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MATHEMATICAL MODELS OF STEM CELL BEHAVIOR (M KOHANDEL, SECTION EDITOR)

# Leukemia's Clonal Evolution in Development, Progression, and Relapse

Jui Wan Loh[1,2,3] · Hossein Khiabanian[1,2,4]

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## Abstract

**Purpose of Review** Advances in high-throughput methods have enabled the molecular characterization of leukemias and have improved our understanding of their clonal evolution from leukemogenesis in hematopoietic stem/progenitor cells to overt diagnosable disease.

**Recent Findings** It has now been revealed that during leukemia's development and progression, genetic alterations accumulate according to the principles of Darwinian evolution. Drug resistance often emerges from changes in evolutionary trajectories of disease through selection of subpopulations that have greater fitness under therapy. In this manuscript, we will review recent data on prevalence of highly branched evolutionary patterns in myeloid and lymphoid leukemias and discuss how different treatment strategies differentially shape leukemia's clonal architecture.

**Summary** Increasing evidence on clinical impact of small pre-malignant clones prior to diagnosis and small resistant clones during treatment strongly suggests that highly sensitive experimental and mathematical models are necessary for accurate dissection of hematopoietic populations and robust identification of predictive markers for disease transformation and relapse.

**Keywords** Leukemia · Clonal evolution · Hematopoietic stem cells · Leukemogenesis · Pre-malignant cells · Tumor dynamics

## Introduction

Hematological malignancies, and cancer in general, are caused by uncontrollable cell growth that is enabled by the gain of genetic and epigenetic alterations. These alterations accumulate after oncogenesis and give rise to a diversified population of tumor cells that result in a diagnosable disease. Chemotherapy and radiotherapy have been the traditional approaches to treat cancer, with the ultimate goal of killing as many tumor cells as possible. More recently, small molecule drugs are being designed to target specific mutated genes or pathways, often to disrupt the mechanisms that tumor cells use to gain oncogenic signals from the microenvironment. These therapeutic strategies have had varying degrees of success in different tumor types; however, there are emerging concerns in treating cancer, including developing hypotheses on the cell of origin for tumor cells, the presence of tumor heterogeneity between and within patients, as well as the role of protective tumor microenvironment. With the advances in biotechnology, increasing numbers of tumors are sequenced with high-throughput methods for better understanding of their genetic and epigenetic heterogeneity, which in turn has demanded rigorous mathematical methods to properly extract biologically significant relationships from the data.

In this paper, we will review recent findings on leukemias' oncogenic origin, their clonal evolution from pre-malignant state to fully transformed tumors, and their evolutionary dynamic under treatment. We will also highlight the application

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✉ Hossein Khiabanian
h.khiabanian@rutgers.edu

Jui Wan Loh
jl1444@scarletmail.rutgers.edu

[1] Rutgers Cancer Institute of New Jersey, Rutgers University, 195 Little Albany Street, New Brunswick, NJ 08903-2681, USA

[2] Center for Systems and Computational Biology, Rutgers Cancer Institute of New Jersey, Rutgers University, New Brunswick, NJ, USA

[3] Graduate Program in Microbiology and Molecular Genetics, Rutgers University, Piscataway, NJ, USA

[4] Department of Pathology and Laboratory Medicine, Rutgers Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ, USA

of mathematical modeling in elucidating patterns of clonal evolution and specifically discuss probabilistic and empirical quantification of leukemia genomes in the context of investigating their response to treatment. We will end with concluding remarks on the significance of molecularly defined clonal analysis of subpopulation structure as a fundamental predictor of prognosis in leukemia.

## Hematopoietic Stem Cells and Leukemogenesis

Cancers of the hematopoietic system are postulated to be associated with the transition of self-renewing hematopoietic stem cells (HSCs) to early progenitor, cancer-initiating cells which, in turn, pass genetic alterations down to their progeny during cell division and differentiation. In this context, Darwinian selective pressures are driven by competition between diversifying pre-malignant populations. Some somatic mutations in HSC often arise under a condition known as clonal hematopoiesis of indeterminate potential (CHIP), which was first identified through genomic profiling of peripheral blood cells from individuals selected without regard to hematologic characteristics [1–5]. CHIP's mutational landscape is dominated by genomic alterations in the *DNMT3A*, *TET2*, and *ASXL1* genes, which are commonly mutated in myeloid leukemias [6•]. Other frequently mutated genes include splicing factor genes *SF3B1*, *SRSF2*, and *U2AF1*, and other cancer genes such as *PPM1D, TP53*, *CBL*, *ATM*, *IDH1/2*, and *JAK2*. CHIP's prevalence increases with age, especially after age 60 when it is detectable in 2–5% of the population, reaching 15–25% by age 80 [3]. CHIP clones, which initiate in HSCs, retain their ability to differentiate and establish mutated myeloid and lymphoid compartments [5]. Pre-leukemic HSCs have been previously identified in myeloid leukemias, including acute myeloid leukemia (AML) [7] and myelodysplastic syndromes (MDS) [8]. More recently, two case-control analyses from long-term follow-up of large population-based cohorts established a direct link between specific CHIP mutations and subsequent development of AML [9••, 10••]. Particularly, all individuals in one study with CHIP clones harboring mutations in the *TP53* or *IDH1/2* genes eventually developed AML within 10 years [10••]. These observations were partially confirmed in the other study, but CHIP mutations affecting the spliceosome genes were also found to be significantly associated with high risk of subsequent evolution to AML [9••]. In addition, both studies reported that higher number of CHIP mutations and larger clone size increased the risk of developing AML. These results highlight the need for longitudinal characterization of extrinsic selection pressures that may drive CHIP's transformation to AML [11•], especially that multiple, exclusive CHIP clones are often observed in single individuals. For example, CHIP is routinely detected in blood and tumor specimens from patients with solid tumors [12–14]. In this context, the association of CHIP mutations in some genes (e.g., *PPM1D* and *TP53*) with prior radiotherapy or chemotherapy [12] and stability of *DNMT3A*-mutated clones during some treatments [15] suggest that distinct CHIP clonal architecture may be exhibited dependent on specific selection pressures imposed.

There is also increasing evidence for the role of pre-malignant stem cells in development of chronic lymphoid leukemias. Hairy cell leukemia (HCL) is a malignancy of B-cells driven by the *BRAF*-V600E mutation [16], which has also been detected in progenitor HSCs collected from HCL patients [17]. Similarly, aberrant HSCs harboring leukemia-associated mutations have been detected in purified cell populations collected from patients with chronic lymphocytic leukemia (CLL) [18]. These results support previous data showing that purified HSCs from CLL patients were capable of generating clonal B cells with CLL-like phenotype [19]. It should be noted that these analyses have relied on fluorescence-activated cell sorting which demands rigorous control of leukemia cell contamination when searching for low abundance mutations in normal HSC fractions [20]. Moreover, presence of unrecognized CHIP in relatively elder HCL or CLL patients might have confounding effects on the results from these studies, especially that xenograft experiments of HSCs collected from these patients were only able to partially recover the aberrant phenotype seen in leukemic cells.

The analysis of unaffected monozygotic twin siblings of individuals with acute lymphoblastic leukemia (ALL) as well as genomic analysis of twins who are both affected with the disease have revealed that oncogenic fusions in pediatric precursor B cell ALL may occur in fetal progenitor or stem cells [21–23]. Genomic and transcriptomic studies have also shown that a specific subset of T cell ALLs are highly similar to normal and myeloid leukemic HSCs [24], suggesting that lymphoid malignancies may initiate with unexpected alterations in less differentiated HSC or hematopoietic progenitor populations. However, considering the genomic landscape of leukemias, these observations raise the notion that the presence of one initiating lesion in HSCs may not be sufficient to induce leukemia transformation and additional genomic and/or epigenetic alterations are required for leukemogenesis. There are indeed mutations that are only observed in leukemia samples and never at pre-leukemic stage. For example, unlike CHIP-associated mutations that confer an increased risk of developing AML and are postulated to be present in pre-leukemic cells and persist during progression and remission [25], *NPM1* mutations are exclusively detected in patients with fully transformed leukemia [26]. The secondary driving alterations in AML have revealed distinct molecular subgroups that reflect discrete evolutionary paths for the disease

[6•], which in turn have allowed genomic stratification of patients' prognoses [27].

Therefore, inferring the temporal order of mutational events in the natural history of disease can inform of alterations that initiate leukemogenesis as well as the secondary hits that are required for full transformation of pre-malignant cells to leukemic ones [28•]. In fact, it has been shown that the order of mutation acquisition directs future evolutionary trajectories of disease, strongly associated with distinct clinical outcomes [6•, 29, 30, 31•, 32, 33]. Statistical integration of longitudinal and cross-sectional genomic data from many patients allows assembling a collection of tumor histories, presents a comprehensive topology of evolutionary landscape, and reveals the underlying paths of tumor progression. For example, a phylogenetic analysis of longitudinal samples from 70 CLL patients collected over 12 years uncovered two mutually exclusive paths for the development of CLL [34]. The first evolutionary path initiates with trisomy 12 and *NOTCH1* alterations. In this path, clonal evolution proceeds toward the development of *TP53* and *BIRC3* abnormalities as late events. The second exclusive evolutionary path initiates with the deletion of 13q14 locus and proceeds with the acquisition of *SF3B1* mutations and *BIRC3* abnormalities. This analysis showed that the molecular lesions of CLL are temporally ordered rather than randomly accumulated, and more importantly, clones with higher fitness often dominate leukemic cell population in later stages of disease [30, 34].

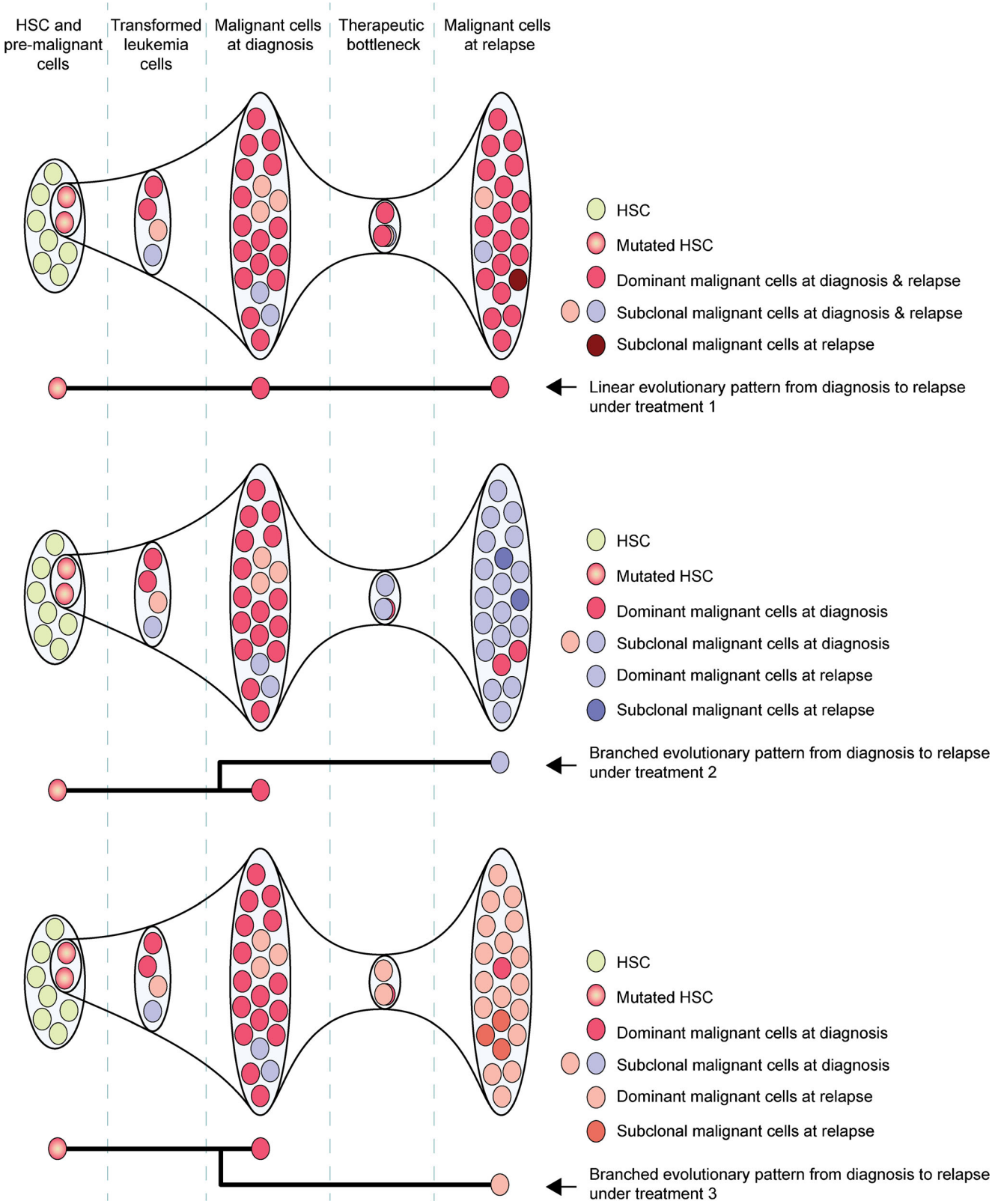
## Leukemia's Clonal Evolution under Treatment

Information on the clinical relevance of mutations that predict therapeutic response has often been limited to clonally represented lesions. However, there is strong evidence that small clones present within heterogeneous tumor cells prior to treatment may have significant impact on patient prognosis dependent on treatment strategy (Fig. 1). This pattern had been previously observed in chronic myeloid leukemia (CML), which is associated with the occurrence of the tyrosine kinase BCR-ABL1 oncoprotein in HSCs [35]. When CML patients are treated with first-, second-, or third-generation kinase inhibitors that target the BCR-ABL1 rearrangement, pre-existing resistant mutations in the kinase domain often emerge [36]. Drugs that inhibit specific pathways may also act as secondary hits and provide a suitable fitness landscape for exclusively mutated clones. For example, in some HCLs treated with the BRAF inhibitor vemurafenib, the expansion of small clones with mutations that reactivate the RAS-RAF-MEK-ERK pathway results in treatment-resistant disease [37, 38].

More recently, a few cells with pathogenic *TP53* mutations present within a thousand CLL cells prior to standard chemoimmunotherapy were shown to become the predominant therapy-resistant population at relapse [29, 39, 40•], indicating that patients harboring small *TP53* mutations show the same risk of failing standard therapy as those with clonal *TP53* defects [39, 41]. The clinical impact of small clones with mutations in other CLL-associated genes such as *NOTCH1*, *SF3B1*, *ATM*, or *BIRC3* appears to be less pronounced than the impact of *TP53*-mutated ones. High-risk patients are candidates for therapeutics designed to interfere with B cell receptor signaling that CLL cells use to gain oncogenic signals from the microenvironment [42]. Ibrutinib is a small molecule that irreversibly inactivates Bruton's tyrosine kinase (BTK), which is involved in connecting antigen stimulation to intracellular responses. Ibrutinib has shown significant activity in CLL. However, in patients treated with ibrutinib, clonal evolution has been reported in 31% of cases within 12 months of treatment [43], and resistance has been associated with the rise of mutations in *BTK* at the drug's binding site, or gain-of-function mutations in *PLCγ2*, a direct downstream of BTK [43, 44•]. Under ibrutinib, and unlike treatment with chemoimmunotherapy, *TP53*-mutated cells do not seem to have significant fitness advantage over *TP53*-wild-type CLL cells. Compared to both of these treatment approaches, in patients managed through watch-and-wait strategy, small mutations at diagnosis do not become dominant or change in abundance during the course of the disease [40•], strongly suggesting that the genomic landscape of resistant phenotype is distinct for each therapeutic approach (Fig. 1).

Similar evolutionary patterns and gain of fitness under therapy have been seen in other leukemias as well. In pediatric ALL, relapse-specific mutations in *NT5C2* are detected in 20% of relapsed T cell ALL and in 3–10% of relapsed precursor B cell ALL cases [45, 46]. These *NT5C2* mutations are predicted to provide gain-of-function, enhancing activity for the enzyme that is responsible for the inactivation of nucleoside-analog chemotherapy drugs [47]. This enhanced activity, however, simultaneously impairs tumor cell growth [48••]. Therefore, it is postulated that activating mutations in *NT5C2* may be negatively selected during ALL initiation and early disease progression when pre-leukemic cells compete with normal hematopoietic cells for resources in the microenvironment, and they only provide necessary evolutionary advantage when purine-based drugs are administered. Nevertheless, not all relapsed cases harbor *NT5C2* mutations, suggesting complex clonal dynamics during treatment. For instance, RAS-MAPK pathway-activating mutations have also been shown to play an important role in relapsed ALL [49–53], where they induce resistance to methotrexate while improving the response to vincristine [53], both of which are commonly used in combination with other drugs to treat ALL.

In contrast to resistant-mutated clones that are selected during treatment, persistence of other mutations may also inform on the risk of disease relapse. Assessing minimal residual disease (MRD) during or after treatment using polymerase




chain reaction and flow cytometry assays [54, 55], or more recently through targeted DNA sequencing of specific driver genes [56], has shown that patients who achieve complete clinical response may have detectable mutations in MRD samples. Deep sequencing of samples from AML patients collected at diagnosis and after induction therapy confirmed that the

◀ **Fig. 1** Schematic clonal evolution of leukemia. Pre-malignant aberrant hematopoietic stem/progenitor cells compete with normal HSC during leukemogenesis and give rise to genomically diverse population of transformed leukemia cells. One clone that can outcompete other leukemia cells becomes the dominant cell population at disease diagnosis, although the leukemic population may retain its heterogeneity. Treatment imposes a therapeutic bottleneck, which in turn shapes clonal architecture of the disease during treatment and at relapse. In this schematic view, treatment 1 does not impose strong selective pressure on diagnostic dominant clone (shown in red) and the tumor follows a linear evolutionary pattern in its progression. This is the scenario observed in relapsed AML after chemotherapy, where the evolution is driven by epigenetic rather than genomic drivers. CLL's progression under watch-and-wait strategy and in the absence of treatment also resembles this pattern. In comparison, treatment 2 imposes a strong selective pressure with a tight therapeutic bottleneck, which provides fitness advantage for a subpopulation of leukemia cells (shown in purple). Treatment 3 also imposes a strong selective pressure; however, it provides fitness advantage for another subpopulation (shown in pink). Under these scenarios, the tumor follows branched evolutionary patterns that are similar in topology but different in their underlying genomics. For example, relapsed CLL after treatment with standard chemoimmunotherapy versus relapsed CLL after treatment with ibrutinib follow similar evolutionary behaviors albeit with different trajectories: under chemoimmunotherapy *TP53*-mutated clones have fitness advantage and under ibrutinib, *BTK*- and *PLCγ2*-mutated clones are selected

detection of some mutations during complete remission was associated with lower rates of relapse-free survival. Remarkably, these data also showed that mutations in the *DNMT3A*, *TET2*, and *ASXL1* genes, which are associated with CHIP and pre-malignant stage of the disease conferred limited value in prognosticating AML relapse [57•, 58]. It should be noted that in analyzing MRD samples using high-throughput sequencing, it is imperative to comprehensively quantify sequencing artifacts and evaluate their impact on accurate detection of mutations at very low abundances [59].

## Modeling Leukemia Progression and Evolution under Treatment

Stochastic modeling and their approximate, deterministic evolutionary models at sufficiently large population sizes have been extensively applied to infer population dynamics in development and progression of hematological malignancies. Similar mathematical models have also been developed to design effective treatment scheduling and dosage. These approaches have been extensively reviewed previously [60, 61••, 62–65]. More recently, a probabilistic model was developed to evaluate CLL's response to gene-specific targeted therapy by ibrutinib [66]. Using available experimental data on CLL dynamics in the absence as well as in the presence of ibrutinib [67, 68], this analysis computationally confirmed that resistant cells are expected to already exist at leukemia diagnosis [44•], and that their numbers are predicted to be too low after treatment initiation to explain rarely negative MRD and lack of complete remission during ibrutinib therapy [55]. These results were complemented by the finding that ibrutinib therapy significantly decreased proliferation rate of CLL cells, while substantially increasing their death rates in blood and tissue compartments [69]. Of interest is evaluating CLL dynamics under lower doses of ibrutinib, which seem to be similarly effective as the currently recommended dosage [70].

Phylogenetic trees are the most common representation of evolutionary processes. In contrast to probabilistic modeling of tumor evolution in which the aim is to infer evolutionary parameters, phylogenetic analyses reconstruct the clonal history of tumors, often based on longitudinal molecular data collected from individual patients. Recently developed methods of analyzing multiple regions sampled from individual solid tumor specimens have been successful in inferring phylogenetic relationships between somatic mutations [71] while jointly analyzing evolutionary behaviors among many patients [72]. In leukemias, however, spatial classification of tumors is limited to circulating cells in peripheral blood versus those in the lymph nodes or in the bone marrow; therefore, phylogenetic analyses need to be performed temporally or at single-cell levels at a single time point. To this end, a large cohort of methodologies has been introduced that is built upon early phylogenetic analyses of taxa; these approaches have been extensively reviewed previously [61••, 73]. When analyzing large number of trees, however, a metric geometry for space of trees, such as the one proposed by Billera, Holmes, and Vogtmann [74], can provide a rigorous mathematical underlying for statistical inference as well as an empirical framework for classification and visualization of evolutionary behaviors that are observed in longitudinal molecular profiles of tumor from different patients [75].

When somatic mutation data are available for primary and relapsed disease, tumor evolutionary histories can be represented by a set of numbers summarizing (1) the mutations in common between primary and relapse, (2) the mutations specific to primary, and (3) the mutations specific to relapse (Fig. 1). Thus, a patient's evolutionary history can be represented as a point in three dimensions. With additional sampling at pre-malignant stage or during treatment, phylogenetic modeling can be performed at higher dimensions with more complex topologies. If the tree branch representing the number of mutations that are specific to the primary tumor is very small relative to other branches, the evolution can be classified as linear, as almost all the mutations in the primary tumor are present in subsequent phases. In contrast, dominant clones in branched evolution share only partial genetic alterations in different phases. The application of these approaches to 55 pairs of diagnosis and relapse samples profiled by whole-exome sequencing demonstrated the underlying mechanism of branching evolution in ALL [53]. In contrast, an analysis of AML's progression to relapse, using eight whole-genome pairs [76], showed linear genomic evolution with very few

**Table 1** Molecular evidence for clonal evolution of leukemia from HSC to relapse under treatment

| Disease | Evidence for pre-malignant HSC | Evidence for clonal evolution under treatment |
|---|---|---|
| AML | Direct evolution of CHIP to leukemia | Epigenetic deregulation in relapse (chemotherapy) |
| ALL | Shared gene fusions in affected twins | *NT5C2* mutations (6-mercaptopurine), RAS-MAPK mutations (methotrexate) |
| HCL | BRAF-V600E mutations in HSC | RAS-RAF-MEK-ERK mutations (vemurafenib) |
| CLL | Activity of HSC from CLL patients in xenograft models | *TP53* mutations (chemoimmunotherapy), *BTK* and *PLCy2* mutations (ibrutinib) |
| CML | Acquisition of BCR-ABL1 fusion in HSC | Mutations in BCR-ABL kinase domain (imatinib and other tyrosine kinase inhibitors) |

mutations found specific to either diagnosis or relapse samples [75]. However, the presence of mutations that could affect global methylation patterns suggests that clinical progression of AML may indeed be driven by epigenetic deregulation [77].

Statistical inference in space of trees can also elucidate the impact of treatment on evolutionary behavior of tumors. An analysis of dominant mutations detected by sequential whole-exome sequencing of 21 CLLs, 6 of which had not received any treatment [29, 78], showed that the distribution of untreated cases formed a tight cluster of linear trees, indicating that tumors from these patients were genetically stable, with few mutations gained from early- to late-phase disease. However, CLLs that received standard treatment presented different mutations at different times [79]. Because of well-defined distances in this space of trees, differences between the clonal histories of tumors from treated versus untreated patients could be statistically assessed [80], demonstrating that treated CLL followed a significantly more branched evolutionary pattern compared to untreated cases. This analysis also suggests that selective pressures can spur tumor evolution and change the mode of leukemia progression from linear to branched, corroborating the findings that highly fit clones become dominant after treatment, often leading to more aggressive and treatment-refractory disease [29, 30, 34].

## Conclusions

The study of clonal expansions began in the 1940s when Salvador Luria and Max Delbrück designed a simple system of single-cell organisms to investigate patterns of mutation accumulation. Their rigorous quantitative methodology led to the discovery that mutations arise randomly and their numbers follow a distinct probability distribution [81]. We now know that cancers follow similar clonal, Darwinian evolution. As genetic alterations accumulate, fitter clones dominate, ultimately leading to macroscopic diagnosable disease. A clonal population diversifies as it expands, enabling it to explore the fitness landscape. Studying the dynamics of genomic heterogeneity during disease development or under treatment can yield insight into when oncogenesis started, how fast the disease evolved, and whether any genomic or epigenetic alterations were selected under specific therapeutic regimes.

Recent analyses of leukemia evolution under different treatment strategies (Table 1), in addition to extensive studies on therapeutic resistance in solid tumors [82], strongly suggest that limiting the knowledge of tumor genetics to the dominant clone may be uninformative for an accurate prediction of outcome and optimal therapeutic decision, as Darwinian selection of ancestral mutated cells that gain fitness under therapy may be the main driver of drug resistance and disease relapse [83]. These emerging patterns stand in contrast to Peter Nowell's proposed linear progression of tumors [84]. Prevalence of highly branched architectures, especially in leukemias, from early stages of pre-malignant cell populations to relapsed disease, raises important hypotheses on the role of subpopulation structure and cells with self-renewing properties in generating and sustaining tumors and demonstrates that they must be incorporated in devising genomic targets for directed therapies. Persistent presence of mutated clones in samples collected prior to or during therapy may also indicate the size of the therapeutic bottleneck that affects leukemic clones. Information theoretic analyses similar to those previously applied to viral evolution by comparing mutation abundances in sequential data [85, 86], can help with assessing mutation-specific fitness and estimating the number of independent mutated clones that establish the therapeutic bottlenecks. As deep sequencing of patient samples becomes a routine part of precision medicine in the clinic, genomic data must be evaluated for presence of small prognostic mutations to inform on the effectiveness of a chosen treatment strategy and to guide novel combination therapies that target both the dominant and the small clones [87•]; hence, establishing the genomic foundation for combating drug resistance and disease relapse in leukemia.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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