Genomic Instability and Clonal Evolution in Chronic Lymphocytic Leukemia: Clinical Relevance

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ABSTRACT

Genomic instability and clonal heterogeneity can influence cancer progression, response to therapy, and relapse. Chronic lymphocytic leukemia (CLL) harbors a variety of clones and subclones that will evolve differently according to intrinsic (microenvironment) and extrinsic (therapy) pressures. Different patterns of clonal evolution have been described, providing insights into the CLL leukemic cell, dynamics, selection, and treatment refractoriness. With the help of genomic technologies allowing a granular resolution of CLL clones, novel synergic therapeutic strategies can be tested with the aim of reaching a genomic–epigenomic ultrapersonalized, tailored approach. These efforts should consider the presence of targetable alterations, continuous cancer reshaping conferring disease refractoriness, and intratumoral clonal equilibrium to possibly avoid clonal selection.

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The concept that cancer results from the accumulation of genetic lesions is widely recognized. At a single-cell level, a large amount of alterations benefiting tumor clones over intrinsic (microenvironment, immune system control) and extrinsic (treatment) selective pressures is promoted during cancer evolution. This underlying genetic heterogeneity confers to cancer the capability of readily reshaping the tumor in an evolutionary fashion by either selecting or suppressing subclones for optimization of cancer progression under adverse conditions.^{1–3} A permissive landscape with higher tolerance to genomic damage due to genetic instability, mainly linked to the disruption of DNA repair pathways or checkpoints (ie, *TP53* or *ATM* mutations), plays a key role in this setting.^{4,5}

Unselected Chronic Lymphocytic Leukemia and Clonal Evolution

The typical genome of unselected chronic lymphocytic leukemia (CLL) harbors approximately 2,000 molecular lesions, with few recurring at a frequency >5%.^{6,7} Conventional karyotyping in the 1990s and fluorescence in situ hybridization (FISH) molecular karyotyping in the 2000s first documented the clonal heterogeneity of CLL by disclosing early, trunk, and fully clonal chromosomal abnormalities, namely deletion 13q14 and trisomy 12, and late, subclonal abnormalities often selected by chemotherapy, namely deletion 11q22–23 and deletion 17p13.^{8,9}

Deletion 13q14 is the most frequent genetic lesion of CLL, occurring in 50% to 60% of cases (Table 1). Deletion of miR15A and miR16A, which favors the constitutive survival and cycling of tumor B cells, is contained within the minimal deleted region on 13q14. These miRNAs physiologically inhibit the expression of key regulators of apoptosis and the cell cycle,¹⁰ with *BCL2* being one of the upregulated genes in CLL as a consequence of 13q14 deletion.¹¹

ATM is a member of the phosphatidylinositol 3-kinase gene family, which regulates DNA damage–induced cell cycle arrest at G1/S and G2/M by activating DNA repair pathways and by inducing apoptosis whenever the DNA damage cannot be repaired. *ATM* maps

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Table 1. Clinical Relevance of Recurrent Genetic Alterations in CLL

Genetic Lesion	Prevalence at Diagnosis	Prevalence at Progression/Relapse
13q14 deletion	50%–60%	~50%–60%
Trisomy 12	15%–20%	~20%
11q22–q23 deletion	8%–9%	30%
17p13 deletion	4%–5%	30%
IGHV lack of mutation	40%	70%–80%
TP53 mutation	5%–10%	30%–40%
NOTCH1 mutation	4%–10%	30%
SF3B1 mutation	6%–9%	20%
ATM mutation	10%–15%	15%–20%

Abbreviations: CLL, chronic lymphocytic leukemia; IGHV, immunoglobulin heavy variable.

on chromosome 11q22–q23 and may be inactivated by both deletion and somatic mutations in CLL.^{12–14} Only 10% to 20% of *ATM*-mutated CLL cases harbor a concomitant 11q22–q23 deletion, and only 20% to 30% of CLL cases with 11q22–q23 deletion also have concurrent *ATM* mutations.¹⁵

Trisomy 12 is reported in approximately 16% of cases of CLL and has a higher prevalence (28%) in atypical phenotypes of CLL and small lymphocytic lymphoma.¹⁶ Trisomy 12 usually correlates to an increased prevalence of *NOTCH1* mutations (25%–30%) and unmutated immunoglobulin heavy variable (IGHV) genes.¹⁷

Deletions of 17p13/*TP53* mutations occur in 10% of newly diagnosed CLL and progressive CLL requiring first treatment, and in 30% to 40% of cases of relapsed CLL (Table 1). The *TP53* gene may be inactivated by deletion and/or somatic mutations.^{18,19} Because of the genetic instability associated with defective DNA damage checkpoints, complex cytogenetic abnormalities (eg, unbalanced translocations) are frequently concurrent with *TP53* abnormalities.^{20–23}

Clustering and Timing of Clonal Evolution

The concept of clonal evolution has been extended within genomic studies based on next-generation sequencing. Recurrent mutations affecting genes involved in the development and progression of CLL can be clustered in a small set of pathways, including microenvironment-dependent signaling (*NOTCH1*, *FBXW7*), inflammatory receptors (*MYD88*), mitogen-activated protein kinase/extracellular signal-regulated kinase (*BRAF*, *KRAS*, *NRAS*, *MAP2K1*), and nuclear factor kappa B (NF- κ B) pathways (*BIRC3*, *TRAF3*, *NFKBIE*), and also as intracellular programs, such as DNA damage and cell cycle control (*ATM*, *TP53*, *SAMHD1*, *POT1*), chromatin modification (*HIST1H1E*, *CHD2*, *ZMYM3*),

transcription (*EGR2*, *IRF4*, *BCOR*, *MED12*), and RNA processing (*XPO1*, *SF3B1*, *RPS15*).^{6,7}

Deletion 13q14 and trisomy 12 are similarly represented in all phases of the disease, and are therefore characterized as first-step genetic abnormalities in CLL (Table 1).^{24–27} The other genetic events usually represent second-hit alterations that are progressively selected or acquired during clonal evolution and that occur in a more advanced phase of the disease. Based on conventional and FISH cytogenetic analysis, clonal evolution mainly consists in the development of 17p13 or 11q22–q23 deletions (Table 1). The development of new *TP53* mutations also contributes to clonal evolution, especially in patients with chemotherapy-refractory CLL and those with Richter syndrome.^{28,29} *SF3B1*, *ATM*, and *BIRC3* mutations, which correlate to a more aggressive clinical phenotype, may emerge during the course of CLL, thus expanding the spectrum of genetic events associated with clonal evolution (Table 1).^{27–29} The greater the inpatient clonal heterogeneity of CLL, the worse the patient's outcome. The unfavorable outcome of patients carrying subclonal driver alterations (mutations and/or copy number alterations [CNAs]) has been consistently documented by both considering single genetic abnormalities and the whole spectrum of coding mutations.^{23,30,31}

Treatment of patients with CLL is currently guided according to different characteristics of the patient (fitness, comorbidities, symptoms) and the disease (treatment timeline, clinical stage, genetics). Some molecular findings are described as predictive biomarkers, providing information on the likely benefit of a specific treatment.³² One of these biomarkers is the IGHV mutational status. Intensive chemoimmunotherapy (CIT; eg, fludarabine/cyclophosphamide/rituximab) is still an option for young (aged <65 years), fit patients with mutated IGHV genes, because it is reported that 50% to 60% of these patients maintain disease remission in the long term—including persistent negativity of minimal residual disease in some instances, showing no relapses beyond 10 years—and have an overall survival similar to that of healthy subjects.^{33–36} Conversely, in the long term, almost all patients with IGHV-unmutated CLL are going to experience disease progression after CIT.^{33–36} Patients with CLL benefit from novel agents independent of IGHV mutation status.^{37–39} *TP53* disruption (deletions of the short arm of chromosome 17 and/or mutations of the *TP53* gene) predicts resistance to CIT.^{40–43} Because their antileukemic efficacy is not exerted through genotoxic mechanisms, novel agents (ibrutinib/acalabrutinib, idelalisib/duvelisib, and venetoclax) are active independently of *TP53* dysfunction.⁴⁴ Accordingly, guidelines for management of CLL recommend testing for 17p13 deletion and *TP53* mutations in patients in need of

treatment,⁴⁵ and targeted therapy is indicated for patients with a disease harboring this predictive biomarker.

Patterns of Clonal Evolution

Sequential sampling of peripheral blood collected at different time points (ie, from pretreatment to relapse after treatment) from patients with CLL helped to describe clonal dynamic patterns over time (Table 2). Some statistical tools have also been developed to identify the subclonal driver events (eg, PhyloPicNDT; Broad Institute of MIT and Harvard), and clonal interaction can be traced by ClonTracer (Novartis Institute for Biomedical Research), GESTALT (University of Washington and Harvard), and COLBERT (The University of Texas at Austin). Different patterns of clonal evolution have been described, along with the associated clinical relevance.^{46–50} Despite efforts to describe the evolutionary events in CLL, the direct influence of these events on an adapted treatment strategy (and vice versa) is not currently fully embraced.

Clonal Equilibrium and Competition

Clonal equilibrium is characterized by the coexistence of a mixed and balanced leukemic population over time. Clonal equilibrium typically occurs in the absence of a strong selection pressure, such as treatment, and during progression from the monoclonal B-cell lymphocytosis to overt CLL (Figure 1).^{23,30} Clonal competition

is characterized by ≥ 1 clones that are more prone to proliferate according to their genetic alterations (Figure 2). In this context, it has recently been shown that the CLL lineage tree shape is characterized by earlier branching and longer branch lengths than normal B cells, reflecting rapid drift after the initial malignant transformation and a greater proliferative history.⁵¹

No Evolution

In most patients, CNAs and/or mutations in putative driver genes were identified before therapy.⁵² Patients lacking clonal evolution with persistent subclones over time and treatment, and thus depicting a stable genetics, usually show a more indolent disease.²³ In one study, 90% of untreated patients with CLL did not show clonal evolution, whereas up to 52% of treated patients developed clonal changes. This observation suggests that treatment facilitates the expansion of clones, which are destined to become dominant by removing their competitors,²⁹ and supports the watch-and-wait approach in the absence of clear treatment indications to avoid accelerating clonal evolution and the selection of more aggressive subclones.

Linear Evolution

Linear evolution is defined as the persistence of a founder clone, with further acquisition of new mutations. Linear evolution is common in Richter syndrome

Table 2. Genomic Studies of Clonal Evolution in CLL

Reference	Patients, n	Description	Insight
Schuh et al, ²⁴ 2012	3	WGS	Different patterns of evolution evident through cycles of therapy
Landau et al, ³¹ 2013	149 (18 longitudinal samples)	WES	Subclonal drivers can anticipate clonal evolution and impact outcome
Landau et al, ⁶ 2015	278	WES	Mutations driving CLL and their evolution in progression and relapse
Ojha et al, ⁵² 2015	12	WES	Genetic heterogeneity and recurrent convergent evolution in CLL
Burger et al, ⁶¹ 2016	5 with relapsed/refractory disease	WES and CNA	CLL progression interrelates with marked clonal evolution
Oakes et al, ⁵⁹ 2016	268	DNA methylation analysis	Epigenetic maturation in CLL is associated with indolent gene expression pattern and favorable clinical outcomes
Ahn et al, ⁶⁴ 2017	84 (15 with disease progression, 69 with no progression)	ddPCR	Emerging mutations in <i>BTK</i> and <i>PLCG2</i> under ibrutinib treatment
Nadeu et al, ⁴⁶ 2018	406 (48 sequential samples)	Access array	Subclonal architecture and evolutionary pathways in CLL
Herling et al, ⁶⁸ 2018	8	WES	Clonal dynamics under venetoclax treatment
Gruber et al, ⁵⁵ 2019	107	WES	Different growing patterns of naturally progressing CLL
Gaiti et al, ⁵¹ 2019	18 (6 healthy donors, 12 patients with CLL)	Multiplexed scRRBS	Evaluation of epimutation rate at single-cell resolution
Pastore et al, ⁶⁰ 2019	22 with primary CLL and 13 healthy donor B-lymphocyte samples	RRBS and ChIP-seq	Epigenetic modifications leading to diverging chromatin states and transcriptional heterogeneity in CLL

Abbreviations: ChIP-seq, chromatin immunoprecipitation sequencing; CLL, chronic lymphocytic leukemia; CNA, copy number analysis; ddPCR, droplet digital PCR; RRBS, reduced-representation bisulfite sequencing; scRRBS, single-cell reduced-representation bisulfite sequencing; WES, whole-exome sequencing; WGS, whole-genome sequencing.

Figure 1. Clonal equilibrium model: a model of CLL progression in the absence of selection representing 3 subclones proportionally growing over time until eventual progression.

Abbreviations: CLL, chronic lymphocytic leukemia; MBL, monoclonal B-cell lymphocytosis.

transformation (90% of cases), but not in CLL progression after CIT (33% of cases) or during ibrutinib therapy (7%).^{29,52,53}

Branching Evolution

Branching evolution is defined as the parallel evolution of competitive clones. Multibranching evolution with ≥ 2 subclones fluctuating over time is common during CIT (67% of patients) or ibrutinib therapy (25% of patients).^{52,53}

Convergent Mutational Evolution

Convergent mutational evolution, defined as the acquisition of ≥ 1 mutation in the same gene, has been reported as a common phenomenon in CLL drivers. Convergent mutational evolution was described in up to 26% of patients and affected nearly 70% of the CLL driver genes.^{46,52,54}

Logistic Growth and Exponential Growth

Gruber et al⁵⁵ described the clonal evolution of 107 patients with untreated CLL after collecting blood samples from diagnosis to first treatment, documenting that different CLL cases can exhibit diverse growth patterns. The logistic-like pattern is sigmoidal and achieves a potentially temporary stable state with $< 1,000 \times 10^9$ cells. Patients showing this growth pattern have a disease that eventually will never require therapy after diagnosis, harboring genetically favorable features such as deletion 13q14 and mutated IGHV status. Conversely, the exponential pattern shows a higher genetic complexity and a faster disease progression, with $> 1,000 \times 10^9$ cells.⁵⁵ This observation mandates caution regarding treatment initiation based on lymphocyte increase or doubling time alone in the context of modest absolute peripheral blood lymphocytosis, because some of those patients are destined to stabilize tumor burden and may not ultimately require therapy.

Figure 2. Clonal competition model: clonal evolution under treatment selection pressure. At diagnosis, the fittest clone for watch and wait predominates over other subclones. After treatment, resistant clones are selected and the MRD clones expand upon relapse. Abbreviation: MRD, minimal residual disease.

Role of Epigenetics in Clonal Reshaping

Epigenetics, including DNA methylation, chromatin remodeling, and posttranslational histone modification, can affect genomic stability by determining the biologic state of the cells. These mechanisms allow cancer cells to quickly adapt to environmental stressors, including therapy and immune editing. Genome-wide arrays and sequencing allowed the definition of the epigenetic landscape of CLL as generally stable over time, sharing common features with normal B-cell differentiation across resting and proliferative compartments.^{56–58} Nevertheless, a more pronounced epigenetic heterogeneity has been described in CLL cells with respect to normal CD19-positive lymphocytes. This heterogeneity could support genetic instability, providing a potential propensity to develop alternative evolutionary trajectories under extrinsic or intrinsic clonal pressure.^{51,58,59} Epigenetic heterogeneity has been reported as correlating with worse outcome and more aggressive disease.^{51,60}

Clonal Evolution Upon Targeted Treatment

Gene mutations are well-known mechanisms of resistance to targeted therapy in CLL. Burger et al⁶¹ suggested that time to clinically detectable relapse after ibrutinib therapy is determined not only by the presence of resistance-conferring mutations within subclonal populations but also by the size and growth rate of the drug-resistant clone at the time of treatment initiation (Figure 3).

Acquisition of *BTk* mutations at the binding site of ibrutinib (ie, Cys481Ser) or in the SH2 domain of *BTk* (T316A), or of gain-of-function mutations in phospholipase $C\gamma 2$ (*PLCG2*) has been described in up to 85% of patients developing resistance to ibrutinib.^{61,62} Detection of such mutations can occur up to 15 months before clinical relapse,⁶¹ conferring a predictive role to these biomarkers, which warrants further prospective data collection. Other lesions associated with ibrutinib resistance include 8p deletion, which encompasses the TRAIL receptor, conferring resistance to TRAIL-induced apoptosis; gain-of-function mutation of *CARD11*, eventually activating the NF- κ B

Figure 3. Clonal architecture of resistance clones: selection of resistant clones under different treatment regimens. (A) *TP53*-mutated subclones leading to resistance/progression under CIT. (B) *BTK*-mutated subclones leading to resistance/progression under ibrutinib. (C) *BCL2*-mutated subclones leading to resistance/progression under venetoclax. Abbreviation: CIT, chemoimmunotherapy.

pathway downstream from Bruton tyrosine kinase; and mutations of *ITPKB*, a central feedback inhibitor of the B-cell receptor signaling pathway.^{39,63–66}

Mutations are a mechanism of resistance to venetoclax therapy, at least in a proportion of cases. A single-nucleotide variant in *BCL2* (Gly101Val), the target of venetoclax, affects the binding of the drug to the protein, occurs in up to 50% of cases developing acquired resistance, and can be detected up to 25 months earlier than standard disease progression criteria are met.⁶⁷ Herling et al⁶⁸ reported that a progressively increasing number of acquired CNAs or aneuploidy occurs upon venetoclax treatment, showing signs of accumulating genomic instability in CLL surviving cells. Mutations in *BTG1* and homozygous deletions of *CDKN2A/B* were reported as recurrent genetic alterations in patients with venetoclax-refractory CLL.¹

Conclusions

A new concept of ultrapersonalized medicine based on knowledge of the genomic–epigenomic structure of CLL could be developed. The goals of such a strategy could be targeting the specific genetic or epigenetic lesions that

Figure 4. Proposal of a genomic-oriented approach targeting clonal versus subclonal lesions. (A) A sequential approach starting with CIT would eventually select *TP53*-disrupted clones, which could be targeted with the introduction of ibrutinib, eventually selecting *BTK*-mutated subclones. Ibrutinib-resistant subclones could be overcome with venetoclax. (B) A fixed-duration combination of different agents (eg, G1Ve) would target a wide range of subpopulations, allowing the possibility to overcome potential resistance mechanisms.

Abbreviations: CIT, chemoimmunotherapy; G1Ve, obinutuzumab/ibrutinib/venetoclax.

can confer an aggressive or resistant phenotype to a clone, even sparing the more stable clones, and reducing the heterogeneity of the disease and the consequent loss of effectiveness of a targeted treatment, applying a debulking approach that may act as an evolutionary restriction point, further resetting interclonal dynamics. A genomic-oriented approach targeting the disease at a clonal–subclonal level is proposed in Figure 4 and could follow a sequential approach (ie, starting with CIT, which would eventually select *TP53*-disrupted clones, then introducing ibrutinib to overcome *TP53* mutations but eventually selecting *BTK* mutations, and finally switching to venetoclax upon development of ibrutinib-resistant mutations). Nevertheless, a combination of different agents would target a wide range of subpopulations, allowing the ability to overcome potential resistance mechanisms.

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