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Single-Cell Genomics to Resolve Tumor Heterogeneity and Predict Therapeutic Resistance in Lymphomas

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Abstract: Lymphomas exhibit considerable genomic complexity and variability, often resulting in differential responses to treatment and variable clinical outcomes. Mutational signatures—distinct patterns of somatic mutations caused by endogenous or exogenous processes—offer critical insights into the underlying mechanisms of lymphomagenesis. These signatures, derived from whole-genome or exome sequencing data, not only reflect the history of DNA damage and repair but also identify actionable pathways that may be amenable to targeted therapies. For instance, the presence of aberrant somatic hypermutation or activation-induced cytidine deaminase (AID)-related signatures is frequently observed in B-cell lymphomas and can influence response to immunochemotherapy or checkpoint inhibitors. Clonality assessments further enhance personalized medicine approaches by determining the evolutionary relationships and dominance hierarchies of tumor subclones. Understanding whether certain driver mutations are clonal (present in all tumor cells) or subclonal (restricted to subsets) can guide the selection and sequencing of targeted therapies. Clonal mutations in pathways such as B-cell receptor signaling, NF-κB, or JAK/STAT may predict robust responses to specific inhibitors, whereas subclonal alterations may require combination regimens or surveillance strategies. Integration of mutational signatures and clonal architecture is especially crucial for identifying early events in tumorigenesis versus late-arising resistance mutations. Advancements in high-throughput sequencing and computational modeling are enabling more precise reconstruction of lymphoma evolution and therapeutic vulnerabilities. Incorporating these molecular metrics into clinical decision-making may improve prognostication, reduce overtreatment, and enable dynamic adaptation of treatment regimens. This review highlights the synergistic utility of mutational signatures and clonality assessments in tailoring precision therapies and overcoming resistance in lymphoma care.

Keywords: Mutational signatures; Clonality assessment; Targeted therapy; Lymphoma, Tumor evolution; Precision medicine

1. INTRODUCTION

1.1 Background on Lymphomas and Clinical Challenges

Lymphomas are a heterogeneous group of hematologic malignancies arising from the clonal proliferation of lymphocytes, primarily affecting lymph nodes and lymphoid tissues. They are broadly classified into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), each with multiple subtypes varying in cellular origin, molecular features, and clinical behavior. NHL comprises over 90% of cases and is further subdivided into B-cell, T-cell, and natural killer (NK) cell lymphomas [1]. Despite advances in diagnostic tools and targeted therapies, treatment outcomes remain suboptimal in several subtypes, particularly aggressive and relapsed forms.

One of the major clinical challenges in lymphoma management lies in its biological complexity and variable response to treatment. For example, while some subtypes such as diffuse large B-cell lymphoma (DLBCL) respond favorably to immunochemotherapy, others like mantle cell lymphoma exhibit intrinsic resistance and high relapse rates [2]. Additionally, many patients present with advanced-stage disease, further complicating therapeutic strategies. Standard treatments, including R-CHOP and autologous stem cell transplantation, have improved survival but are not universally effective, especially in refractory disease [3].

Further complicating the landscape is the limited predictive power of conventional biomarkers. Histopathological evaluation and flow cytometry often fail to capture the full spectrum of molecular diversity within tumors [4]. In the era of precision medicine, this lack of granularity hinders the ability to stratify patients effectively and personalize treatments. Consequently, there is a pressing need for deeper molecular characterization to understand disease mechanisms and optimize therapeutic decisions. This necessity underscores the relevance of novel approaches that can dissect cellular-level variations and unveil hidden subpopulations driving progression and resistance [5]. Recognizing and targeting these underlying complexities could significantly enhance prognostication, therapy design, and ultimately, patient outcomes.

1.2 Importance of Tumor Heterogeneity in Therapy Resistance

Tumor heterogeneity refers to the existence of genetically, epigenetically, and phenotypically distinct subpopulations of cancer cells within a single tumor. In lymphomas, both interpatient and intratumoral heterogeneity are recognized as key factors influencing disease trajectory and treatment resistance. Interpatient heterogeneity accounts for variable clinical outcomes across individuals with the same histological subtype, while intratumoral heterogeneity

underlies differential responses within the same tumor microenvironment [6].

This heterogeneity poses a major obstacle to effective therapy, as minor subclones resistant to treatment can survive initial interventions and drive disease relapse. For instance, subclonal mutations in genes regulating B-cell receptor signaling or apoptosis can confer survival advantages, promoting therapeutic evasion [7]. Such dynamics are particularly problematic in aggressive lymphomas like DLBCL and follicular lymphoma, where resistant clones often emerge under therapeutic pressure, limiting long-term remission rates.

The tumor microenvironment also contributes to heterogeneity by providing supportive niches for certain subpopulations. Interactions with immune cells, stromal elements, and cytokine gradients can induce adaptive phenotypes, allowing malignant cells to persist despite cytotoxic therapy [8]. These influences not only promote resistance but also complicate the development of universally effective treatment regimens.

Moreover, conventional bulk sequencing approaches fail to resolve these intricate patterns, as they average signals across diverse cell populations. As a result, rare but clinically significant clones may remain undetected, delaying recognition of emerging resistance mechanisms [9]. Understanding and mapping this complexity are thus critical for developing adaptive treatment strategies. Targeting tumor heterogeneity could enable dynamic therapy adjustments that preempt resistance and improve survival. In recent years, the focus has shifted toward high-resolution tools that allow dissection of these subclonal structures and their evolutionary trajectories within the tumor ecosystem [10].

1.3 Emergence of Single-Cell Genomics in Cancer Biology

Single-cell genomics has emerged as a transformative technology in cancer research, offering unprecedented resolution to study the cellular and molecular architecture of tumors. By enabling the analysis of DNA, RNA, and epigenetic modifications at the individual cell level, this approach overcomes the limitations of traditional bulk sequencing and reveals the full extent of tumor heterogeneity [11]. In lymphomas, where clonal evolution and dynamic microenvironmental interactions shape disease progression, single-cell profiling provides critical insights into lineage hierarchies, therapy-resistant clones, and functional diversity among malignant cells.

Technological innovations such as single-cell RNA sequencing (scRNA-seq) and single-cell ATAC-seq now allow researchers to dissect gene expression patterns, chromatin accessibility, and mutational landscapes with exceptional granularity [12]. These methods have already uncovered distinct transcriptional states associated with resistance, immune evasion, and proliferation, even within immunophenotypically similar lymphoma cells. Furthermore, integrating multi-omics data at the single-cell level facilitates the identification of biomarkers predictive of therapy response, helping to stratify patients more accurately and personalize treatment plans [13].

Single-cell genomics thus represents a pivotal advancement in the era of precision oncology, particularly for lymphomas characterized by spatial, temporal, and functional diversity. It holds promise not only for better understanding disease biology but also for guiding the design of next-generation therapies [14].

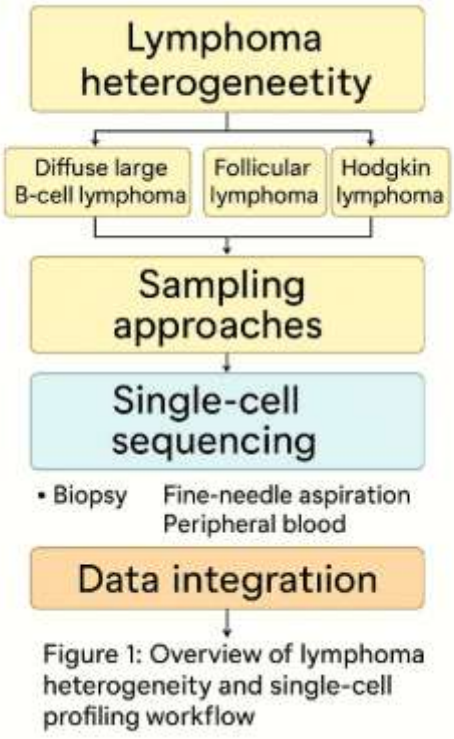

Figure 1: Overview of lymphoma heterogeneity and single-cell profiling workflow

A schematic summarizing lymphoma subclonal diversity, sampling approaches, and single-cell sequencing pipelines leading to data integration and therapeutic insights.

2. UNDERSTANDING TUMOR HETEROGENEITY IN LYMPHOMAS

2.1 Genetic and Epigenetic Diversity in Lymphoid Tumors

Genetic and epigenetic heterogeneity forms the molecular basis of diverse clinical behaviors observed in lymphoid malignancies. Unlike solid tumors, lymphomas often demonstrate widespread dissemination at diagnosis, making it difficult to pinpoint a single dominant mutation or lesion. This is further complicated by branched evolutionary trajectories, where multiple subclones accumulate distinct genetic alterations over time [5]. Commonly mutated genes in lymphoid tumors include *TP53*, *EZH2*, *MYD88*, and *BCL2*, but the pattern and combinations vary significantly between patients and across timepoints.

Somatic hypermutation and class switch recombination—processes intrinsic to normal B-cell development—introduce additional variability that can foster lymphomagenesis when deregulated [6]. These processes can result in a broad spectrum of mutations, some of which are drivers of malignant transformation, while others represent passenger events. Additionally, structural variants such as chromosomal translocations, notably t(14;18) in follicular lymphoma, further contribute to intertumoral genomic complexity [7].

In parallel, epigenetic alterations—heritable changes in gene expression that occur without changes to the DNA sequence—play an equally important role. Aberrant DNA methylation, histone modifications, and altered chromatin remodeling have been implicated in transcriptional reprogramming and lineage plasticity in lymphomas [8]. For example, mutations in epigenetic regulators such as *CREBBP* and *KMT2D* have been associated with immune evasion and therapy resistance in B-cell lymphomas.

Importantly, genetic and epigenetic diversity is not merely a reflection of stochastic variation but often represents adaptive responses to environmental and therapeutic pressures [9]. Tumor cells may acquire or select for advantageous mutations that confer survival benefits under chemotherapy or immunotherapy. This dynamic process leads to clonal expansion of resistant populations, fueling relapse.

Dissecting these molecular layers is crucial to understanding lymphoma pathogenesis, identifying biomarkers of response, and designing effective combination therapies. Recent integrative studies suggest that epigenetic reprogramming can precede genetic divergence, hinting at the primacy of regulatory plasticity in driving phenotypic diversity [10].

2.2 Functional and Phenotypic Intratumoral Diversity

Beyond genotypic variability, lymphoid tumors exhibit remarkable functional and phenotypic heterogeneity at the cellular level. This refers to the coexistence of malignant cells with distinct proliferative capacities, survival pathways, and immunophenotypes within the same tumor mass. In diffuse large B-cell lymphoma (DLBCL), for instance, tumor cells may differ in B-cell receptor signaling intensity, metabolic profile, and response to microenvironmental stimuli, even when harboring similar genetic mutations [11].

This functional heterogeneity can be attributed in part to the hierarchical organization of malignant clones. Some subpopulations may behave like lymphoma-initiating cells with self-renewal and multilineage potential, whereas others display more differentiated phenotypes [12]. Moreover, varying expression of surface markers such as CD19, CD20, and CD38, along with intracellular signaling proteins like NF-κB and BCL-6, further delineate discrete subclones with divergent behaviors [13].

A significant contributor to phenotypic diversity is the tumor microenvironment. Cytokines, stromal interactions, hypoxia, and immune infiltration create selective niches that support specific cellular states. For example, interactions with follicular dendritic cells in the lymph node may preserve a quiescent state in certain lymphoma clones, while others exposed to inflammatory cytokines may exhibit enhanced proliferation and resistance [14].

Single-cell RNA sequencing studies have confirmed that transcriptional states associated with stress responses, proliferation, or immune modulation often coexist within the same tumor sample. These states may be reversible, indicating that phenotype is not strictly determined by genotype but is shaped by dynamic microenvironmental feedback [15]. Such plasticity poses significant challenges for therapeutic targeting, as interventions directed at a dominant clone may fail to eliminate less abundant, yet therapy-resistant, subclones.

Furthermore, therapy-induced reprogramming can alter the phenotypic landscape of tumors. Exposure to chemotherapeutic agents may not only select for pre-existing resistant clones but also induce dedifferentiation or phenotypic switching, giving rise to new cellular states [16]. As a result, tumors evolve into a more heterogeneous and therapy-refractory state following treatment.

Understanding the functional spectrum of lymphoma cells at diagnosis and during therapy is critical for identifying robust therapeutic targets. It also underscores the need for adaptive treatment strategies capable of addressing cellular diversity, minimizing clonal escape, and preventing disease recurrence [17].

2.3 Limitations of Bulk Sequencing in Capturing Heterogeneity

Conventional bulk sequencing has played a vital role in cataloging mutations and gene expression profiles in lymphomas. However, its inherent averaging effect across cell populations masks intratumoral heterogeneity, providing only a snapshot of dominant clones. This limits the capacity to identify rare but clinically significant subpopulations that may contribute to disease progression or therapy resistance [18].

In lymphoid malignancies characterized by cellular diversity and dynamic clonal evolution, the inability to resolve subclonal architecture restricts the interpretation of genomic data. For instance, two subclones with opposing transcriptional signatures may cancel each other's signal in bulk RNA sequencing, leading to misleading conclusions regarding gene expression patterns [19]. Additionally, temporal sampling—especially post-treatment—may not capture the emergence of new or minor resistant populations that drive relapse.

Bulk sequencing also falls short in assessing clonal dynamics in the tumor microenvironment. Immune cells, stromal components, and malignant cells are often intermingled in biopsy specimens, and bulk analyses cannot reliably

distinguish their contributions [20]. Even with computational deconvolution, these approaches lack the precision and resolution offered by single-cell platforms.

Another drawback is the inability to detect cell-state transitions or lineage plasticity, which are critical to understanding phenotypic switching and treatment adaptation. By contrast, single-cell technologies enable direct observation of such processes, allowing more accurate modeling of disease trajectories [21].

While bulk sequencing remains a valuable tool for identifying shared mutations, its limitations necessitate complementary strategies like single-cell genomics to fully capture the complexity of lymphoid tumors [22].

2.4 Clinical Impact of Undetected Clonal Subpopulations

Undetected clonal subpopulations pose a significant clinical threat in lymphoma management. These minor clones may harbor mutations or epigenetic profiles that confer resistance to frontline therapies, allowing them to survive initial treatment and drive relapse. Because they are often below the detection threshold of bulk sequencing, clinicians may underestimate the risk of disease progression [23].

In DLBCL and other aggressive lymphomas, resistant subclones have been shown to expand under therapeutic pressure, replacing the dominant clone at relapse. This clonal evolution can result in altered phenotypes that no longer respond to previously effective treatments, necessitating second-line strategies with diminished efficacy [24].

Moreover, these subpopulations can modulate the immune microenvironment, suppressing cytotoxic responses and fostering immune escape. For example, minor clones may upregulate immune checkpoint molecules like PD-L1, reducing T-cell-mediated clearance [25].

Early detection of these subpopulations could allow clinicians to implement preemptive interventions or design combination therapies that target both dominant and minor clones. Without such insights, treatment remains reactive rather than proactive, contributing to poorer long-term outcomes. As precision oncology advances, integrating high-resolution tools capable of capturing clonal complexity at diagnosis and during follow-up will be pivotal for improving patient stratification and therapeutic success [26].

Table 1. Comparative Features of Bulk vs. Single-Cell Sequencing in Lymphoma Studies

Feature	Bulk Sequencing	Single-Cell Sequencing
Resolution	Population-averaged	Individual cell-level
Detection of rare clones	Limited	High
Cell-state transition analysis	Not possible	Possible
Immune and stromal deconvolution	Requires computational modeling	Direct measurement
Clonal evolution tracking	Indirect	High-resolution lineage tracing
Cost and computational complexity	Lower	Higher
Clinical applicability (current state)	Widely used	Emerging but rapidly growing

3. SINGLE-CELL GENOMICS TECHNOLOGIES: TOOLS AND METHODOLOGIES

3.1 Single-Cell RNA Sequencing (scRNA-seq)

Single-cell RNA sequencing (scRNA-seq) has emerged as a pivotal technology in cancer biology, enabling researchers to profile the transcriptomes of individual cells and resolve the heterogeneity masked by bulk approaches. In lymphomas, where malignant clones are interspersed with non-malignant immune and stromal cells, scRNA-seq allows precise identification and functional characterization of distinct cellular populations [11].

The process begins with dissociation of tumor tissue into single-cell suspensions, followed by isolation and barcoding of individual cells. Reverse transcription and amplification steps convert cellular mRNA into cDNA, which is then sequenced to generate transcriptomic data for each cell [12]. The resulting datasets contain gene expression matrices with thousands of features per cell, which can be analyzed to infer cellular states, lineage relationships, and transcriptional programs.

One of the most powerful applications of scRNA-seq in lymphoma is its ability to detect rare subpopulations that may evade conventional therapies. For instance, studies have uncovered transcriptionally quiescent clones that resist chemotherapy and later drive relapse [13]. Additionally,

immune landscape profiling has revealed diverse populations of T cells, macrophages, and natural killer cells within tumor samples, offering insights into immune evasion and checkpoint inhibitor responsiveness [14].

Unlike bulk RNA sequencing, scRNA-seq enables reconstruction of gene expression trajectories using pseudotime analysis, which models the progression of cellular differentiation or reprogramming. This is particularly relevant in lymphomas with plastic cellular phenotypes, such as those undergoing transformation from indolent to aggressive states [15].

Moreover, integration of scRNA-seq with T-cell receptor (TCR) or B-cell receptor (BCR) sequencing allows clonotype tracking and functional mapping of lymphocyte subsets. These combined modalities provide a comprehensive picture of tumor-immune dynamics in the lymphoma microenvironment [16].

Despite its transformative potential, scRNA-seq is not without limitations. High dropout rates, technical noise, and batch effects can confound data interpretation. Nevertheless, ongoing advancements in library preparation, computational pipelines, and normalization methods continue to enhance data quality and analytical resolution [17]. As scRNA-seq becomes more accessible, its integration into clinical workflows may refine patient stratification, therapy selection, and minimal residual disease monitoring in lymphomas.

3.2 Single-Cell ATAC-seq and Epigenomic Profiling

While scRNA-seq captures gene expression patterns, it does not directly reflect regulatory mechanisms controlling transcription. To bridge this gap, single-cell Assay for Transposase-Accessible Chromatin using sequencing (scATAC-seq) has been developed to map chromatin accessibility at single-cell resolution. This technique provides insights into the epigenetic landscape and regulatory elements that drive cell identity and state transitions in lymphomas [18].

scATAC-seq works by using a hyperactive Tn5 transposase to insert sequencing adapters into open chromatin regions. These accessible regions, often corresponding to promoters, enhancers, and transcription factor binding sites, are sequenced to generate cell-by-region matrices [19]. This data can be used to identify regulatory programs specific to malignant subclones or immune infiltrates.

In lymphomas, scATAC-seq has helped delineate differences in chromatin accessibility across malignant and non-malignant B cells, revealing subtype-specific regulatory signatures and enhancer usage. For example, distinct accessibility profiles in germinal center-derived versus activated B-cell-like DLBCLs suggest divergent epigenetic regulation [20]. Moreover, integration of scATAC-seq with scRNA-seq has allowed simultaneous assessment of transcriptional and regulatory heterogeneity within the same tumor ecosystem [21].

Beyond tumor profiling, scATAC-seq offers potential for understanding resistance mechanisms. Epigenetic changes may precede transcriptional reprogramming, positioning chromatin accessibility as an early indicator of therapeutic adaptation. This epigenomic insight is particularly valuable in cases where genetic mutations are absent, but phenotypic plasticity persists [22].

Despite its advantages, scATAC-seq faces technical challenges such as low signal-to-noise ratios and sparsity of data, making interpretation complex. However, novel computational tools for peak calling, motif analysis, and trajectory reconstruction are rapidly evolving, enhancing its utility in dissecting lymphoma biology [23].

3.3 Multi-omics Integration at the Single-Cell Level

To fully understand the multifaceted nature of lymphoid tumors, integrating multiple layers of single-cell data—such as transcriptomics, epigenomics, and proteomics—is essential. Multi-omics single-cell platforms now enable simultaneous capture of different molecular modalities from the same cell, offering an unparalleled view of tumor heterogeneity and clonal architecture [24].

Approaches such as SHARE-seq, Paired-seq, and SNARE-seq combine scRNA-seq with scATAC-seq, allowing researchers to link chromatin accessibility to gene expression in individual cells. These integrative technologies have been instrumental in identifying regulatory networks driving malignant transformation, immune evasion, and therapy resistance in lymphoma subtypes [25]. By overlaying transcriptional programs with cis-regulatory landscapes, researchers can pinpoint master regulators and candidate therapeutic targets unique to specific subclones.

Moreover, the integration of single-cell proteomics—using methods like CITE-seq or REAP-seq—adds another layer of granularity. These approaches pair surface protein quantification with RNA profiles, enabling refined cellular annotation and lineage tracing, particularly in phenotypically plastic tumors [26]. For instance, CITE-seq has revealed immunophenotypic transitions in DLBCL subclones following chemotherapy, offering insight into tumor adaptation and immune escape mechanisms.

One critical benefit of single-cell multi-omics is its ability to resolve ambiguous cell states that cannot be fully characterized using transcriptomic data alone. Transcriptionally similar cells may differ epigenetically or proteomically, influencing their behavior and therapeutic response [27].

However, multi-omics datasets pose significant analytical challenges, including high dimensionality, integration bias, and increased computational demands. New algorithms based on canonical correlation analysis, mutual nearest neighbors, and deep learning are now being developed to address these

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complexities and enable robust biological inference from high-throughput single-cell studies [28].

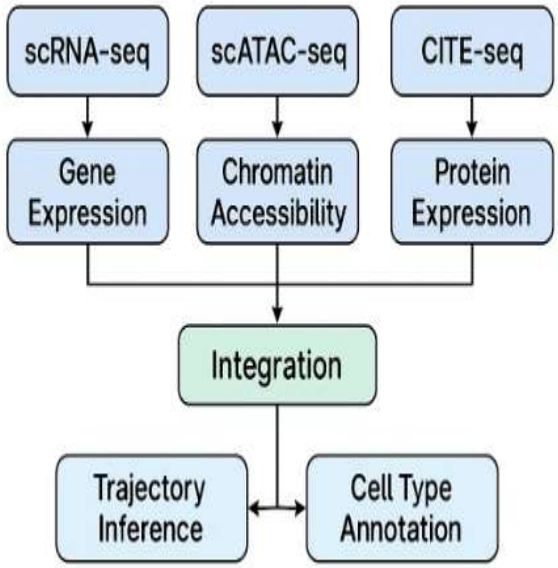

Figure 2 Multi-Omics Integration in Lymphomas

3.4 Challenges in Single-Cell Data Processing and Analysis

Despite the transformative capabilities of single-cell technologies, data processing and interpretation remain challenging. One of the foremost issues is technical noise, which arises from low input material, amplification bias, and dropout events. These factors result in sparsity—where a large fraction of gene expression values are zero—and can obscure meaningful biological signals if not properly corrected [29].

Batch effects represent another major obstacle. Differences in sample processing times, reagent lots, or sequencing runs can introduce artificial variation. Without proper normalization, these artifacts may be misinterpreted as biological differences. Several batch correction algorithms, such as Harmony, ComBat, and Seurat's integration pipeline, have been developed to address this issue, but each comes with trade-offs in sensitivity and interpretability [30].

Cell-type annotation in complex tissues like lymphomas is also non-trivial. Automated clustering methods such as Louvain or Leiden can group cells based on transcriptomic similarity, but the resulting clusters often require expert curation and validation using known markers. Misclassification is especially problematic in tumors with high plasticity or transitional cell states [31].

Computational scalability is a further limitation, particularly for multi-omics datasets. Analyzing hundreds of thousands of cells with integrated modalities demands substantial processing power, memory, and time. Cloud-based solutions and GPU-accelerated frameworks are increasingly adopted, but accessibility remains uneven across research institutions [32].

Finally, interpretation of biological relevance from data-driven clusters or trajectories can be challenging. Many analytical pipelines yield results that are statistically robust but biologically ambiguous without validation. Integration with external datasets, such as reference atlases or patient outcomes, is often necessary to derive actionable insights [33].

Addressing these challenges will require a combination of methodological improvements, cross-disciplinary collaboration, and community-driven standards for benchmarking, validation, and data sharing in single-cell lymphoma research.

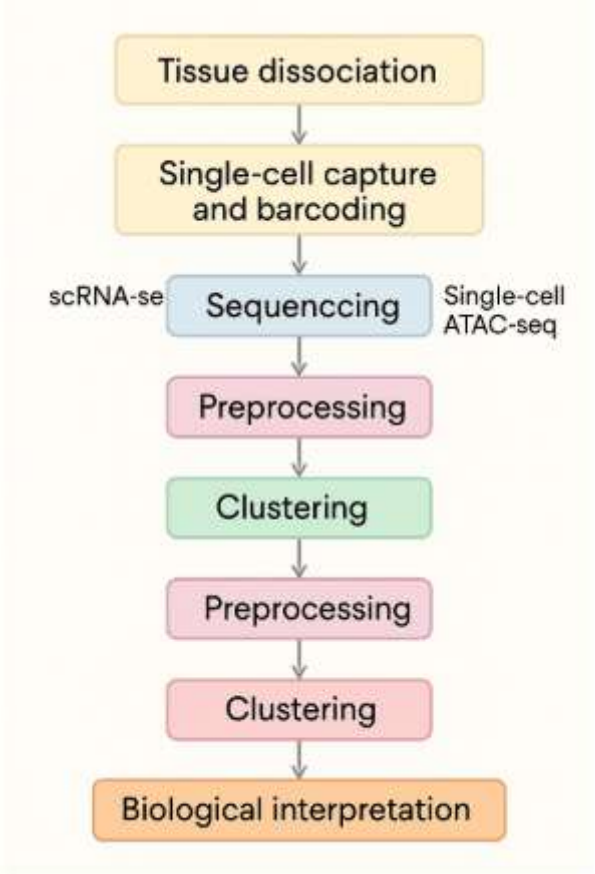

Figure 3 *Schematic of scRNA-seq and single-cell ATAC-seq analysis pipelines*

A flow diagram showing parallel tissue dissociation, single-cell capture, barcoding, sequencing, preprocessing, clustering, and downstream biological interpretation steps.

4. INSIGHTS GAINED FROM SINGLE-CELL ANALYSES IN LYMPHOMAS

4.1 Identification of Rare Clonal Subtypes Driving Relapse

Relapse in lymphoma is frequently attributed to minor clonal populations that survive frontline therapy and expand under selective pressure. These rare subtypes often exhibit distinct genomic, transcriptomic, or epigenomic features that confer

intrinsic resistance. Single-cell technologies have proven invaluable for detecting such clones, which are typically masked in bulk analyses due to their low abundance [15].

Studies using scRNA-seq in relapsed diffuse large B-cell lymphoma (DLBCL) have revealed small populations with stem-like characteristics, including quiescence and drug efflux capacity, which persist after chemotherapy [16]. These cells often lack conventional biomarkers and are transcriptionally distinct, displaying elevated expression of stress response and DNA repair genes. Such features enable survival under cytotoxic conditions, making them likely initiators of relapse.

Additionally, rare subclones may harbor specific genetic mutations or epigenetic marks that provide survival advantages. For instance, scATAC-seq profiling has uncovered enhancer reprogramming in minor clones resistant to BCL2 inhibitors in follicular lymphoma, suggesting early chromatin remodeling events as resistance drivers [17]. These resistant populations frequently occupy unique niches within the tumor microenvironment, where interactions with stromal cells or immune regulators further support their persistence.

The ability to track clonal hierarchies at single-cell resolution has revealed that relapse is not necessarily driven by the dominant diagnostic clone but often by a previously undetected minor lineage [18]. This finding underscores the importance of preemptive identification and therapeutic targeting of rare malignant populations at diagnosis or during minimal residual disease monitoring.

Moreover, combining single-cell transcriptomics with lineage tracing and mutational profiling allows for temporal tracking of subclonal behavior from diagnosis through relapse [19]. Such integrative approaches have led to the discovery of transitional clones that initially appear benign but later acquire aggressive features.

Understanding the biology of these rare subtypes can inform adaptive treatment strategies that eliminate potential sources of recurrence. Future clinical implementation may include real-time single-cell surveillance to detect emerging resistant clones and guide treatment intensification or modification before overt relapse occurs [20].

4.2 Mapping Tumor Evolutionary Trajectories and Clonal Dynamics

Tumor evolution in lymphomas follows non-linear and often branched patterns, driven by the accumulation of genetic, epigenetic, and environmental changes. Single-cell technologies offer a powerful lens for reconstructing these evolutionary trajectories by capturing the temporal and spatial heterogeneity within malignant populations [21].

Using scRNA-seq and scATAC-seq, researchers can infer pseudotime trajectories that model transitions from early to advanced cellular states. In mantle cell lymphoma, for example, such analyses have revealed progression from proliferative to immune-evading subpopulations, with transcriptional programs reflecting increasing therapy resistance over time [22]. These models provide insight into the sequence of regulatory events underlying clonal expansion and transformation.

Phylogenetic reconstructions based on mutational data from single-cell DNA sequencing also allow for mapping of clonal lineages. These trees often demonstrate early diversification and parallel evolution, challenging the notion of linear disease progression [23]. Importantly, some subclones undergo convergent evolution, independently acquiring similar resistance-conferring features, which emphasizes the importance of understanding clonal fitness landscapes.

Longitudinal sampling in patients has shown that treatment pressures reshape clonal composition. In follicular lymphoma, chemotherapy induces selective bottlenecks where only specific clones survive and dominate the post-treatment population [24]. Such bottlenecks can obscure the presence of minor, therapy-adapted subclones that re-emerge at relapse.

Single-cell lineage tracing tools, including mitochondrial barcode tracking and CRISPR-based fate mapping, have added further resolution to dynamic clonal studies [25]. These tools enable visualization of cell fate decisions, lineage plasticity, and the influence of microenvironmental cues on clonal behavior.

Moreover, spatially resolved single-cell approaches, such as spatial transcriptomics and multiplexed immunofluorescence, allow for mapping of clonal architecture across tissue compartments. These methods have revealed regional heterogeneity within lymph nodes, where aggressive clones preferentially localize to hypoxic or immune-privileged niches [26].

Ultimately, understanding the evolutionary dynamics of lymphoma at the single-cell level will improve prognostication and therapeutic planning. Predicting which clones are likely to dominate or evolve under specific treatments can enable precision intervention strategies, preventing disease progression through proactive targeting of high-risk trajectories [27].

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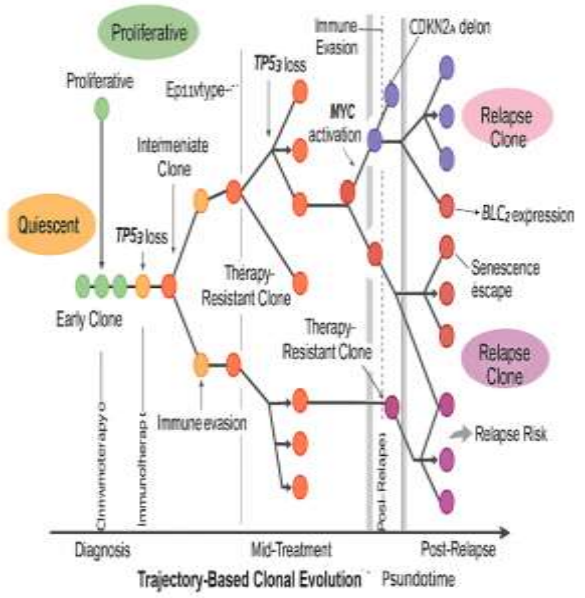

Figure 4 Tumor Evolution

4.3 Decoding the Tumor Microenvironment Interactions

The tumor microenvironment (TME) in lymphomas is a complex ecosystem comprising T cells, macrophages, stromal fibroblasts, dendritic cells, and extracellular matrix components. These non-malignant cells dynamically interact with malignant clones, shaping disease behavior, immune evasion, and therapeutic response. Single-cell profiling has revolutionized our understanding of these interactions by deconvoluting the cellular diversity and functional states within the TME [28].

scRNA-seq has uncovered discrete immune cell subsets within lymphoma tissues, including exhausted cytotoxic T cells, immunosuppressive regulatory T cells (Tregs), and tumor-associated macrophages with M2-like phenotypes [29]. These populations often localize in close proximity to malignant cells and contribute to immune tolerance through cytokine secretion, checkpoint expression, or antigen presentation deficits.

Moreover, malignant lymphoma cells themselves can modulate the TME by expressing chemokines and ligands that recruit suppressive cells or inhibit effector cell function. For instance, upregulation of PD-L1 and HLA-G in certain subclones correlates with T cell exclusion and poor prognosis [30].

Single-cell analyses have also shown that stromal cells within the TME are not passive scaffolds but actively support lymphoma survival through integrin-mediated signaling, angiogenic factor secretion, and metabolic cooperation. These interactions create protective niches that shield malignant cells from therapeutic insult [31].

Spatial single-cell methods, such as imaging mass cytometry and spatial transcriptomics, have further confirmed that cellular organization within the TME is non-random and functionally relevant. Certain immune-evading clusters are preferentially located at tumor-stroma boundaries, suggesting spatial coordination of resistance mechanisms [32].

Understanding the crosstalk between lymphoma cells and their microenvironment at high resolution offers opportunities for combinatorial therapies. Disrupting supportive interactions or reprogramming suppressive immune subsets could restore anti-tumor immunity and sensitize tumors to standard treatments [33].

4.4 Predictive Biomarkers for Immunotherapy and Chemoresistance

The heterogeneity of lymphomas complicates the identification of robust biomarkers predictive of treatment response. Single-cell technologies, by resolving functional states and molecular signatures at high resolution, offer a new paradigm for biomarker discovery in both immunotherapy and chemotherapy contexts [34].

In the immunotherapy space, scRNA-seq has enabled profiling of exhausted versus active T-cell states within tumors. Expression of exhaustion markers such as PD-1, LAG-3, and TIM-3 correlates with poor responsiveness to checkpoint inhibitors. However, these markers are heterogeneously expressed and may coexist with proliferative or memory signatures, necessitating nuanced interpretation [35].

Additionally, malignant subclones exhibiting high expression of immune checkpoint ligands (e.g., PD-L1, CD47) have been associated with immunotherapy resistance. Single-cell profiling allows for simultaneous evaluation of both immune and tumor compartments, offering a holistic view of immune escape mechanisms [36]. This dual profiling may guide selection of patients likely to benefit from immune-based therapies.

In chemotherapy, certain transcriptional programs detected by scRNA-seq—including those governing cell cycle arrest, DNA repair, and oxidative stress responses—have been linked to resistance. These programs often emerge in rare subpopulations that expand post-treatment [37]. For example, resistance to R-CHOP has been associated with enrichment of NF-κB-driven transcriptional states and metabolic reprogramming in relapsed DLBCL cases.

Moreover, epigenomic profiling via scATAC-seq can identify accessible chromatin regions associated with drug resistance, including enhancers of anti-apoptotic genes or transporters. These findings offer potential biomarkers that are not apparent at the mRNA level [38].

Incorporating these single-cell biomarkers into clinical practice could enable stratified therapy, real-time monitoring, and dynamic treatment adjustments. As validation studies

expand, such approaches may redefine predictive modeling in lymphoma care, reducing overtreatment and improving therapeutic outcomes [39].

Table 2. Single-cell studies in major lymphoma subtypes and their key findings

Lymphoma Subtype	Technology Used	Key Findings
DLBCL	scRNA-seq	Identification of stem-like resistant subclones [16]
Follicular Lymphoma	scATAC-seq	Enhancer reprogramming linked to BCL2 inhibitor resistance [17]
Mantle Cell Lymphoma	Multi-omics (scRNA + ATAC)	Evolution from proliferative to immune-evading states [22]
T-cell Lymphomas	CITE-seq	Immune checkpoint profiling of T-cell phenotypes [35]
Hodgkin Lymphoma	Spatial Transcriptomics	Localization of immune suppressive niches at tumor-stroma boundaries [32]

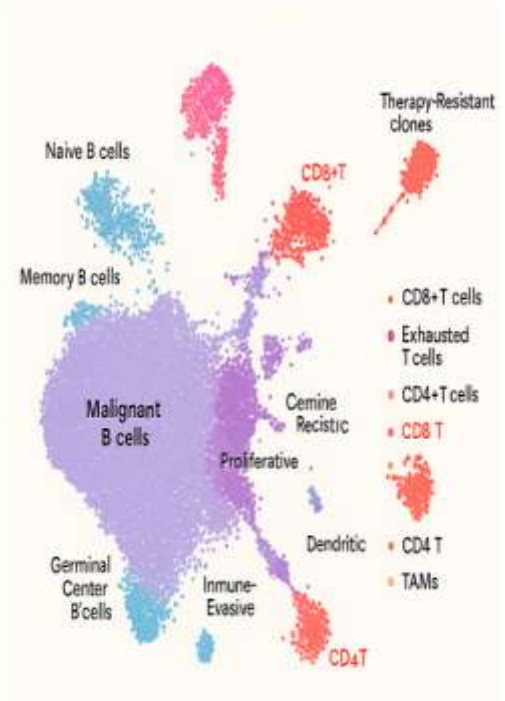

Figure 5 UMAP or t-SNE plot of lymphoma single-cell clusters with annotated subtypes

A visualization displaying discrete clusters of malignant and non-malignant cells in a lymphoma tissue sample, annotated with B-cell subtypes, immune infiltrates, and therapy-resistant clones.

5. PREDICTING AND MONITORING THERAPEUTIC RESISTANCE

5.1 Resistance Mechanisms in Hodgkin and Non-Hodgkin Lymphomas

Therapeutic resistance remains a significant barrier in the treatment of both Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), often leading to relapse and poor long-term survival. While initial response rates can be high, particularly with combination regimens like ABVD or R-CHOP, a subset of patients develops resistance that is poorly understood using conventional diagnostic tools [19]. Single-cell studies have begun to elucidate the underlying mechanisms contributing to this resistance by capturing functional and molecular heterogeneity at unprecedented resolution.

In Hodgkin lymphoma, a distinguishing feature is the scarcity of malignant Reed-Sternberg cells amidst a rich inflammatory milieu. These tumor cells frequently express PD-L1 and other immune checkpoint ligands, allowing them to evade immune surveillance [20]. scRNA-seq has revealed that these cells exhibit high plasticity and upregulation of stress-response pathways that may contribute to their resilience under cytotoxic stress [21]. Moreover, changes in their interaction with surrounding immune cells have been shown to dampen cytotoxic T-cell activity and promote immune tolerance.

In contrast, NHL encompasses a more diverse set of diseases with distinct resistance profiles. In DLBCL, resistant clones often emerge through activation of NF-κB signaling, B-cell receptor (BCR) pathway amplification, and upregulation of anti-apoptotic genes such as *BCL2* and *MCL1* [22]. These features may preexist in minor subclones or be acquired following treatment exposure. Additionally, epigenetic remodeling—particularly involving enhancer reprogramming—has been linked to lineage plasticity and drug escape in follicular and mantle cell lymphomas [23].

Resistance can also be microenvironmentally driven. Tumor-associated macrophages and stromal cells have been shown to secrete cytokines like IL-6 and TNF-α, promoting survival pathways in malignant cells even during therapy [24]. This paracrine support complicates therapeutic targeting and highlights the need for strategies that disrupt cellular crosstalk.

Collectively, these insights underscore the multifactorial nature of resistance in lymphomas. A deeper understanding through single-cell technologies may guide the development of rational combination therapies capable of overcoming both cell-intrinsic and microenvironmental resistance mechanisms [25].

5.2 Single-Cell Tracing of Resistant Cell States During Treatment

Single-cell sequencing technologies have enabled dynamic tracing of resistant cell states as they emerge during therapy. Unlike bulk analyses, which provide only an averaged snapshot, single-cell resolution captures the diversity of transcriptional and regulatory responses within heterogeneous tumors. This granularity is particularly valuable for understanding how therapy reshapes clonal architecture and promotes resistant phenotypes [26].

In DLBCL, longitudinal scRNA-seq profiling has shown that chemotherapy induces shifts in cellular composition, favoring the expansion of stress-tolerant and quiescent populations [27]. These surviving cells often display high expression of antioxidant genes, unfolded protein response (UPR) elements, and chromatin remodelers. Such features not only promote survival under oxidative or genotoxic stress but may also enable reactivation and proliferation after treatment cessation.

Some studies have combined scRNA-seq with mitochondrial mutation mapping to trace cell lineages across treatment timepoints. This integration has demonstrated that resistant clones can either preexist as minor subpopulations or arise de novo through transcriptional reprogramming [28]. Notably, resistant cells often show reduced antigen presentation and immune checkpoint engagement, facilitating immune escape even under immunotherapy.

In Hodgkin lymphoma, where the tumor mass is largely composed of reactive immune cells, single-cell tracking has revealed dynamic remodeling of the immune compartment in response to checkpoint inhibitors. A shift from cytotoxic to exhausted T-cell phenotypes occurs over treatment cycles, corresponding with diminishing therapeutic efficacy [29]. Simultaneously, malignant Reed-Sternberg cells adopt transcriptional programs enriched in JAK-STAT signaling, contributing to immune resistance and cell survival.

Advanced pseudotime and RNA velocity analyses have further elucidated the trajectory of resistant cell development. In some lymphomas, malignant cells follow bifurcating pathways—one leading to apoptosis and clearance, and the other toward quiescence and adaptation [30]. This dichotomy helps explain mixed responses observed clinically and highlights the importance of capturing early divergence events.

These insights open opportunities for therapeutic intervention before full resistance develops. For instance, targeting stress-adaptive pathways or re-sensitizing quiescent clones through epigenetic drugs may prevent clonal expansion. The use of single-cell tracing in real-time, especially during induction and consolidation phases, could revolutionize resistance prediction and guide timely treatment adjustments [31].

5.3 Longitudinal Single-Cell Profiling for Early Detection of Resistance

Early detection of treatment resistance is crucial for preventing disease progression and optimizing therapeutic strategies. Longitudinal single-cell profiling offers a unique window into evolving tumor ecosystems, allowing the identification of early resistance markers long before clinical relapse becomes evident [32].

By sampling peripheral blood, bone marrow, or lymph node tissue at multiple treatment stages, clinicians can observe changes in clonal composition and transcriptional states at high resolution. This approach has revealed early expansions of subpopulations characterized by stress-response genes, apoptotic suppression, and immune evasion markers [33]. These features often precede measurable changes in tumor size or biomarkers and can serve as early indicators of therapeutic failure.

For example, in patients treated with CAR-T therapy, scRNA-seq of circulating tumor cells identified transcriptionally distinct relapse-initiating clones that emerged during the initial response phase. These clones exhibited altered surface antigen expression and reduced interferon signaling, predicting treatment escape weeks before radiological relapse [34].

Moreover, integration of longitudinal single-cell data with machine learning classifiers has improved prediction accuracy for relapse risk and resistance trajectories. These predictive models can inform dynamic risk stratification, allowing for earlier therapeutic escalation or modification in high-risk patients [35].

Importantly, the use of minimally invasive sampling techniques, such as liquid biopsies coupled with scRNA-seq, is expanding the feasibility of serial monitoring in clinical settings. As technical and computational methods evolve, longitudinal single-cell profiling may become a cornerstone of personalized lymphoma care, enabling preemptive responses to emerging resistance.

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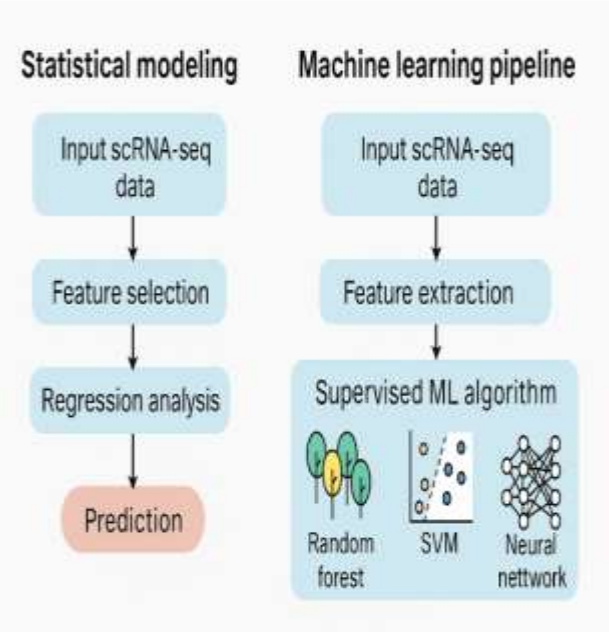

Figure 6 Predictive Modeling

5.4 Implications for Adaptive Therapy Design

The insights gained from single-cell profiling of lymphoma resistance have profound implications for the development of adaptive therapy frameworks. Traditional therapeutic regimens are largely static, assuming uniform tumor response and fixed clonal landscapes. However, resistance frequently emerges due to dynamic clonal adaptation and selection pressures exerted by treatment [36].

Adaptive therapy aims to modulate treatment based on real-time monitoring of tumor evolution, with the goal of maintaining disease control while minimizing resistance emergence. Single-cell technologies enable high-resolution tracking of subclonal shifts, allowing clinicians to detect the early rise of resistant phenotypes and adjust therapy accordingly [37].

For instance, detecting the enrichment of quiescent or stress-adapted clones may prompt the addition of differentiation agents or metabolic inhibitors. Similarly, the emergence of immune-evasive subclones might trigger the initiation of checkpoint blockade or cytokine modulation. These interventions, guided by single-cell insights, can suppress clonal dominance and prolong therapeutic efficacy.

Furthermore, temporal de-escalation of therapy based on reduced clonal diversity could reduce toxicity without compromising disease control. As longitudinal single-cell profiling becomes more integrated into clinical decision-making, adaptive therapy designs are likely to replace rigid protocols, offering more personalized and evolution-informed lymphoma treatment paradigms [38].

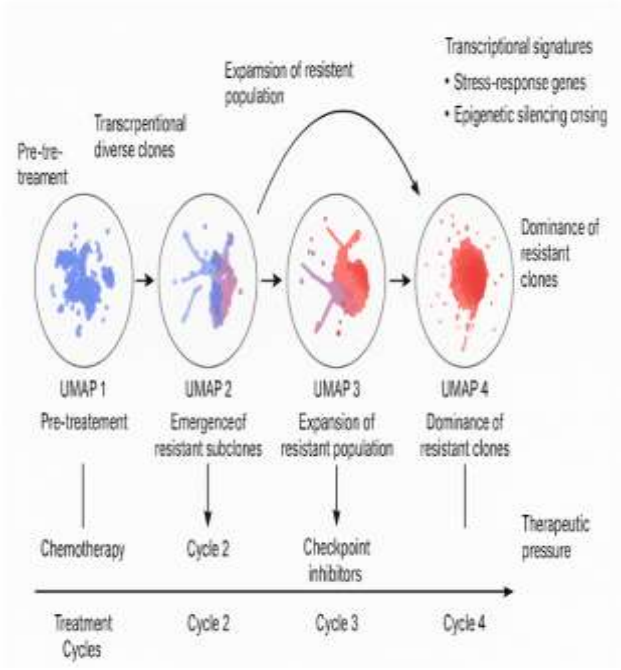

Figure 7 Longitudinal single-cell sampling and resistance progression in treated lymphoma patients

A conceptual timeline diagram showing serial biopsies across treatment cycles with UMAP clusters revealing emergence and expansion of resistant subclones over time, supported by transcriptional and epigenomic trajectory mapping.

6. COMPUTATIONAL FRAMEWORKS AND AI IN SINGLE-CELL DATA INTERPRETATION

6.1 Machine Learning in Dimensionality Reduction and Cell Type Annotation

Single-cell datasets are inherently high-dimensional, consisting of thousands of features across tens of thousands of cells. Machine learning techniques, particularly for dimensionality reduction and cell type annotation, are critical in making these datasets interpretable. Traditional methods like Principal Component Analysis (PCA) and t-Distributed Stochastic Neighbor Embedding (t-SNE) have been widely used to project high-dimensional data into lower-dimensional spaces for visualization and clustering [24].

More recently, Uniform Manifold Approximation and Projection (UMAP) has become the preferred method for capturing the global structure of data while preserving local relationships. UMAP provides faster computation and better preservation of continuum relationships between cell states compared to t-SNE [25]. These projections help uncover cellular hierarchies and subtle phenotypic transitions in lymphoma tissues, including identification of precursor or transitional states not evident through supervised analysis.

Deep learning models, such as autoencoders and variational autoencoders (VAEs), are now being used to enhance feature extraction from noisy, sparse single-cell data. These models can denoise and compress data without losing biologically relevant information, enabling more accurate downstream analyses [26]. Convolutional neural networks (CNNs) and graph neural networks (GNNs) have also shown promise in mapping spatial transcriptomic data for cell-type inference in tissue architecture contexts.

For automated annotation, supervised machine learning classifiers like support vector machines (SVM), random forests, and ensemble models have been employed to assign cell identities based on labeled training data [27]. These tools can rapidly classify thousands of cells, reducing manual curation and improving reproducibility. Tools like SingleR and Garnett are widely adopted in the single-cell community for reference-based annotation.

Machine learning continues to play a central role in transforming high-throughput single-cell data into actionable insights. As algorithmic sophistication grows, these methods will become even more integral to real-time clinical diagnostics and cellular taxonomy in lymphomas [28].

6.2 Clonal Inference Algorithms for Lymphoma Evolution Studies

Clonal inference is a foundational task in single-cell analysis, particularly in lymphomas, where the understanding of subclonal dynamics directly informs therapeutic decisions. Reconstructing the evolutionary history of tumors requires distinguishing between genetically or epigenetically related cell populations and modeling their divergence over time. Computational algorithms have been developed to infer clonal structure from various single-cell modalities, including DNA, RNA, and chromatin data [29].

Phylogenetic approaches, such as SCITE (Single Cell Inference of Tumor Evolution) and PhISCS, use mutational data from single-cell DNA sequencing to reconstruct lineage trees by mapping shared and private mutations [30]. These methods help visualize clonal hierarchies and estimate the order of mutation acquisition. In lymphomas, this has been particularly useful in understanding treatment-induced bottlenecks and relapse initiation points.

For transcriptomic data, algorithms like Monocle, Slingshot, and PAGA infer pseudotemporal trajectories that represent differentiation paths or phenotypic transitions. These trajectories often reflect clonal evolution in the absence of direct mutation data and have been used to track the emergence of resistant states in follicular and DLBCL subtypes [31].

Integrated tools such as CloneAlign and Cardelino bridge transcriptomic and genotypic data to assign cells to specific clones while accounting for technical noise. These tools have enabled researchers to associate gene expression programs with specific subclonal identities, enhancing the biological interpretability of resistance mechanisms [32].

When applied to scATAC-seq, tools like ArchR and cisTopic enable clustering of chromatin accessibility profiles and identification of regulatory divergence between clones. These methods capture enhancer dynamics and transcription factor activity that often precede phenotypic shifts.

As single-cell technologies generate increasingly multimodal datasets, clonal inference algorithms are evolving to support multi-omics integration. This allows for simultaneous tracking of genomic, transcriptomic, and epigenomic evolution—offering a holistic view of lymphoma progression [33].

Algorithm	Methods	Key Features
SCITE	Maximum likelihood	Probabilistic mutation ordering
PhISCS	Integer linear programming	Genotype conflict resolution
ArchR	Bayesian hierarchical model	Mutation data and chromatin accessibility
CloneAlign	Linear combination model	Gene expression and genotype data

Figure 8 Clonal Inference Algorithms

6.3 Predictive Modeling of Treatment Response from Single-Cell Profiles

Predicting treatment response remains one of the most clinically impactful goals of single-cell analytics. Machine learning models trained on single-cell transcriptomic and epigenomic profiles have shown growing potential in forecasting therapeutic outcomes in lymphomas. These models can detect predictive cellular states, transcriptional programs, and resistance signatures even before clinical progression is apparent [34].

Supervised learning approaches, including logistic regression, gradient-boosted trees, and deep neural networks, have been used to classify patients into responders and non-responders based on pre-treatment single-cell data. Such models have incorporated features like gene module scores, transcription factor activity, and pathway enrichment to increase interpretability and biological relevance [35].

For example, in CAR-T therapy, scRNA-seq profiling has identified T-cell exhaustion markers and cytokine profiles predictive of durable response. When integrated into machine

learning pipelines, these features have improved stratification accuracy over clinical variables alone [36]. Similarly, expression of interferon response genes in malignant cells has been linked to resistance to immunotherapy and chemotherapy in follicular lymphoma.

Multimodal approaches that combine scRNA-seq, scATAC-seq, and spatial data further enhance predictive power. Algorithms such as MOFA+ and DeepMAPS enable feature fusion and latent space modeling, capturing interdependencies between omics layers [37].

Importantly, the interpretability of predictive models is critical for clinical acceptance. Recent frameworks integrate SHAP (SHapley Additive exPlanations) values and attention mechanisms to identify key drivers of prediction, facilitating transparency and biological validation [38].

As clinical datasets grow and model performance improves, predictive analytics from single-cell data will become indispensable in guiding therapy decisions and tailoring treatment to individual lymphoma patients.

6.4 Integrating Real-Time Analytics into Clinical Workflows

Bringing single-cell analytics into real-time clinical workflows requires streamlined integration of wet-lab processing, computational pipelines, and decision-making frameworks. Automated platforms are now being developed to perform sample processing, sequencing, and cloud-based analytics within clinically actionable timelines [39].

For instance, cloud-based systems utilizing scalable machine learning frameworks can process single-cell RNA and ATAC datasets within hours of biopsy acquisition. Dashboards powered by visualization libraries like Plotly or Streamlit present UMAP plots, resistance scores, and clonal hierarchies to clinicians in interpretable formats [40].

Furthermore, APIs and electronic health record (EHR) integration enable cross-platform interoperability, allowing physicians to overlay single-cell insights with clinical history, lab data, and imaging results. Such real-time feedback loops can support adaptive therapy design, early intervention, and biomarker-driven clinical trials.

While regulatory, computational, and ethical challenges remain, the fusion of single-cell science and clinical decision-making is becoming increasingly tangible. It signals a shift toward predictive, data-rich, and personalized oncology care in lymphoma treatment [41].

Table 3. Comparison of Computational Tools for Clonal Inference and Resistance Prediction

Tool Name	Application Domain	Input Modality	Strengths	Limitation
SCITE	Clonal tree reconstruction	Single-cell DNA	High mutation accuracy	Requires high coverage
Monocle	Pseudotime trajectory	Single-cell RNA	Models lineage transitions	Limited clonal resolution
CloneAlign	Genotype-to-phenotype map	DNA + RNA	Links expression to clone identity	Sensitive to dropout
ArchR	Epigenomic clustering	Single-cell ATAC	Integrates chromatin and gene scores	High RAM requirements
DeepMAPS	Multimodal prediction	RNA + ATAC + spatial	Strong performance in resistance models	Complex implementation
MOFA+	Multi-omics integration	Multi-omics	Captures latent regulatory factors	Assumes linearity in factors

7. CLINICAL TRANSLATION AND PRECISION ONCOLOGY APPLICATIONS

7.1 Current Clinical Trials Leveraging Single-Cell Technologies

Single-cell technologies are increasingly being incorporated into clinical trial frameworks to enhance response prediction, monitor disease progression, and refine treatment strategies in lymphomas. Several ongoing studies are integrating single-cell RNA sequencing (scRNA-seq), single-cell ATAC-seq (scATAC-seq), and spatial transcriptomics as part of exploratory endpoints to uncover biomarkers of efficacy and resistance [28].

For instance, clinical trial NCT04896062 is exploring the use of single-cell transcriptomic profiling in patients with

relapsed/refractory diffuse large B-cell lymphoma (DLBCL) undergoing CAR-T therapy. The trial aims to correlate pre-infusion T-cell phenotypes with treatment outcomes using scRNA-seq, enabling identification of signatures predictive of durable responses [29]. Another study, NCT04460248, focuses on single-cell epigenomic analysis of follicular lymphoma biopsies to investigate the chromatin accessibility changes associated with therapy adaptation.

In Hodgkin lymphoma, a pilot trial at the Dana-Farber Cancer Institute is utilizing spatially resolved single-cell technologies to understand how Reed-Sternberg cells interact with immune infiltrates during checkpoint inhibitor therapy. The insights gained from these spatial data are expected to improve stratification and therapeutic selection for immunorefractory patients [30].

Moreover, single-cell data from these trials are feeding into collaborative consortiums such as the Human Tumor Atlas Network (HTAN), which aims to generate comprehensive multi-omics atlases across various cancer types, including lymphomas. These atlases provide reference frameworks for interpreting patient-specific data and developing generalized predictive models [31].

Importantly, several trials now mandate longitudinal sample collection for serial single-cell profiling. This temporal design enables tracking of clonal dynamics and identification of emerging resistance markers, facilitating real-time treatment adjustment and adaptive trial design [32]. As these trials progress, they are expected to establish robust pipelines for integrating single-cell outputs into regulatory and therapeutic decision-making processes.

7.2 Personalized Treatment Design Based on Clonal Composition

Personalized treatment approaches in lymphomas are shifting from mutation-centric strategies to those informed by cellular states, clonal architecture, and microenvironmental context—insights uniquely afforded by single-cell technologies. By identifying resistant subclones, therapy-sensitive populations, and immunosuppressive elements at the time of diagnosis, clinicians can now formulate individualized treatment regimens that target the full spectrum of tumor complexity [33].

In practice, personalized design begins with biopsy-based single-cell sequencing to delineate the transcriptional and epigenetic profiles of malignant and non-malignant cells. Clonal composition is assessed using computational tools that map lineage hierarchies and define high-risk subpopulations [34]. These subclones are then characterized for druggable features, such as overexpressed survival pathways, immune checkpoint ligands, or metabolic dependencies.

For example, patients with DLBCL harboring NF-κB-active resistant clones may receive up-front inclusion of IκB kinase (IKK) inhibitors alongside standard R-CHOP. Alternatively, those with a clonal dominance of PD-L1–expressing lymphoma cells may benefit from early integration of checkpoint blockade therapy [35]. Importantly, such strategies are based not only on the presence of specific mutations but also on dynamic cell-state features and transcriptional programs.

Furthermore, some treatment algorithms now utilize decision-support systems that integrate single-cell data with clinical parameters to recommend therapy options. These tools continuously learn from updated datasets, improving personalization accuracy over time [36].

As more data become available from clinical trials and patient registries, clonal composition–guided therapy selection is likely to become a routine component of lymphoma care, particularly in refractory or high-risk patients.

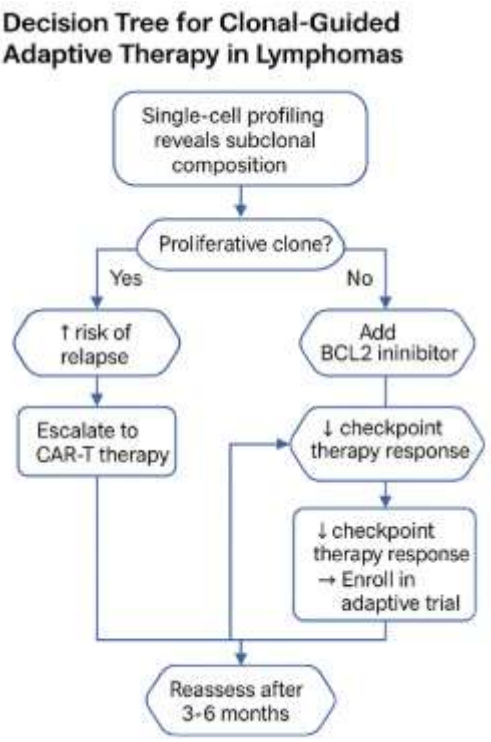

Figure 9 Personalized Treatment Design

7.3 Challenges in Clinical Adoption and Regulatory Considerations

Despite promising advances, several barriers hinder the widespread clinical adoption of single-cell technologies in lymphoma management. One major challenge is the lack of standardized protocols for tissue dissociation, sequencing, and data preprocessing, leading to variability across institutions and studies [37]. These inconsistencies limit the reproducibility and comparability of results, making it difficult to derive universal clinical guidelines.

Another issue is the interpretation of complex, high-dimensional datasets. While computational tools exist to process and analyze single-cell outputs, their application often

requires specialized bioinformatics expertise not readily available in routine clinical settings [38]. This challenge is compounded by the current lack of integration between single-cell platforms and electronic health record (EHR) systems, limiting seamless decision-making.

Cost and turnaround time also remain significant constraints. Although technologies are becoming more accessible, the infrastructure required for real-time processing—including high-throughput sequencing and computational resources—is still prohibitive for many healthcare institutions [39]. Moreover, regulatory frameworks for the clinical validation of single-cell assays are still evolving. Regulatory bodies such as the FDA and EMA currently classify these technologies as research-use only, with limited pathways for diagnostic approval.

To overcome these barriers, efforts are underway to establish standardized pipelines, accredited laboratories, and validated clinical-grade single-cell assays. In parallel, multi-institutional initiatives are developing benchmark datasets and reference atlases to facilitate regulatory harmonization and risk assessment [40].

Addressing these challenges will be crucial for transitioning single-cell profiling from experimental use into certified diagnostic and therapeutic tools in lymphoma care.

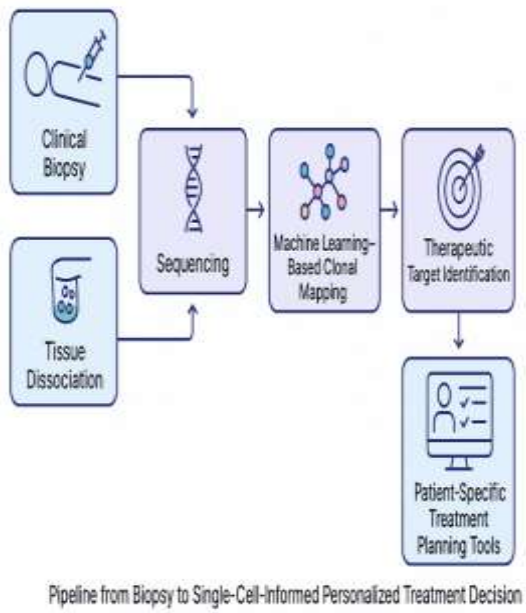

Figure 10 Pipeline from Biopsy to Single-Cell-Informed Personalized Treatment Decision
(A schematic flowchart showing clinical biopsy collection, tissue dissociation, sequencing, machine learning–based clonal mapping, therapeutic target identification, and integration into patient-specific treatment planning tools.)

8. FUTURE PERSPECTIVES AND RESEARCH DIRECTIONS

8.1 Expanding Beyond Transcriptomics: Proteomics and Metabolomics in Single Cells

While transcriptomics has dominated single-cell research due to its relatively mature methodologies and analytical frameworks, proteomics and metabolomics are rapidly emerging as complementary layers to further decode cellular behavior in lymphomas. Single-cell proteomics enables the quantification of surface and intracellular proteins, providing a direct readout of functional states not always inferred from RNA expression alone [32]. For example, surface markers such as CD19, CD20, and PD-L1 often serve as therapeutic targets and are critical for defining cell identity in immunophenotypic classification.

Technologies like mass cytometry (CyTOF) and oligonucleotide-tagged antibody platforms (e.g., CITE-seq, REAP-seq) have expanded the capacity to profile dozens to hundreds of proteins in thousands of individual cells [33]. These approaches have been used to characterize tumor-infiltrating lymphocytes and malignant B cells, identifying signaling adaptations that contribute to immune escape or drug resistance.

In parallel, single-cell metabolomics—although still in its infancy—has begun to reveal metabolic heterogeneity in cancer. Techniques such as mass spectrometry imaging and nanoDESI allow spatially resolved detection of metabolites, providing insight into nutrient uptake, redox states, and bioenergetic profiles at the single-cell level [34]. This is particularly relevant in lymphoma, where metabolic rewiring plays a role in supporting rapid proliferation and therapy resistance.

The integration of transcriptomic, proteomic, and metabolomic data allows for multi-dimensional profiling of lymphoma ecosystems. This holistic view captures not only the potential (RNA) but also the activity (protein) and function (metabolites) of each cell. As technology advances, these multi-omics approaches will likely become central to understanding functional diversity, therapeutic vulnerabilities, and adaptive responses in lymphoid malignancies [35].

8.2 Spatial Single-Cell Genomics for Tumor Architecture Insights

Understanding the spatial context of cellular interactions is essential for interpreting the behavior of malignant and non-malignant cells within the lymphoma microenvironment. Spatial single-cell genomics merges gene expression profiling with tissue localization, allowing researchers to preserve architectural information while interrogating cell identity and function [36].

Technologies such as spatial transcriptomics (10x Genomics Visium), multiplexed error-robust fluorescence in situ

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hybridization (MERFISH), and Slide-seq enable high-resolution mapping of transcriptomic features across tissue sections. These tools have been applied to lymphomas to uncover how malignant clones are distributed relative to immune cell niches and stromal structures [37]. For example, spatial transcriptomics in Hodgkin lymphoma has shown that Reed-Sternberg cells often cluster in regions with diminished T-cell infiltration, supporting spatially organized immune evasion.

Moreover, spatial profiling has revealed gradients of gene expression associated with hypoxia, cytokine gradients, and clonal zonation. These features often correlate with treatment resistance or immune modulation, underscoring the relevance of location-specific signaling [38]. In follicular lymphoma, for instance, the spatial exclusion of cytotoxic T cells from germinal centers has been linked to suboptimal immunotherapy response.

The integration of spatial data with conventional single-cell sequencing and imaging technologies enhances our understanding of how microenvironmental architecture supports or suppresses disease progression. These insights are crucial for designing therapies that not only target cellular subtypes but also disrupt the spatial dependencies that sustain them [39].

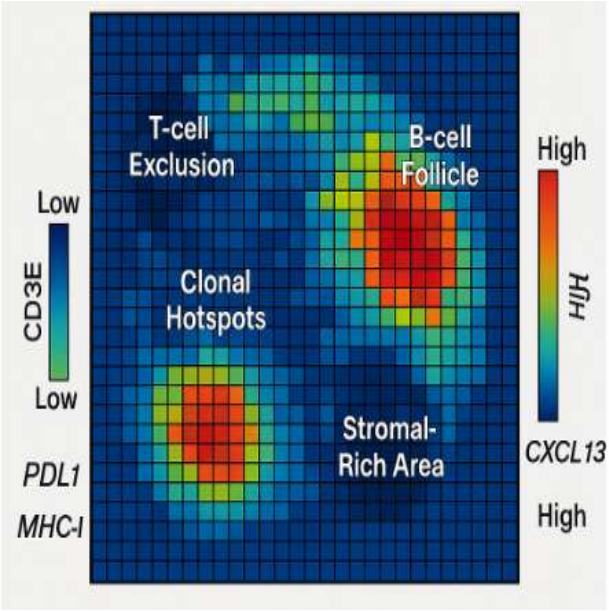

Figure 11 Spatial Genomics

8.3 Ethical and Data-Sharing Considerations in Large-Scale Single-Cell Studies

As single-cell studies expand in scale and scope, ethical considerations surrounding data privacy, informed consent, and equitable access to data have gained increasing importance. Single-cell sequencing often generates high-dimensional, personally identifiable information, particularly when integrated with clinical metadata, imaging, and genomic sequencing [40]. Ensuring that patients understand how their data will be used, stored, and potentially reanalyzed in future studies is essential for maintaining ethical standards.

Consent procedures must now account for open-ended data sharing, longitudinal re-contacting for follow-up studies, and potential re-identification risks posed by multi-omics data. Institutional Review Boards (IRBs) and data governance committees are being urged to develop new frameworks tailored specifically to the ethical demands of high-resolution single-cell datasets [41].

On the data-sharing front, the need for open-access repositories that support cross-study comparison and meta-analysis is critical. Platforms like the Human Cell Atlas (HCA), Cancer Research Data Commons (CRDC), and Single Cell Portal provide valuable infrastructure for storing, accessing, and annotating large-scale datasets [42]. However, challenges remain in harmonizing formats, ensuring interoperability, and addressing disparities in data contribution from underrepresented populations.

Furthermore, researchers and institutions must balance open science with the protection of intellectual property, particularly when findings are linked to clinical applications or biomarker development [44]. As single-cell approaches become more embedded in translational research, continuous dialogue between scientists, clinicians, ethicists, and policymakers will be essential to ensure that these technologies are used responsibly and inclusively [43].

9. CONCLUSION

9.1 Summary of Key Findings and Conceptual Advances

This review has explored the transformative role of single-cell technologies in unraveling the complexity of lymphomas. By moving beyond population-averaged analyses, single-cell profiling has enabled a detailed dissection of tumor heterogeneity, clonal evolution, and microenvironmental dynamics. One of the most significant conceptual advances lies in the ability to identify rare subclonal populations that contribute to relapse and treatment resistance. These insights have redefined our understanding of intratumoral diversity, emphasizing that therapeutic failure is often driven not by dominant clones but by resilient, transcriptionally distinct minor populations.

Advances in single-cell RNA sequencing, ATAC-seq, and multi-omics integration have also provided new frameworks for modeling tumor progression and cell-state transitions. These modalities allow for real-time tracking of therapy-induced changes, offering predictive insight into emerging resistance before it becomes clinically evident. Spatial single-cell technologies further contextualize these findings by mapping cellular interactions and niche-dependent behavior within the tumor microenvironment.

Machine learning and bioinformatics tools have played a crucial role in managing and interpreting the vast complexity

of single-cell data. From dimensionality reduction to clonal inference and treatment prediction, computational innovations have expanded the utility of single-cell platforms in translational research.

Importantly, several clinical trials are now incorporating single-cell endpoints, signaling a shift from research novelty to clinical relevance. Despite logistical and regulatory challenges, the field is moving toward personalized lymphoma care informed by high-resolution molecular insights. Collectively, these findings establish single-cell approaches as central to future diagnostic, prognostic, and therapeutic strategies in hematologic oncology.

9.2 Call for Integrative and Clinically Embedded Single-Cell Research

To fully realize the clinical potential of single-cell technologies in lymphoma care, there is a pressing need for more integrative and clinically embedded research frameworks. While experimental and computational tools have matured considerably, their translation into standard oncology workflows remains limited. Bridging this gap will require collaborative models that bring together clinicians, researchers, data scientists, and regulatory experts within shared ecosystems.

One critical priority is the development of clinically validated single-cell assays and interpretation platforms. This includes creating standardized protocols for tissue processing, sequencing, and data annotation that are compatible with clinical timelines. Equally important is the incorporation of bioinformatics tools into electronic health records and decision-support systems, enabling real-time application of single-cell insights to patient management.

Integrative research must also address biological diversity and population-level disparities. Many current datasets are biased toward specific subtypes or demographics, limiting the generalizability of findings. Embedding single-cell approaches within multicenter clinical trials and international consortiums can promote data inclusivity and accelerate biomarker discovery across diverse populations.

Furthermore, training programs must be expanded to equip clinicians and translational researchers with the skills needed to interpret and apply single-cell data. This cultural shift in clinical oncology will be instrumental in ensuring that technological advances translate into meaningful patient outcomes.

Ultimately, the future of lymphoma treatment lies in the convergence of high-resolution molecular profiling and personalized care. Clinically embedded single-cell research represents a pivotal step toward this goal, offering a roadmap for adaptive, precision-guided oncology in the years ahead.

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