Hematological Disorders in the Single-Cell Era
eISSN: 3120-3833
https://hdsce.cultechpub.com/index.php/hdsce

Tumor Heterogeneity in Leukemia and Lymphoma: Insights from Single-Cell Analyses

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Abstract

Tumor heterogeneity is a fundamental property of leukemia and lymphoma that drives disease progression, relapse, and therapy resistance. For decades, bulk genomic and transcriptomic analyses have provided a foundational understanding of these malignancies but have obscured the cellular diversity within the tumor ecosystem. The advent of high-throughput single-cell technologies, particularly single-cell RNA sequencing (scRNA-seq), has revolutionized our capacity to deconvolute this complexity. This review synthesizes how single-cell analyses are reshaping our understanding of tumor heterogeneity in hematological cancers. We explore the role of intra-clonal genetic diversity in Acute Myeloid Leukemia (AML) and B-cell Acute Lymphoblastic Leukemia (B-ALL), the functional and transcriptional plasticity of subpopulations, and the dynamic interplay between malignant cells and the immune microenvironment in both leukemia and lymphoma. We further highlight how the tumor microenvironment in lymphomas fosters heterogeneity and immune evasion. Furthermore, the integration of single-cell data with spatial transcriptomics and computational models is beginning to predict evolutionary trajectories, offering a window into future clinical behaviors. Critically, we discuss how single-cell approaches have identified pre-existing or emergent resistant subclones under therapeutic pressure, providing a mechanistic basis for treatment failure. The clinical implications are profound, paving the way for single-cell diagnostics for minimal residual disease (MRD) monitoring and the identification of novel therapeutic targets. We conclude that single-cell multi-omics is an indispensable tool for unraveling the multifaceted nature of tumor heterogeneity, with the potential to propel the field toward more precise and effective therapeutic strategies.

Keywords

Tumor Heterogeneity, Single-Cell Rna Sequencing, Leukemia, Lymphoma, Clonal Evolution, Tumor Microenvironment, Therapy Resistance, Minimal Residual Disease

1. Introduction

Leukemia and lymphoma, the principal hematological malignancies, originate from the malignant transformation of hematopoietic stem, progenitor, or mature immune cells. Despite significant advances in treatment, including targeted therapies and immunotherapies, relapse and therapeutic resistance remain major clinical challenges. A central tenet explaining these outcomes is tumor heterogeneity-the genetic, epigenetic, transcriptomic, and functional variation among cancer cells within a patient. This diversity is not merely a static feature but a dynamic ecosystem shaped by evolutionary pressures [1].

Traditional bulk sequencing methods have been instrumental in identifying recurrent driver mutations and classifying disease subtypes. However, they provide only an averaged signal, masking the existence of rare subpopulations, mixed cell states, and the complex cellular interactions that constitute the tumor microenvironment (TME). This limitation has hindered a complete understanding of disease initiation, progression, and relapse.

The emergence of single-cell technologies, particularly scRNA-seq, coupled with assays for transposase-accessible chromatin (scATAC-seq), and cellular indexing of transcriptomes and epitopes (CITE-seq), has ushered in a new era of resolution. These tools allow for the unbiased dissection of cellular composition, the inference of developmental trajectories, and the characterization of cell-to-cell communication networks at an unprecedented scale [2]. The clinical urgency of this paradigm shift cannot be overstated. The failure to eradicate minor, resistant subclones or to modify a pro-tumorigenic TME often dictates poor long-term outcomes. Single-cell technologies offer the resolution needed to move beyond population-level averages and confront the root causes of therapeutic failure: diversity and adaptation.

This article aims to provide a comprehensive overview of the insights gained from single-cell analyses into the nature and consequences of tumor heterogeneity in leukemia and lymphoma. We will delve into the genetic and non-genetic sources of diversity, explore the role of the TME, and elucidate the mechanisms of therapy resistance uncovered at

single-cell resolution. Finally, we will discuss the translational potential of these findings in shaping future diagnostics and therapeutics [3].

2. Methodological Foundations of Single-Cell Analysis in Hematology

The application of single-cell technologies to hematological malignancies is particularly powerful due to the natural suspension state of blood and bone marrow cells, which simplifies sample preparation.

2.1 Core Single-Cell Technologies

• **Single-Cell RNA Sequencing (scRNA-seq):** This is the workhorse of the field. It quantifies the transcriptome of thousands of individual cells, enabling the identification of distinct cell states, subpopulations, and their transcriptional signatures. It can reveal previously unappreciated diversity within seemingly homogeneous diagnostic categories.

• **Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq):** This method simultaneously measures transcriptome and surface protein expression (using oligonucleotide-tagged antibodies), providing a robust bridge between transcriptomic data and conventional immunophenotyping by flow cytometry [4].

• **Single-Cell Assay for Transposase-Accessible Chromatin using sequencing (scATAC-seq):** This technique profiles chromatin accessibility, providing insights into the epigenetic regulation and transcriptional regulatory networks that define cell identity and state.

• **Single-Cell DNA Sequencing (scDNA-seq):** While more challenging, scDNA-seq allows for the direct interrogation of genetic heterogeneity and the reconstruction of clonal phylogenies by identifying mutations in individual cells [5].

2.2 Computational and Bioinformatic Approaches

The power of single-cell data is unlocked through sophisticated computational pipelines:

• **Dimensionality Reduction and Clustering:** Tools like UMAP and t-SNE are used to visualize high-dimensional data, where cells with similar expression profiles cluster together, revealing distinct subpopulations.

• **Lineage Tracing and Pseudotime Analysis:** Algorithms such as Monocle and PAGA can order cells along a pseudotemporal trajectory, inferring the developmental pathways and cellular transitions that occur during differentiation or malignant transformation.

• **Cell-Cell Communication Analysis:** Tools like CellChat and NicheNet infer potential ligand-receptor interactions between different cell types within the TME, predicting key signaling pathways.

2.3 The Power of Multi-Omic Integration

The true potential of single-cell analysis is realized through the integration of multiple modalities from the same cell or the same sample. Techniques like scRNA-seq + scATAC-seq (multiome) allow researchers to directly link a cell's open chromatin landscape to its transcriptional output, revealing the regulatory logic behind cell states. For instance, this can identify the specific transcription factors driving a resistant subpopulation. Furthermore, the computational integration of datasets from different patients or time points enables the identification of conserved cell states and signaling pathways across a heterogeneous cohort, distinguishing patient-specific variation from universal disease mechanisms. This holistic, multi-omic view is transforming single-cell biology from a descriptive to a predictive science, capable of modeling the regulatory networks that sustain malignancy.

The integration of these multi-omic modalities at the single-cell level provides a holistic view of the rules governing cellular identity and behavior in cancer.

3. Deconstructing Genetic and Clonal Heterogeneity in Leukemia

Single-cell studies have fundamentally altered our understanding of the clonal architecture of leukemias [6].

3.1 Acute Myeloid Leukemia (AML)

AML is a paradigm of clonal heterogeneity. Single-cell DNA and RNA sequencing studies have revealed that the founding clone acquires subsequent mutations in a branching, evolutionary pattern.

• **Subclonal Architecture:** scRNA-seq studies have shown that distinct genetic subclones can occupy unique transcriptional states. For example, a subclone with a *FLT3-ITD* mutation might exhibit a proliferative signature, while another with *IDH2* mutations shows a more differentiated state. This genetic and transcriptional divergence contributes to functional heterogeneity.

• **Leukemia Stem Cells (LSCs):** The LSC model posits a hierarchical organization sustained by a rare population of self-renewing cells. scRNA-seq has enabled the prospective identification of LSCs based on their transcriptional signatures, often resembling primitive progenitor states, and has revealed their extensive heterogeneity both between and within patients. This heterogeneity underlies the variable responses to therapies designed to eradicate LSCs [7].

3.2 B-Cell Acute Lymphoblastic Leukemia (B-ALL)

In B-ALL, single-cell analyses have uncovered diversity that impacts diagnosis and treatment.

• **Intra- and Inter-Leukemic Heterogeneity:** scRNA-seq has identified multiple, coexisting subpopulations within individual B-ALL patients that reflect different stages of B-cell development (e.g., pro-B, pre-B, and early B-cell states). This developmental arrest is not uniform, and the presence of "differentiated" subclones can influence prognosis.

• **Clonal Evolution under Therapy:** Longitudinal tracking of patients using single-cell methods has visualized the dynamic process of clonal evolution. Treatment often eradicates the dominant clone, only for a minor, pre-existing resistant subclone to expand and cause relapse. This has been clearly demonstrated in cases of Philadelphia chromosome-like B-ALL, where scRNA-seq can pinpoint the specific subclone harboring resistance mutations [8].

3.3 Chronic Lymphocytic Leukemia (CLL): A Model of Clinical Progression

CLL provides a powerful model for studying how clonal dynamics dictate clinical course. Single-cell analyses have revealed that the progression from indolent to aggressive disease is often driven by the expansion of subclones with specific genetic lesions, such as mutations in *TP53* or *NOTCH1*. scRNA-seq has further shown that these high-risk subclones can reside in specific proliferative niches and exhibit distinct signaling pathway activation (e.g., B-cell receptor signaling). This allows for a molecular explanation of clinical heterogeneity: patients with a more diverse and dynamic clonal architecture at diagnosis, as revealed by single-cell sequencing, are more likely to experience rapid progression and therapy resistance. Thus, single-cell profiling in CLL is not just a research tool but is approaching clinical utility for risk stratification [9].

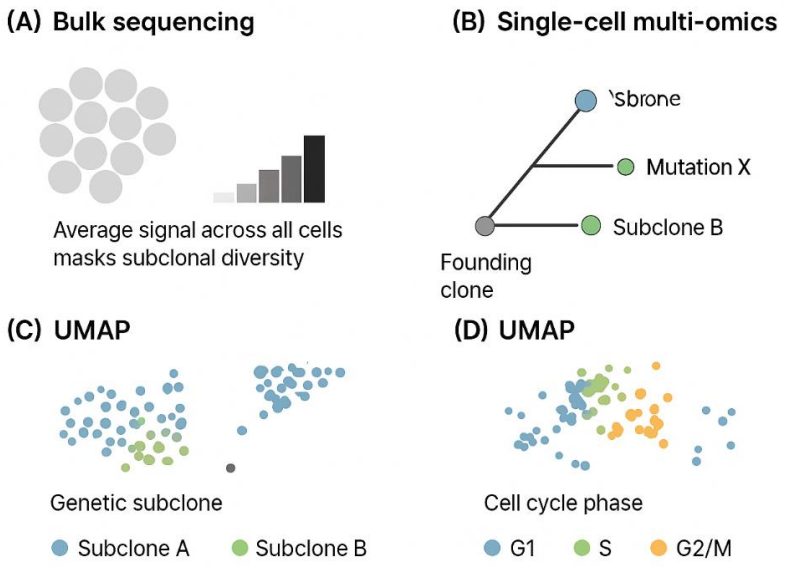

Figure 1. Single-Cell Resolution of Clonal Architecture in Acute Leukemia

Figure 1 compares traditional bulk sequencing and single-cell multi-omics, illustrating their differences in resolving cellular heterogeneity, gene mutations, and cell states.

(A) Bulk sequencing: The left side of the image shows a cluster of gray cells, and the right side shows a bar chart. Explanation: Bulk sequencing averages the signals from all cells together, causing differences between subclones (different cell populations) to be "masked." In other words, if a sample consists of different types of cells, bulk sequencing mixes them together, making it impossible to distinguish which cells have which mutations or states.

(B) Single-cell multi-omics: Displays a clonal lineage. Each point represents a subclone, each a different color. It differentiates into Subclone A (blue) and Subclone B (green) Subclone A acquires a mutation X. Key point: Single-cell methods can track mutations, gene expression, epigenome, etc., in each cell, thus accurately identifying clonal structures.

(C) UMAP – Genetic Subclone: UMAP is a visualization method that reduces high-dimensional data to 2D. The dots in the graph represent individual cells. Colors represent different genetic subclones: Blue: Subclone A, Green: Subclone B. The graph shows cells with different gene mutation backgrounds spatially clustered into two groups [10].

(D) UMAP – Cell cycle phase: Same single-cell data, but this time the colors represent cell cycle phases: Blue: G1, Green: S, Yellow: G2/M, You can see that cells are divided into different functional states, not just genetic background.

Essentially, single-cell technology can reveal biological heterogeneity hidden by bulk sequencing.

4. Non-Genetic Heterogeneity and Cellular Plasticity

Not all heterogeneity is driven by genetic mutations. Single-cell transcriptomics has highlighted the profound role of non-genetic plasticity.

4.1 Transcriptional and Metabolic States

scRNA-seq analyses routinely reveal continuous gradients of cell states within leukemic populations, defined by variations in:

- **Cell Cycle Status:** Proliferating versus quiescent cells.
- **Metabolic Dependencies:** Subpopulations reliant on oxidative phosphorylation versus glycolysis.
- **Stress Response Pathways:** Cells exhibiting signatures of endoplasmic reticulum stress or apoptosis priming.
- This functional heterogeneity creates a "bet-hedging" strategy for the tumor, ensuring that a subset of cells is pre-adapted to withstand therapeutic insults.

4.2 Lineage Plasticity

A striking finding from single-cell studies is the phenomenon of lineage plasticity, where leukemic cells demonstrate a capacity to shift their identity.

- **AML with Monocytic Differentiation:** scRNA-seq trajectories have shown that leukemic stem and progenitor cells can retain a latent potential for monocytic or granulocytic differentiation, which can be influenced by external signals from the TME. This plasticity may contribute to immune evasion and therapy resistance.

4.3 Epigenetic Heterogeneity as a Precursor to Resistance

scATAC-seq has uncovered a layer of heterogeneity that precedes transcriptional changes: variability in chromatin accessibility. In AML, for example, scATAC-seq can identify subpopulations of cells with an open chromatin configuration at the loci of genes involved in drug efflux or survival pathways, even before those genes are highly expressed. This "epigenetic priming" represents a pre-existing, non-genetic reservoir of resistance potential [11]. Upon drug exposure, these primed cells can rapidly activate the transcriptional programs necessary for survival. This explains how resistance can emerge uniformly and quickly across a population without the need for new mutations, highlighting the need for therapies that target both genetic and epigenetic drivers.

5. The Tumor Microenvironment: A Nexus of Cellular Crosstalk

The TME is not a passive bystander but an active participant in shaping tumor heterogeneity. Single-cell analyses provide a complete census of the TME and its interactions.

5.1 The Bone Marrow Microenvironment in Leukemia

In AML, scRNA-seq of primary patient bone marrow has dissected the non-hematopoietic stromal and immune components.

- **Mesenchymal Stromal Cells (MSCs):** Single-cell studies have revealed functionally distinct subclasses of MSCs in the leukemic bone marrow. Some subsets may provide niche factors that support LSC quiescence and survival, while others may have tumor-suppressive functions [12].
- **Immune Cell Dysfunction:** scRNA-seq has detailed the immunosuppressive landscape in AML, characterized by the expansion of exhausted T-cell subsets, regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs). Cell-cell communication analysis predicts that leukemic cells actively secrete factors that sculpt this immunosuppressive niche.

5.2 The Immune Microenvironment in Lymphoma

The application of single-cell technologies to solid lymphomas, such as Diffuse Large B-Cell Lymphoma (DLBCL), has been equally revealing.

- **Cellular Composition and Outcomes:** Integrated scRNA-seq and CITE-seq have correlated specific TME compositions with patient outcomes. For instance, a TME rich in CD8+ T cells and CD4+ T-helper cells is associated with a better prognosis, while one dominated by Tregs and pro-tumorigenic macrophages is linked to treatment failure.
- **Spatial Context:** When combined with spatial transcriptomics, single-cell data can map these interactions to specific anatomical locations, such as the lymphoma niche, revealing how spatial organization contributes to functional heterogeneity and immune evasion.

5.3 The Vascular Niche and Metabolic Symbiosis

A newly appreciated aspect of the TME is the metabolic crosstalk between tumor cells and stromal components. scRNA-seq analyses have shown that leukemic cells can induce a pro-angiogenic signature in endothelial cells, creating

a "vascular niche" that not only supplies nutrients but also provides survival signals. Furthermore, metabolic symbiosis has been observed, where subpopulations of cancer cells with different metabolic dependencies (e.g., glycolytic vs. oxidative) exchange metabolites to mutual benefit [13]. For instance, one subpopulation might break down glucose to lactate, which is then taken up and used as a fuel by a neighboring subpopulation. This metabolic cooperation, deconvoluted by single-cell analysis, creates a robust ecosystem that is highly resistant to metabolic inhibitors targeting a single pathway.

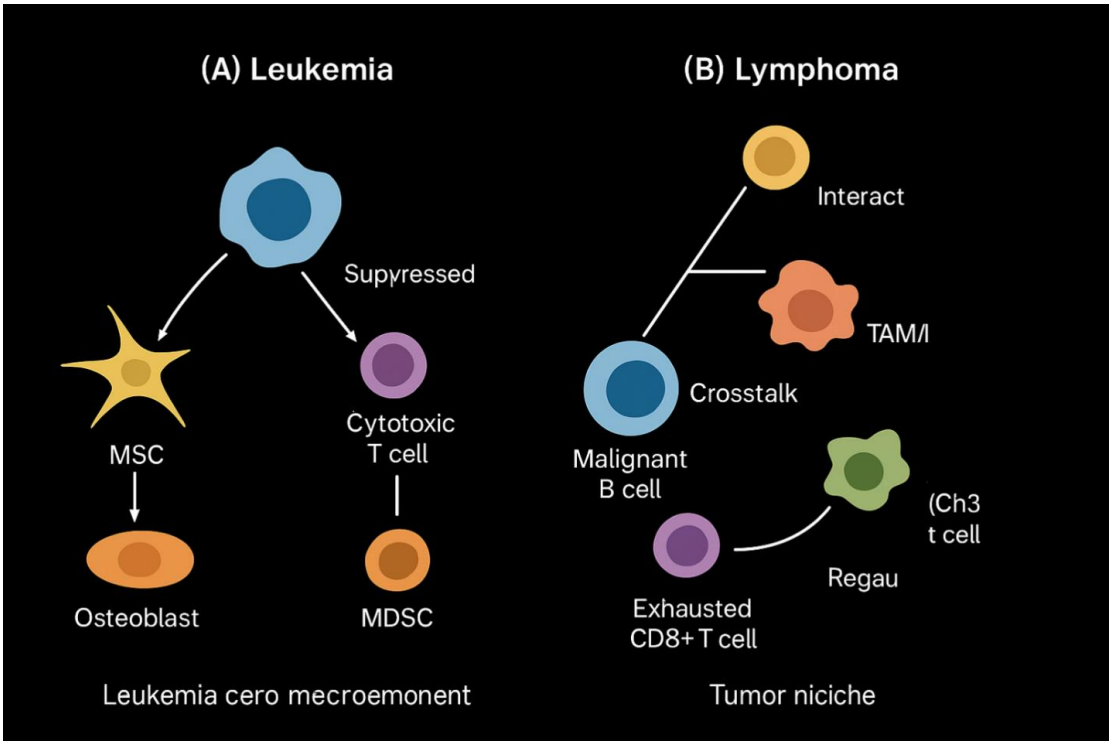

Figure 2. The Tumor Microenvironment in Hematological Malignancies

Figure 2 is divided into two parts, illustrating how the tumor microenvironment works in leukemia and lymphoma. Although some words are misspelled, the overall meaning is clear: tumors do not exist in isolation, but rather grow within a microenvironment composed of various cells that either help the tumor, are controlled by the tumor, or are suppressed by the tumor. This diagram illustrates that both leukemia and lymphoma "remodel" their surrounding microenvironment, promoting tumor survival and spread by suppressing the immune system and manipulating surrounding cells.

6. Insights into Therapy Resistance and Relapse

The primary clinical challenge in leukemia and lymphoma is therapy resistance, a direct consequence of tumor heterogeneity.

6.1 Pre-Existing versus Acquired Resistance

Single-cell studies of paired diagnosis-relapse samples have delineated two primary routes to resistance:

• **Selection of Pre-Existing Minor Subclones:** In many cases of relapsed AML and ALL, the dominant relapse clone was present as a minor, often genetically distinct, subpopulation at diagnosis. This subclone was inherently resistant to the therapy and gained a competitive advantage upon the eradication of the sensitive majority.

• **Acquired Evolution:** In other cases, the relapse clone evolves from the founding clone, acquiring new mutations under therapeutic pressure. Single-cell multi-omics can track the acquisition of these mutations and the accompanying transcriptional rewiring [14].

6.2 Non-Genetic Resistance Mechanisms

Resistance is not always genetic. scRNA-seq has identified:

• **Persister Cells:** A transient, drug-tolerant state characterized by a distinct transcriptional program, often involving metabolic quiescence and upregulation of survival pathways. These cells are not genetically distinct but can serve as a reservoir for the eventual emergence of genetic resistance.

• **Cell State Switching:** Therapy can induce a shift in the transcriptional state of malignant cells. For example, venetoclax treatment in AML can select for cells with a monocytic-like signature, which are inherently less dependent on BCL-2 for survival [15].

6.3 The Protective Role of the Microenvironment in Therapy Failure

Single-cell analyses have clarified that resistance is not solely a cell-autonomous phenomenon. The TME actively protects malignant cells from therapy. For example, scRNA-seq studies have shown that bone marrow MSCs and macrophages can upregulate anti-apoptotic signals (e.g., cytokines like IL-6) in response to chemotherapy, creating a protective niche for residual cells. In lymphoma, cancer-associated fibroblasts can form a physical barrier and secrete factors that reduce drug penetration. Cell-cell communication inference from single-cell data consistently predicts these pro-survival interactions, identifying the TME as a co-conspirator in relapse. This underscores the necessity of combining targeted therapies with agents that disrupt these protective niche interactions to achieve more durable responses.

7. Clinical Translation and Future Perspectives

The insights from single-cell analyses are rapidly moving toward clinical application.

7.1 Redefining Minimal Residual Disease (MRD)

The detection of MRD is a critical prognostic factor. scRNA-seq and CITE-seq offer a highly sensitive and multidimensional approach to MRD monitoring. They can detect rare, persistent cells not only by their immunophenotype but also by their leukemia-specific transcriptional or metabolic signatures, which may be more predictive of impending relapse than current methods.

7.2 Identifying Novel Therapeutic Vulnerabilities

By revealing the full spectrum of cellular states and interactions within tumors, single-cell analyses uncover new targets. These could include:

- **Surface Proteins** uniquely expressed on resistant subclones or LSCs.
- **Key Ligand-Receptor Pairs** mediating immunosuppressive crosstalk in the TME.
- **Dependency Pathways** active in specific cell states, such as persister cells.

7.3 Challenges and Future Directions

While promising, challenges remain, including cost, sample processing complexity, and the computational burden of data analysis. Future efforts will focus on:

- **Spatial Multi-omics:** Integrating single-cell data with spatial context to understand the geographical rules of heterogeneity.
- **Longitudinal Tracking:** Routine sampling of patients to build dynamic maps of clonal evolution in response to therapy.
- **Algorithm Development:** Creating better tools to integrate genetic, transcriptomic, and epigenetic data from the same cell to build complete causal models of cellular behavior.

7.4 Towards Single-Cell Guided Clinical Trials and Patient Stratification

The future of oncology clinical trials lies in patient stratification based on deep molecular profiling. Single-cell technologies are poised to play a central role by identifying biomarkers beyond single mutations-such as specific cell state signatures or TME compositions-that predict response to therapy. For example, a trial for a new immunotherapy could stratify patients based on the abundance of exhausted T cells versus cytotoxic T cells in their TME, as measured by CITE-seq. Furthermore, single-cell data can guide rational drug combinations by simultaneously identifying a driver mutation in a subclone and an immunosuppressive pathway in the TME, suggesting a combination of a targeted agent and an immune modulator. As costs decrease and protocols standardize, the integration of single-cell analysis into clinical trial design will be crucial for demonstrating its value in improving patient outcomes.

8. Conclusion

Single-cell technologies have dismantled the view of leukemia and lymphoma as monolithic diseases, revealing them instead as complex, adaptive ecosystems governed by principles of evolution and cellular plasticity. The dissection of tumor heterogeneity at genetic, transcriptional, and functional levels has provided fundamental insights into the mechanisms of disease maintenance and therapy resistance. The tumor microenvironment emerges as a critical architect of this heterogeneity, fostering protective niches and suppressing anti-tumor immunity. As these technologies mature and become more accessible, their integration into clinical trials and routine diagnostics is inevitable. The ultimate promise lies in leveraging this deep, single-cell understanding to design smarter combination therapies that anticipate and preempt evolutionary escape routes, thereby achieving more durable remissions and cures for patients with hematological malignancies.

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