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Single-Cell RNA Sequencing: A Powerful Tool for Advancing Precision Oncology Drug Development

Md Atiq Ashhab

Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi 6205, Bangladesh; atiq.ashhab1996@gmail.com
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Abstract: Single-cell RNA sequencing (scRNA-seq) has transformed precision oncology by allowing for high-resolution transcriptome investigation at the individual cell level. Unlike bulk RNA sequencing, which yields averaged gene expression data, scRNA-seq exposes cellular heterogeneity inside tumors, detecting rare cancer subpopulations, stem-like cells, and drug-resistant clones. This skill has major implications for tumor drug discovery, enabling researchers to identify new therapeutic targets, anticipate patient-specific medication responses, and devise more accurate treatment plans. Furthermore, scRNA-seq allows for a better knowledge of tumor microenvironment interactions, revealing information on the roles of immune and stromal cells in cancer growth and therapeutic resistance. Recent advances in scRNA-seq technologies, including as droplet-based sequencing systems and spatial transcriptomics, have increased their usefulness in oncology research. Droplet-based technologies allow scientists to study tumor differences in much greater detail than ever before, utilizing platforms created by 10× Genomics and Drop-seq, which allow the high-throughput collection and barcoding of thousands of individual cells. These systems enable researchers to find rare cell groups that are frequently undetectable with bulk RNA-seq, specifically, cancer stem cells or drug-resistant clones. Spatial transcriptomics, on the other hand, maps different cellular subpopulations directly within the tumor microenvironment by fusing tissue architecture and gene expression profiling. Understanding cancer growth and treatment resistance requires knowledge of immune infiltration patterns, cell-cell interactions, and tumor evolution dynamics, all of which are crucially revealed by this technique. All of these developments have combined to make scRNA-seq an effective tool for identifying new biomarkers, predicting treatment outcomes, and directing the creation of precision oncology plans. Furthermore, the combination of artificial intelligence (AI) and machine learning has improved the interpretation of scRNA-seq datasets, allowing for the discovery of major oncogenic pathways and possible therapeutic candidates. Despite its transformative promise, scRNA-seq faces significant barriers to widespread use in clinical cancer, including high sequencing costs, technical constraints in single-cell isolation, and the complexity of bioinformatics analysis. This study investigates the present applications of scRNA-seq in tumor drug discovery, focusing on recent advances in discovering druggable targets, tracking tumor progression, and overcoming therapeutic resistance. We also highlight new tactics for overcoming current difficulties, including as advancements in multi-omics integration and computational modeling. As scRNA-seq advances, it is predicted to play a critical role in bringing precision oncology into clinical practice, ultimately improving cancer treatment outcomes through more effective and tailored therapeutic interventions.

Keywords: scRNA-seq; precision oncology; drug discovery

1. Background

Cancer research has experienced a dramatic transition since the introduction of single-cell RNA sequencing (scRNA-seq) technology. This sophisticated technology has given researchers unparalleled insights into the genetic diversity and complexity of cancer cells, paving the path for advances in precision oncology treatment development [1,2]. Precision oncology is a tailored approach to cancer therapy that tailors medicines to the genetic and molecular characteristics of a patient's tumor. This is critical for customized medicine because it enables for the development of specific therapies that are more successful and have fewer negative effects than standard, one-size-fits-all treatments [3,4]. Precision oncology uses insights from single-cell RNA sequencing to identify specific genetic abnormalities inside a patient's cancer cells, allowing for the selection of the most appropriate and effective therapy [5].

While bulk RNA sequencing and conventional drug screening in tumors have proven useful, they are restricted in their ability to capture the full complexity and heterogeneity of cancer cells. Bulk sequencing averages a population's gene expression profiles, hiding important variations between individual cells. Similarly, traditional drug screening in bulk tumor samples fails to account for the varied subpopulations of cells within a tumor, potentially excluding viable medicines that target specific cell types [6]. In contrast, single-cell RNA sequencing provides a high-resolution view of the genetic and transcriptional landscape of individual cancer cells, allowing for the identification of uncommon, drug-resistant cell subsets and the development of more customized, successful therapies [2,7]. This innovation in cancer research has paved the door for more tailored and effective treatment development, resulting in better patient outcomes and quality of life.

Single-cell RNA sequencing is a powerful approach for analyzing gene expression levels in individual cells, offering previously unknown insights into cancer cells' genetic diversity and complexity [8]. This groundbreaking technology has transformed cancer research, allowing for the discovery of distinct genetic abnormalities inside a patient's cancer cells and the development of more customized and effective medicines, ultimately enhancing patient outcomes and quality of life. Despite this, tumor heterogeneity is a major problem in oncology since it affects drug resistance and calls for sophisticated therapeutic intervention techniques [9]. The innate genetic and phenotypic diversity of cells within a single tumor makes precision cancer treatments less successful and frequently results in the selection and enrichment of drug-resistant subclones [10,11]. Therefore, it is essential to comprehend the intricate molecular processes causing this variability in order to create methods that effectively overcome therapeutic resistance and enhance patient outcomes [12]. A potent method for analyzing this heterogeneity is single-cell RNA sequencing (scRNA-seq), which makes it possible to find new treatment targets and create plans to counteract medication resistance [13].

Even though scRNA-seq analysis has advanced significantly, some issues still exist that need more research. Clustering, which is essential for describing cellular heterogeneity, locating uncommon cell types, and examining cell–cell interactions, is one such difficulty [14]. Another significant issue is data sparsity, which reduces the accuracy of downstream analysis like clustering and trajectory inference since a significant amount of the gene–cell expression matrix comprises zeros as a result of either technological dropouts or real biological absence [15]. Batch effects, or non-biological differences introduced during sample preparation or sequencing, are another major problem. They frequently cause biologically comparable cells to cluster apart, leading to inaccurate interpretations of cell diversity [15]. Addressing these challenges will be critical for accurate identification of cell types, rare populations, and for translating scRNA-seq findings into clinical applications.

This review will look at how single-cell RNA sequencing has transformed cancer research by allowing the identification of unique genetic aberrations within a patient's cancer cells. This has facilitated the development of more personalized and effective therapies.

2. Overview of Single-Cell RNA Sequencing Technology

Single-cell RNA sequencing (scRNA-seq) is an advanced genomic technology that allows for the investigation of gene expression levels in individual cells. This method involves isolating individual cells, extracting and sequencing their RNA content, and then evaluating the results to provide insight into the genetic diversity and transcriptional patterns of distinct cell types within a sample [16,17]. The procedure for single-cell RNA sequencing (scRNA-seq) includes sample preparation, cell isolation, sequencing, and data interpretation. The sample is first prepared by separating the tissue into a single-cell solution. Individual cells are subsequently isolated, commonly utilizing microfluidic devices or fluorescence-activated cell sorting. Next, each cell's RNA is harvested and transformed into a sequencing library [18]. High-throughput sequencing is performed on these libraries, resulting in massive volumes of gene expression data for individual cells [19]. Finally, bioinformatic analysis of single-cell RNA sequencing data enables the identification of discrete cell subpopulations and gene expression patterns, offering a level of clarity and depth unavailable with bulk RNA sequencing.

Bulk RNA sequencing averages gene expression levels over a population of cells, hiding important variations between individual cells [20]. In contrast, single-cell RNA sequencing allows researchers to characterize cellular heterogeneity within a sample, allowing them to identify rare cell types and subpopulations that may be critical for disease development and therapy response [21,22].

The discipline of single-cell RNA sequencing (scRNA-seq) has made remarkable progress in recent years, thanks to the development of numerous platforms and technologies that have transformed the way we research cellular heterogeneity. Some of the major platforms utilized for scRNA-seq include Table 1 [23]:

1. 10× Genomics: This platform utilizes microfluidics to capture and barcode individual cells, enabling the analysis of thousands of cells in a single experiment.
2. Smart-seq: This method uses a template-switching approach to generate full-length cDNA from single cells, providing comprehensive transcriptome coverage.
3. Drop-seq: This platform employs a droplet-based system to encapsulate single cells and perform barcoded RNA sequencing.
4. InDrop: This microfluidic-based technique enables the capture and analysis of individual cells in nanoliter-scale droplets.
5. Fluidigm C1: This system uses an integrated fluidic circuit to isolate and process individual cells for scRNA-seq.
6. Seq-Well: This platform utilizes a simple and scalable microwell-based approach for scRNA-seq.
7. BD Rhapsody: This system combines microfluidics and molecular barcoding to enable high-throughput scRNA-seq analysis.
8. Chromium: Developed by 10× Genomics, this platform utilizes a microfluidic-based approach to capture and barcode individual cells.

Table 1. Comparison of different single cell RNA-sequencing technologies.

Platforms	Targets RNA Type	Isolation	Cell Number	Amplification	Transcript Region	UMI	Advantage	References
10× Genomics	No poly(A) minus RNA detection	Microdroplets	$<10^5$	PCR	3' end	Yes	cost-effective per cell, well-established computational pipelines, excellent for capturing cell diversity	[24]
Smart-seq	-	FACS	10^4	PCR	Full length	No	Full-length transcript coverage, high sensitivity for low RNA content cells, excellent for isoform and splicing analysis.	[25]
InDrop	No poly(A) minus RNA detection	Microdroplets	$<10^5$	In-Vitro Transcription	3' end	Yes	Flexible barcoding design, high cell capture efficiency, good for custom experimental designs.	[26]
Fluidigm C1	No poly(A) minus RNA detection	Microfluidic	10^4	PCR	Full Length	No	Precise single-cell capture and imaging, allows selection of high-quality cells, excellent for small-scale, high-quality studies.	[27]
Seq-Well	No poly(A) minus RNA detection	Microfluidic	$<10^5$	PCR	3' end	Yes	Portable, low-cost platform, compatible with fragile or frozen samples, good for low-resource settings.	[28]
BD Rhapsody	-	Microfluidic	10^4	PCR	3' end	Yes	High reproducibility, robust targeted gene expression profiling, integrated workflow from capture to analysis.	[29]
Chromium	-	Microfluidic	Large number of Cells	PCR	3' end	Yes	Standardized workflow with strong bioinformatics support, Excellent for tumor heterogeneity, immune profiling, and drug discovery research, High reproducibility and low technical variability.	[30]

Abbreviations: FACS, fluorescence-activated cell sorting; PCR, polymerase chain reaction; UMI, unique molecular identifier; No poly(A) minus RNA detection, cannot detect RNAs without poly(A) tails, such as many non-coding RNAs and only captures poly(A)$^+$ RNAs like most messenger RNAs (mRNAs).

These advances in scRNA-seq technology have given researchers new insights into cancer cells' genomic diversity and complexity, paving the way for more tailored and effective precision oncology treatment development.

2.1. Role of scRNA-seq in Understanding Tumor Heterogeneity

A significant barrier in cancer research is the tremendous heterogeneity exhibited within tumors, where individual cancer cells might exhibit distinct genetic and transcriptional profiles [2,17,31].

2.2. Tumor Heterogeneity & Cancer Evolution

Traditional bulk RNA sequencing approaches are limited in their capacity to capture the full degree of this variability since they provide an average gene expression profile across a population of cells [32]. Cancer cell is extremely heterogeneous, which means that individual cancer cells within a tumor might have different genetic and molecular profiles. This is owing to cancer cells' fast and uncontrolled division, which can result in the accumulation of genetic mutations and epigenetic alterations over time. As the tumor progresses, several

subpopulations of cancer cells form, each with the potential to respond differently to treatment [33]. Tumor heterogeneity is a significant obstacle in cancer therapy because it can lead to drug resistance, which occurs when some cancer cells are able to evade the effects of a specific medicine or treatment. Understanding and addressing this heterogeneity is critical for developing more effective and tailored cancer treatments [34].

2.3. Identification of Cancer Cell Subpopulations

Single-cell RNA sequencing (scRNA-seq) is an effective method for detecting and characterizing various subpopulations of cells inside a tumor. This approach can assist distinguish between cancer stem cells, immune cells, and any resistant clones that may exist [35]. Cancer stem cells are a tiny subset of cells in a tumor that may self-renew and specialize into the many cell types that make up the majority of the tumor. scRNA-seq enables researchers to detect these rare CSCs and comprehend their distinct transcriptional profiles, which can aid in the development of targeted therapeutics [36].

Similarly, scRNA-seq can assist identify the presence and features of immune cells in the tumor microenvironment, including T cells, B cells, and myeloid cells. This knowledge is critical for understanding the tumor's immune landscape and developing immunotherapies that can effectively target the disease [37]. Finally, scRNA-seq can detect the presence of resistant clones, which are cancer cells that have developed ways to dodge the effects of specific treatments. By identifying these resistant subpopulations, researchers can devise tactics to combat medication resistance and enhance patient outcomes [38].

2.4. Spatial Transcriptomics & Tumor Microenvironment

When single-cell RNA sequencing is paired with spatial transcriptomics, it becomes a useful tool for understanding the complicated interactions between cancer cells and their microenvironments. Spatial transcriptomics allows for the localization of gene expression profiles to specific regions inside a tumor, revealing spatial organization and communication between distinct cell types [39]. By combining scRNA-seq data with geographical information, researchers can learn how the tumor microenvironment, which includes immune cells, stromal cells, and extracellular matrix components, influences the behavior and phenotype of individual cancer cells. The geographical and functional context is critical for identifying important signaling pathways and cell-cell interactions that govern tumor growth and treatment response [40]. The combination of single-cell resolution and spatial information allows for a more comprehensive understanding of the tumor ecology, resulting in the creation of more effective and tailored cancer therapies [13].

3. scRNA-seq in Tumor Drug Discovery & Target Identification

3.1. Discovering Novel Drug Targets

Single-cell RNA sequencing (scRNA-seq) has emerged as a transformational method for discovering new cancer therapeutic targets by identifying oncogenic pathways and signaling networks in the varied tumor landscape. By evaluating the transcriptional patterns of individual cancer cells, scRNA-seq can show the activation of specific signaling cascades, the presence of rare but potentially targetable cell subpopulations, and the intricate connections between cancer cells and their environment [41]. This high-resolution, single-cell knowledge enables researchers to identify important drivers of tumor growth, metastasis, and treatment resistance, ultimately leading to the creation of more effective and customized cancer medicines [42,43].

3.2. Predicting Drug Response

Single-cell RNA sequencing data can also be used to predict which patients would respond to certain medications. By examining the transcriptional patterns of individual cancer cells, researchers can find genetic signatures or biomarkers linked with treatment susceptibility or resistance [5]. This data can then be utilized to stratify patient populations and determine the best treatment for each person, which is a basic principle of precision oncology. Furthermore, scRNA-seq can assist identify the processes behind medication response, allowing for the development of more effective combination therapies capable of overcoming resistance [44]. Integrating scRNA-seq data with clinical results allows researchers to create prediction models that guide the selection of optimal drug regimens for each patient, thereby enhancing treatment efficacy and patient outcomes [45]. Using scRNA-seq data to predict treatment response and resistance is a critical application for improving precision oncology [46].

3.3. Case Studies

Single-cell RNA sequencing (scRNA-seq) has enabled the identification of novel cancer treatment targets and biomarkers in a variety of tumor types. For example, in lung cancer, scRNA-seq analysis identified a small subpopulation of cancer cells expressing high quantities of the protein DLL3, prompting the development of rovalpituzumab tesirine, a targeted antibody-drug combination. Similarly, in breast cancer, scRNA-seq identified a subset of estrogen receptor-positive tumors with different transcriptional signatures, paving the path for personalized therapy [47]. In colorectal cancer, scRNA-seq identified an uncommon mesenchymal-like cancer cell state linked with a poor prognosis, which guided the development of combination therapy to combat this aggressive phenotype [48]. These examples show how scRNA-seq can disclose previously unknown tumor cell types and weaknesses, which can then be used to further precision oncology drug development.

3.4. AI & Machine Learning Integration

Integrating single-cell RNA sequencing data with advanced computational approaches, such as artificial intelligence and machine learning, can boost scRNA-seq's potential for precision cancer medication development [17]. Machine learning algorithms can be trained on scRNA-seq datasets to extract strong biomarker signatures, find new therapeutic targets, and predict treatment response at the individual patient level [49].

Additionally, Complex single-cell RNA sequencing (scRNA-seq) datasets (Table 2) are currently being analyzed using AI-driven algorithms, which improves the accuracy of finding unusual cell types and novel biomarkers compared to conventional techniques [50]. To speed up the drug development pipeline, AI and ML are being used more and more in clinical trial settings for patient stratification, drug response prediction, and trial design optimization [51]. Deep learning models, for example, can identify possible responders to targeted medicines and forecast therapeutic results by integrating scRNA-seq data with patient clinical records. Furthermore, in order to increase the repeatability and scalability of single-cell investigations, AI-powered systems are being attempted to automate data preprocessing tasks including batch effect correction and normalization [52]. These developments highlight how AI and ML have the potential to revolutionize precision medicine as well as basic research, making them essential instruments in contemporary biomedical research and clinical practice.

Table 2. A survey of databases for scRNA-seq.

Database	Link (Data Source of scRNA-seq)
PanglaoDB	https://panglaodb.se/markers/PanglaoDB_markers_27_Mar_2020.tsv.gz
Hierarchical PanglaoDB	https://github.com/JiaLiVUMC/scMRMA/tree/main/data
SCSig	https://data.broadinstitute.org/gsea-msigdb/msigdb/supplemental/scsig/1.0/scsig.all.v1.0.symbols.gmt
Cellmarker	http://bio-bigdata.hrbmu.edu.cn/CellMarker/download/all_cell_markers.txt
CellMatch	https://github.com/ZJUFanLab/scCATCH/raw/master/data/cellmatch.rda

For example, to facilitate accurate cell-type annotation from the scRNA-seq data, there are several publicly available marker databases. A comprehensive summary of these resources, including their primary data sources and access links, is provided in Table 2, and several supervised cell annotation techniques that concentrate on the correlation between the target and reference datasets have been created especially for single cell RNA sequencing (scRNA-seq) data (Table 3). SingleR [53], Cell Assign [54], CHETAH [55], and scmap [56] are notable techniques. SingleR uses a built-in reference transcriptome of pure cell types derived from microarray or bulk RNA-sequencing data to assign cellular identities to single-cell transcription tomes. Cell Assign uses a marker-based reference force ll type assignment in a probabilistic model. Through a stepwise traversal of the classification tree, cells can be allocated to intermediate or unassigned kinds using CHETAH's hierarchical chemical categorization technique. Lastly, scmap uses a variety of correlation metrics to categorize query cells according to how similar they are to reference cell types.

These computational tools (Table 3) enable researchers to identify previously unknown weaknesses in the tumor landscape and create more focused and individualized cancer medicines.

Table 3. Supervised machine learning methods for cell annotation.

Tool	Reference Database	Algorithm	Use Case/Practical Example	Reference
SingleR	Built-in cell dex (transcriptome of pure cell types)	Spearman	Automatically annotates new scRNA-seq cells by comparing their expression profiles with reference pure cell types. Example: Identifying immune cell populations in tumor samples.	[53]
Cell Assign	Markergenes	Probabilistic Bayesian model	Uses predefined marker genes to assign cell types probabilistically. Example: Assigning T cell subtypes based on canonical markers like CD4/CD8.	[54]
CHETAH	Annotated transcriptome	Spearman and confidence	Provides hierarchical and confidence-based cell type annotation. Example: Distinguishing closely related epithelial cell subtypes in single-cell datasets.	[55]
scmap	Annotated transcriptome	K-nearest neighbor (KNN)	Maps new cells to reference datasets using nearest neighbors. Example: Mapping scRNA-seq cells from a new mouse tissue dataset to a reference atlas.	[56]

4. scRNA-seq in Drug Resistance Mechanisms & Personalized Therapy

4.1. Mechanisms of Drug Resistance

Single-cell RNA sequencing (scRNA-seq) is a valuable method for identifying drug resistance mechanisms in cancer. ScRNA-seq can detect resistant cell clones that persist and multiply in the absence of therapy by evaluating the transcriptional profiles of individual tumor cells before and after treatment [57]. This method identifies the particular gene expression alterations and signaling pathways that allow resistant subpopulations to survive and thrive in the face of pharmacological pressure. Several key genes associated with these resistance mechanisms across various cancer types are detailed in Table 4. Importantly, scRNA-seq provides a high-resolution view of tumor heterogeneity, enabling researchers to identify uncommon resistant cells that bulk sequencing approaches may miss [5]. This in-depth understanding of resistance mechanisms at the single-cell level is critical for developing individualized combination therapies that can effectively target and overcome drug resistance in each patient.

Table 4. Drug Resistance-Related Genes Identified via scRNA-seq and Other Studies.

Gene	Function	Associated Drug Resistance	Cancer Type	Reference
ABCB1 (MDR1)	Efflux pump (ATP-binding cassette transporter)—actively exports drugs out of cancer cells	Paclitaxel, Doxorubicin, Cisplatin	Breast, Ovarian, Colon	[58]
BCL2	Anti-apoptotic regulator—prevents programmed cell death, promoting survival of drug-resistant cells	Venetoclax	Leukemia (CLL, AML)	[59]
EGFR T790M	Mutation in receptor tyrosine kinase—leads to resistance to targeted EGFR inhibitors	Gefitinib, Erlotinib (EGFR inhibitors)	Non–small cell lung	[60]
TP53	Tumor suppressor gene—mutations cause impaired apoptosis and multi-drug resistance	Multi-drug resistance	Breast, Lung, Colon, Ovarian	[61]

4.2. Adaptive Responses to Therapy

Researchers can use single-cell RNA sequencing to learn about how cancers change in response to chemotherapy, immunotherapy, or targeted therapy. ScRNA-seq, which analyzes the transcriptional patterns of individual cancer cells before and after therapy, can reveal the specific genetic and molecular modifications that allow certain cell subpopulations to survive and prosper despite therapeutic pressure [62]. This high-resolution perspective of tumor heterogeneity and adaptation is critical for understanding drug resistance mechanisms and creating targeted combination therapy to overcome them.

4.3. Personalized Drug Screening

Researchers can use patient-derived single-cell RNA sequencing data to match individuals to the most successful treatment combinations. This personalized method to drug screening enables the identification of tailored medicines that target the distinct molecular profiles of each patient's tumor, hence enhancing treatment efficacy and patient outcomes [63].

5. Challenges & Limitations of scRNA-seq in Drug Discovery

5.1. Technical Challenges

Despite substantial advancements in single-cell RNA sequencing technology, various technical obstacles have to be addressed before it can fully realize its potential for precision cancer medication development. Efficient and unbiased library generation from a small amount of RNA in a single cell, as well as transcript detection sensitivity and accuracy, continue to be significant challenges. Furthermore, powerful bioinformatics pipelines are required for computational processing of scRNA-seq data because to its high-dimensional and sparse nature [64]. Also, many research labs may find it difficult to handle the enormous magnitude of scRNA-seq datasets since it requires a significant amount of processing power and storage space. Because of their complexity, activities like trajectory inference, batch effect correction, and clustering frequently call for sophisticated algorithms and scalable computer frameworks. To increase the accuracy and efficiency of data processing, ongoing research is looking into options like cloud-based platforms, GPU-accelerated computing, and machine learning-driven algorithms [54,65]. It will be essential to incorporate these developments into scRNA-seq analysis pipelines in order to overcome present constraints and guarantee repeatable, superior outcomes, eventually opening the door for more extensive clinical applications.

5.2. Tumor Heterogeneity & Complexity

The enormous heterogeneity and complexity of solid tumors make it difficult to apply scRNA-seq effectively in cancer research. Tumors are made up of a variety of cell types, including cancer cells, immune cells, stromal cells, and endothelial cells, each having their own transcriptional profile [9]. This diversity is further complicated by spatial organization, tumor microenvironment interactions, and dynamic changes during tumor progression or treatment response. Fully collecting and integrating this cellular variability in the context of drug response is a difficult undertaking that necessitates advanced computational and experimental methods [66].

Algorithms that deconvolute intricate tumor ecosystems and find uncommon subpopulations associated with treatment resistance have been developed recently; however, these methods require substantial computational resources and thorough validation [67]. To use scRNA-seq to inform precision oncology and individualized treatment plans, these obstacles must be overcome.

5.3. Integration with Other Omics Data

To fully realize the potential of single-cell RNA sequencing for precision oncology, it is critical to combine scRNA-seq data with other high-throughput omics technologies, such as single-cell genomics, epigenomics, and proteomics [68]. Combining these disparate data sources can provide a more complete understanding of the underlying biological mechanisms governing medication response and resistance, ultimately allowing for the development of more effective tailored cancer therapies [69].

5.4. High Cost & Scalability Issues

Single-cell RNA sequencing's high cost and restricted scalability may also prevent its broad use in clinical and research contexts. Significant technological and infrastructure investments are necessary to enable the frequent use of scRNA-seq in drug development pipelines [70].

Although recent developments like droplet-based sequencing technologies and combinatorial indexing seek to increase scalability and lower per-cell costs, more advancements are required before scRNA-seq can be regularly used in precision oncology and extensive clinical trials [65,71].

5.5. Ethical Considerations & Data Privacy

The collection and analysis of single-cell data from patient samples raises fundamental ethical concerns, including informed consent, data privacy, and the responsible use of sensitive genomic information [13]. Even when data are anonymized, there remains a risk of patient re-identification because scRNA-seq generates highly precise molecular profiles, capturing unique genetic variations and rare cellular subpopulations [72]. This level of resolution makes scRNA-seq data particularly sensitive compared to bulk sequencing approaches.

To ensure ethical integrity, it is imperative to obtain comprehensive and transparent informed consent, clearly outlining how patient samples and data will be used—not only for current research but also for future applications, such as drug discovery, commercial development, or integration into precision oncology platforms. Consent

protocols should follow a dynamic consent model, allowing patients to update their preferences over time as technology and data-sharing practices evolve.

As scRNA-seq datasets are increasingly shared through open-access repositories, the need for robust data governance systems becomes urgent. This includes advanced encryption standards, strict access controls, and data de-identification techniques to mitigate the risk of unauthorized use or data breaches [73]. Additionally, regulatory challenges arise due to variations in privacy laws across jurisdictions. For instance, compliance with GDPR (General Data Protection Regulation) in Europe or HIPAA (Health Insurance Portability and Accountability Act) in the U.S. is critical to maintaining legal and ethical standards when handling genomic data. Establishing harmonized international guidelines, such as those advocated by the Global Alliance for Genomics and Health (GA4GH), can help balance the protection of individual rights with scientific innovation [74].

A deeper ethical challenge lies in equitable access and representation. Most scRNA-seq datasets are currently derived from patients in developed countries, potentially reinforcing health disparities and biasing precision oncology models. Addressing this requires diverse, globally representative datasets and equitable policies to ensure all populations benefit from technological advances.

By proactively addressing these ethical and regulatory challenges, the scientific community can build public trust and foster responsible integration of scRNA-seq into clinical research and precision medicine. Despite these hurdles, ongoing advances in scRNA-seq technology, coupled with sophisticated computational methodologies, hold enormous promise for accelerating the development of personalized cancer therapies while safeguarding patient rights.

6. Future Perspectives & Clinical Applications

Our basic understanding of biology is being revolutionized by scRNA-seq, and this technology has led to new study areas beyond descriptive studies of cell states. One can envision a plethora of fascinating medicinal uses for this technology. We anticipate that scRNA-seq can be used to shed light on undiscovered tumor traits that are not detectable by conventional bulk transcriptome studies. Tumor heterogeneity is a prevalent phenomena that can occur both within and between tumors. This method might be used, for instance, to examine the expression patterns of certain pathways and to evaluate transcriptional heterogeneity during the development of drug tolerance in cancer cells [75]. Thus, scRNA-seq could contribute to the creation of cancer evolution models. The clinical potential and current status of these applications across various cancer types are summarized in Table 5.

Table 5. Clinical applications of scRNA-seq.

Application	Cancer Type	Key Insight	Therapeutic Impact	Clinical Status/Outcome	Reference
CAR-T Optimization	Leukemia	Identified exhausted CAR-T subpopulations	Guided design of next-generation CAR-T therapies	Early-stage clinical trials; promising preclinical data but more validation needed	[76]
Combination Immunotherapy	Melanoma	T-cell exclusion gene program discovered	Suggested checkpoint + targeted therapy combos	Some combinations are in phase II/III trials; early results show improved response rates	[77]
Targeted Therapy Resistance	NSCLC (Lung Cancer)	Identified drug-tolerant persister cells	Prevented relapse through dual targeting	Preclinical studies successful; clinical validation ongoing in pilot trials	[41]

Additionally, combining scRNA-seq with proteomics, metabolomics, and epigenomics can lead to a more thorough understanding of the molecular mechanisms behind treatment response and resistance, allowing for the development of more effective customized cancer therapies [78]. The integration of multiple orthogonal data sources can assist researchers in discovering previously unknown vulnerabilities in the tumor landscape and identifying personalized medication combinations that target the distinct molecular profiles of each patient's tumor [79]. For example, combining scRNA-seq with other large-scale genetic screening technologies will increase the technology's uses. One such combinational technology is combining scRNA-seq and CRISPR-based genome-scale genetic screening, such as Perturb-seq, which enables the assessment of transcriptional effects of knocking out several genes with CRISPR [80] and LinTIMaT, which integrates single-cell transcriptome data and mutation data for lineage tracing [81]. In addition to CRISPR-mediated mutagenesis, it is also conceivable to combine scRNA-seq with CRISPR-mediated gene activation or interference [82,83]. These combinational applications enable us to explore the genetic effect on the cellular transcriptome and functions at a wide scale. With the ongoing advancement of both single cell RNA sequencing and CRISPR gene editing, such as prime editing [84]. More

such combinational technologies and applications are expected to be arrived and contributed to the better understanding of gene and cell functions.

Prognostic testing is another example of how genetic datasets are used in clinical decision-making. Oncotype Dx and Mammaprint are exemplary assays for assessing the recurrence risk of early-stage breast cancer [85]. Oncotype Dx calculates a recurrence score from RT-PCR-based expression data of 21 genes, whereas Mammaprint calculates the probability of distant recurrence 10 years after diagnosis using a microarray-based gene expression score of 70 genes [86]. These assays are suggested in clinical guidelines by the National Comprehensive Cancer Network (NCCN) and the American Society of Clinical Oncology (ASCO) as prognostic and therapy-predictive (Oncotype Dx only) assays to supplement other clinical data. In multiple myeloma, MyPRS [87] and SKY-92 [88] give microarray-based gene expression signature scores for risk stratification. However, these remain investigational, and their clinical implementation requires further validation.

Additionally, future advancements in single-cell RNA sequencing technology, such as improved library preparation, transcript detection sensitivity, and computational analysis pipelines, will increase the efficiency and accuracy of this powerful tool for precision cancer medication development [89].

7. Conclusions

Single-cell RNA sequencing has emerged as an effective method for precision cancer medication development. Although scRNA-Seq technology is widely used in a variety of basic research disciplines and clinical trials, there are still several key technical challenges that must be addressed. First, the single-cell capture process disrupts cell integrity and activity, and there is still room for improvement in cell throughput and precision. Second, sequencing generates high-dimensional data with a high level of noise, and reducing the impact of noise on high-throughput data analysis is a critical issue that must be addressed in order to improve the accuracy of scRNA-Seq. Furthermore, cell differentiation and reproduction are dynamic processes, and current scRNA-Seq methods make it difficult to discriminate between cell types. In addition to technical issues, the cost of scRNA-Seq analysis remains high when compared to other analytical methods, which limits the development of scRNA-Seq as a routine analytical tool. Although significant technological progress has been made, technical hurdles such as efficient library preparation, sensitive transcript detection, and robust computational analysis remain. Tumor heterogeneity and complexity, the need to combine multi-omics data, and cost, scalability, and ethical concerns all contribute to scRNA-seq's limited use in precision oncology. However, continuous technological developments and integration with other methodologies show enormous promise for advancing the development of more effective tailored cancer medicines. Finally, the development of scRNA-Seq and its integration with other multiomics technologies is critical not only for understanding individual cell growth and differentiation, as well as the regulatory relationships between different genes, but also for understanding the heterogeneity of cells in complex tissues, and thus for understanding disease pathogenesis and developing novel drugs.

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