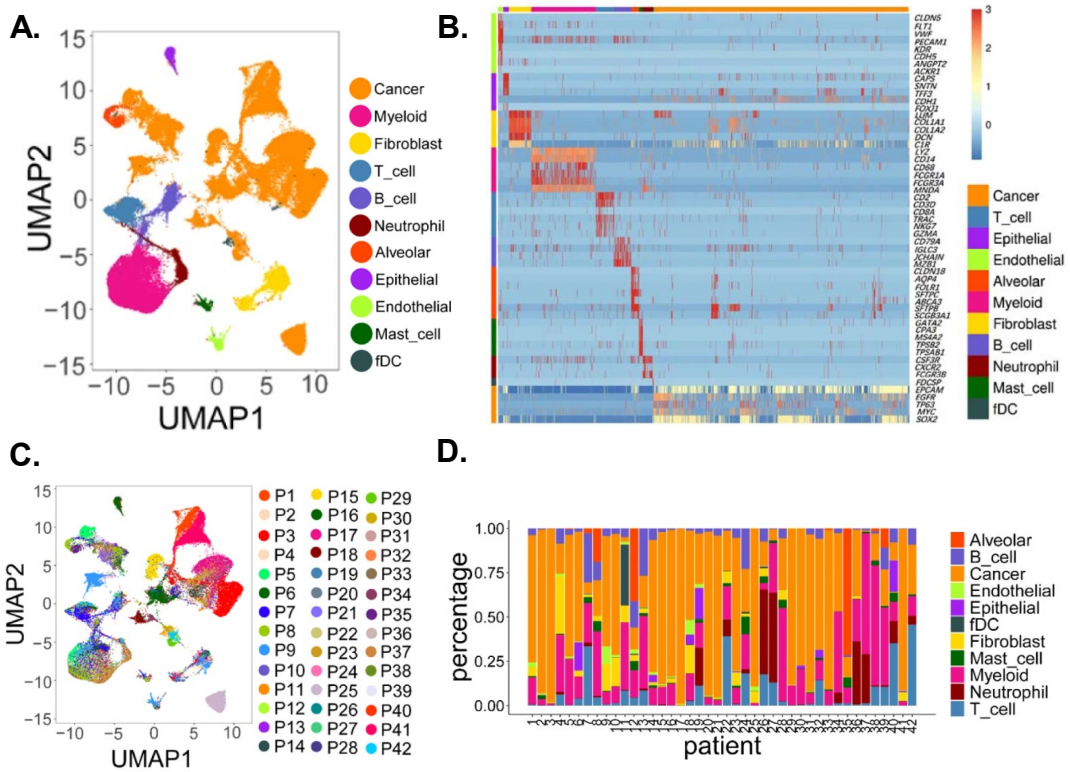


**Figure 3. A.** UMAP plot of 90,406 cells from 42 patients, colored by their 11 major cell types. **B.** Heatmap of canonical cell-type markers of 11 major cell types. **C.** UMAP plot of all cells, colored by patients. **D.** Major cell-type composition of each patient. Biopsies were all taken from the primary lung tumors. Image from Wu et al. (2021). CC BY 4.0

## Reference

Wu, F., Fan, J., He, Y. *et al (2021).* Single cell profiling of tumor heterogeneity and the microenvironment in advanced non-small cell lung cancer. *Nat Commun* **12**, 2540. https://doi.org/10.1038/s41467-021-22801-0

## Case Study 3. Identifying Gene Mutation Phenotypes

A study by Shen et al. (2023) in ***Cell Biochemistry & Function*** utilized Singleron targeted scRNA-seq (FocuSCOPE) workflow to examine the bone marrow (BM) cells of patients with Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML). A targeted oncogenic gene panel was used to overcome the limitation of conventional scRNA-seq in detecting oncogenic mutations, aiming to identify how specific oncogenic mutations correlate with different cellular lineages.

The distribution of oncogenic IDH1, IDH2 and KRAS mutations in each cell type was identified in the BM samples of each patient. Progression via these mutations caused hematopoietic maturation arrest, providing a rationale for using primary BM cells for personalized treatment in clinical settings.

**Case study: Targeted scRNA-seq analysis in hematologic malignancies studies**

Scan to learn more in *Cell Biochemistry & Function* →

### Data Showcase:

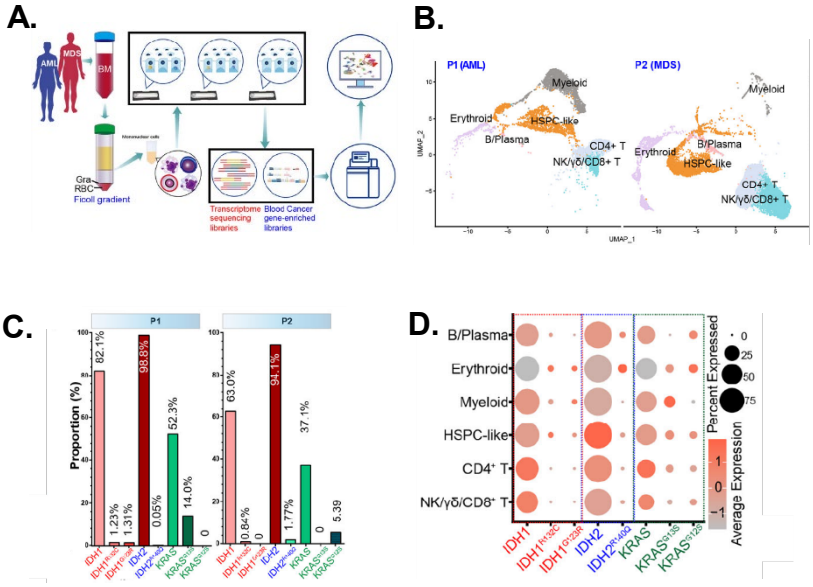


**Figure 4.** Distribution of oncogenic mutations in clonal BM disorders. **A.** Singleron targeted scRNA-seq sample preparation, library construction, and analysis workflow. **B.** UMAP visualization of the cell populations in each sample. **C.** Percentages of the selected variant-expressing cells in each sample. IDH1, IDH2, and KRAS exhibit all the detected transcripts from scRNA-seq libraries. Transcriptomic structural variants of oncogenic IDH1, IDH2, and KRAS were detected in single-cell panel-enriched libraries. **D.** Dot plots showing the expression of selected genes and their variants in different cell subsets. Image from Shen et al. (2023). CC BY 4.0

## Reference

Shen, X., et al. (2023). Targeted single cell RNA sequencing analysis reveals metabolic reprogramming and the ferroptosis-resistant state in hematologic malignancies. *Cell Biochemistry & Function*. doi: 10.1002/cbf.3869.

EUROPE
Singleron Biotechnologies GmbH
+49 (0) 221 16824777
Gottfried-Hagen-Strasse 60
51105 Cologne, Germany
info@singleron.bio

ASIA-PACIFIC
Singleron Biotechnologies Pte. Ltd
+65 6990 5987
#02-08, 61 Science Park Rd,
Singapore 117525
sales.sg@singleron.bio

NORTH AMERICA
Singleron Biotechnologies Inc.
+1 203-298-0604 (CT)
+1 734-249-0883 (MI)
1 Bradley Road, Suite 401 Woodbridge, CT 06525
333 Jackson Plaza, Ann Arbor, MI 48103