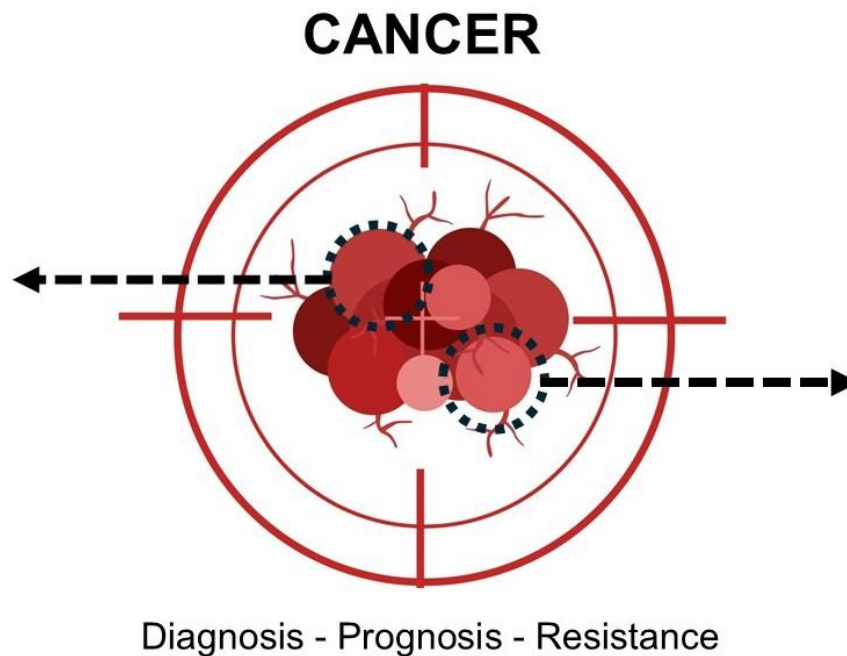


Single-Cell Analysis in Cancer Therapy: Advances in Diagnosis, Prognosis, and Prediction of Drug Resistance



Introduction

Cancer is fundamentally a disease of cellular heterogeneity. Tumors are composed of genetically and phenotypically diverse malignant cells, surrounded by stromal and immune populations that dynamically interact to influence disease progression and therapeutic response. This intra- and intertumoral heterogeneity underlies many of the challenges faced in oncology, from accurate diagnosis to predicting patient prognosis and anticipating drug resistance. Single-cell analysis has emerged as a powerful approach that can be used clinically for cancer patients. Since the advent of single-cell RNA sequencing (scRNA-seq) over a decade ago, the field has rapidly expanded to encompass single-cell DNA sequencing (scDNA-seq), chromatin accessibility profiling (scATAC-seq), single-cell proteomics, and multi-omic methods that simultaneously capture multiple molecular modalities from the same cell. These technologies, now increasingly combined with spatially resolved transcriptomics and proteomics, have enabled insight into the biology of cancer at the resolution of individual cells within their microenvironment.

Recently, studies have demonstrated that single-cell technologies are not merely descriptive but can be applied to clinically meaningful endpoints: identifying diagnostic biomarkers, stratifying patients for prognosis, and predicting or even preempting the emergence of therapeutic resistance. This review focuses on the translational applications of single-cell analysis in cancer therapy. By examining diagnostic, prognostic, and resistance-predictive applications alongside advances in molecular approaches, we provide a comprehensive overview of the current landscape and future directions of single-cell oncology.

Single-Cell Analysis for Cancer Diagnosis

Cancer diagnosis refers to the identification and characterization of malignant disease, including its type, stage, and progression, and it is essential for guiding treatment strategies, monitoring disease course, and predicting outcomes. Conventional technologies for diagnosis, such as tissue biopsies, immunohistochemistry, imaging, and bulk sequencing, remain widely used but have significant limitations, particularly in detecting rare cancer cell populations and subtle molecular changes. Diagnostic conclusions are typically based on integrating histopathological features, tumor markers, imaging results, and molecular assays, but bulk approaches average signals across millions of cells, masking important heterogeneity and preventing the detection of minimal residual disease, resistant clones, and immune cell shifts.

To overcome these limitations, single-cell analysis has emerged as a transformative molecular approach for cancer diagnostics, with single-cell RNA sequencing (scRNA-seq) being the leading technology. New single-cell analysis platforms allow high-throughput transcriptomic profiling of thousands of individual cells, offering unmatched resolution of tumor heterogeneity. Complementary methods, including single-cell DNA sequencing and spatial transcriptomics, further enhance this approach by adding genomic and spatial context to cancer cell populations. Through these methods, single-cell analysis provides detailed diagnostic information on gene expression profiles, molecular signatures, circulating tumor cells, tumor microenvironment composition, and mechanisms of drug resistance. This enables the identification of cancer stem cells, resistant clones, and immune subsets within tumors, and links key molecular signatures to malignant conversion, tumor progression, metastasis potential, therapy resistance, and relapse risk, all providing valuable information to allow an accurate diagnosis. Importantly, the sensitivity of single-cell analysis allows the detection of rare malignant cells and minimal residual disease that bulk sequencing often misses, making it valuable for early detection, patient monitoring, and personalized therapy planning.

Comparison of conventional cancer diagnosis techniques to Single-cell RNA Sequencing (scRNA-seq)

Approach	Cancer Diagnosis	Cancer Type, Stage, Progression	Advantages	Disadvantages
Immuno histochemistry (IHC)	Detects protein expression patterns in tissue sections; identifies presence/absence of tumor markers.	Determines cancer type and progression Limited for precise staging.	Widely used in clinics; relatively inexpensive; provides spatial context of cells within tissue.	Limited molecular resolution; dependent on antibody specificity; semi-quantitative;.
Imaging (e.g., CT, MRI, PET)	Provides anatomical and functional visualization of tumors; detects tumor size, location, and spread.	Excellent for staging and progression (tumor size, metastasis).	Non-invasive; widely available; essential for monitoring tumor burden and treatment response.	Limited molecular information; may miss microscopic disease; resolution depends on modality;
Bulk RNA Sequencing	Measures average gene expression across all cells in a tumor sample. Identifies dysregulated pathways and cancer subtypes.	Useful for cancer type classification and prognosis. Limited for stage and progression	Provides genome-wide transcriptomic data; can identify subtypes and potential therapeutic targets.	Masks cellular heterogeneity; dominated by most abundant cell populations.
Single-cell RNA Sequencing (scRNA-seq)	Profiles gene expression at the resolution of individual cells; reveals tumor heterogeneity, rare populations, and microenvironment interactions.	Can distinguish cancer cell subtypes, identify tumor evolution, and provide insights into progression. Still emerging in clinical staging.	High resolution; identifies rare resistant or stem-like cells; informs tumor microenvironment and immune infiltration.	Technically complex; expensive; requires specialized analysis; limited availability in routine clinical practice.

Clinical applications are still emerging, but several trials demonstrate the diagnostic value of this technology. For example, pediatric leukemia and melanoma studies have successfully integrated scRNA-seq data with clinical outcomes to refine treatment selection and disease monitoring, while analyses of breast cancer subtypes and renal carcinoma have revealed gene expression signatures and immune cell changes that predict malignant progression, resistance, and relapse risk. Together, these findings illustrate that single-cell analysis, particularly scRNA-seq—offers unprecedented precision for cancer diagnosis by capturing rare and clinically significant molecular features, though further validation is required to fully standardize its accuracy and minimize false positives and negatives.

Examples of different cancer types diagnosed using scRNA-seq, including diagnostic data characteristics, and substantiation status

Cancer Type	Data Characteristics Allowing Diagnosis	Diagnosis Substantiation Status
Melanoma	Distinct gene expression signatures unique to melanoma cells; identification of immune cell subtypes within the tumor microenvironment	Supported by correlation with clinical outcomes and immunotherapy response; validated in clinical trial contexts
Renal Cell Carcinoma	Identification of tumor cell subpopulations with unique molecular signatures and chemotherapy resistance profiles	Substantiated through patient prognosis correlation and treatment response data
Glioma	Transcriptomic profiles revealing immune cell infiltration explaining radiological progression beyond tumor growth	Supported by pathological diagnosis and patient clinical follow-up showing sustained response
Breast Cancer	Malignant cells detected by large-scale chromosomal variations, molecular signature refinement correlating with malignancy and subtype	Validated using multiple patient datasets confirming subtype associations and clinical relevance

This table exemplifies how single-cell analysis reveals tumor heterogeneity, immune microenvironment interactions, and genetic alterations to aid cancer diagnosis, with clinical validation supporting its diagnostic value across these cancer types.

Prognostic Applications of Single-Cell Analysis for Cancer

Beyond diagnosis, single-cell approaches have proven powerful in stratifying patients according to prognosis. Prognostic biomarkers are essential for guiding therapeutic decisions, and single-cell analysis provides unique insight into cellular states and interactions that influence outcomes. Cancer prognosis refers to the prediction of



disease progression and patient outcomes, including survival probability and the likelihood of recurrence. Traditional prognostic methods rely on clinicopathological variables such as tumor stage, histological grade, and the presence of specific molecular markers measured in bulk tissue. These approaches, while clinically useful, provide only averaged signals across heterogeneous cell populations, which can obscure critical cellular states influencing disease progression. Prognostic conclusions are typically determined by correlating these molecular and clinical variables with long-term survival data, therapeutic response rates, or recurrence risk in large patient cohorts.

The specific information gained from single-cell analysis includes the identification of stem-like or proliferative immune subsets, characterization of tumor-infiltrating lymphocytes, delineation of stromal subtypes, and mapping of aggressive cellular phenotypes. These data can be directly used to stratify patients into prognostic categories, such as predicting which individuals are more likely to respond to immune checkpoint blockade or which tumors exhibit invasive trajectories associated with high recurrence risk.

The use of single-profiling in melanoma and other cancers has revealed that the presence of stem-like or proliferative T-cell subsets strongly correlates with improved response to checkpoint blockade therapies and better long-term outcomes. In pancreatic cancer, integrated single-cell and spatial transcriptomic analyses identified cellular subtypes responsible for neural invasion, a feature strongly associated with poor prognosis and reduced survival. Together, these studies illustrate how single-cell technologies are advancing cancer prognosis by uncovering cellular states and interactions that cannot be detected by conventional bulk methods, ultimately enabling more precise patient stratification and guiding personalized therapeutic decisions.

Prediction of Drug Resistance with Single-Cell Analysis

Therapeutic resistance remains one of the most formidable barriers in oncology, leading to relapse and treatment failure. Genomic and transcriptomic profiling through next-generation sequencing (NGS) is currently the most established approach for predicting drug resistance in cancer therapy. By detecting mutations, copy number variations, and expression signatures, this strategy reveals mechanisms such as secondary *EGFR* or *BCR-ABL* mutations, activation of bypass pathways, or upregulation of drug efflux pumps. Clinically, FDA-approved assays are already in use to guide therapy selection and resistance monitoring.

Single-cell RNA sequencing, ATAC-seq, and proteomics enable high-resolution mapping of resistant subclones and drug-tolerant populations, which are often undetectable in bulk analyses. This approach uncovers transcriptional reprogramming and lineage plasticity that drive therapy escape. While highly informative in research,

clinical use remains limited due to cost, scalability, and lack of standardized workflows. Nevertheless, pilot trials are testing single-cell technologies to predict resistance in hematologic malignancies and solid tumors, suggesting future translational potential.

Approaches, mechanisms, and the type of information they provide to guide resistance prediction.

Approach	Mechanism	Type of Information for Drug Resistance Prediction
scRNA-seq	Profiles transcriptomes of individual tumor cells to identify rare resistant clones defined by activation of stress-response and survival pathways.	Detects pre-existing resistant subpopulations within tumors before clinical relapse.
Machine learning applied to scRNA-seq data	Uses computational models to analyze high-dimensional single-cell transcriptomic data, enabling prediction of therapeutic outcomes.	Predicts patient-level therapy responses and resistance more accurately than bulk transcriptomics.
Spatial multiomics	Integrates spatial transcriptomics and proteomics to map tumor–stroma–immune interactions within the microenvironment.	Reveals resistant niches shaped by cell–cell interactions, particularly under immunotherapy, and identifies intervention points.
Immune cell analysis	Characterizes phenotypic and functional states of immune cells such as T cells and macrophages within tumors.	Links immune cell states to therapy sensitivity or resistance, allowing identification of immune features predictive of patient outcomes.
Computational frameworks	Develops models to track gene regulatory context shifts (e.g., gene context drift) under therapy using single-cell data.	Identifies dynamic changes in regulatory networks, uncovers new drug targets, and enables predictive modeling of resistance evolution.

In summary, single-cell approaches provide complementary layers of information, from intrinsic tumor cell programs to microenvironmental and immune influences, that collectively enhance our ability to predict and counteract drug resistance. These methods not only inform prognosis but also guide the development of adaptive therapeutic strategies.

Leading Single-Cell Methods in Cancer Diagnosis, Prognosis, and Prediction of Drug Resistance

Several unique approaches are being employed to interrogate single cancer cells. Below is a sampling of contemporary technologies.

Single-cell RNA sequencing (scRNA-seq):

- Distinguishes multiple cancer cell types, reveals rare subpopulations, and enables comprehensive gene expression profiling, critical for precise tumor classification and guiding individualized therapies.

Single-cell DNA sequencing (scDNA-seq):

- Identifies cancer-driving mutations, copy number alterations (CNAs), and tumor phylogeny to accurately detect primary and metastatic tumors, track clonal evolution, and uncover genetic heterogeneity.

Single-cell ATAC sequencing (scATAC-seq):

- Maps chromatin accessibility at single-cell resolution, revealing regulatory elements and epigenetic changes unique to cancer cells versus normal or stromal compartments.

scCITE-seq (Cellular Indexing of Transcriptomes and Epitopes by sequencing):

- Simultaneously profiles surface protein markers and the transcriptome, dramatically improving immune cell phenotyping to identify tumor-reactive cells, and prognostic biomarkers.

Single-cell proteomics (Mass cytometry, CyTOF):

- Measures protein expression directly on individual cells, supporting rapid classification of malignancy, assessment of treatment resistance, and real-time monitoring of circulating tumor cells (CTCs).

Single-cell spatial transcriptomics and in situ sequencing (FISSEQ, MERFISH, seqFISH):

- Locates cancerous and immune cells in tissue samples, preserving spatial relationships and linking genomic changes to histopathology, advancing tissue-based diagnostics and prognosis.

Computational tools (eg., Cancer-Finder, PanClassif):

- Deep learning algorithms rapidly annotate malignant cells in single-cell datasets, with 95%+ accuracy in malignant cell detection, bringing advanced bioinformatics to clinical diagnostic pipelines.

These methods pinpoint molecular heterogeneity, detect rare cell populations, and identify actionable mutations or protein markers. They facilitate robust tumor classification, improve early detection of minimal residual disease, and enable prediction of treatment response far beyond traditional histology or bulk sequencing.



Conclusion

Single-cell analysis has transformed our understanding of cancer heterogeneity, providing insight into the molecular and cellular ecosystems that drive disease. High-impact studies have demonstrated the translational value of single-cell technologies in diagnosis, prognosis, and prediction of therapeutic resistance. Advances in transcriptomics, genomics, epigenomics, proteomics, and multi-omics at single-cell resolution are converging to generate comprehensive maps of tumors and their microenvironments.

Looking forward, integration of single-cell data into clinical decision-making pipelines is likely to reshape oncology. Diagnostic workflows will increasingly incorporate single-cell and spatial signatures, prognostic models will stratify patients based on cellular states, and predictive pipelines will guide therapy selection and resistance monitoring. Ultimately, single-cell technologies hold the potential to enable personalized cancer treatment strategies, improve patient outcomes, and bring us closer to the long-sought goal of precision oncology.