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Genomic and Epigenomic Alterations in Chronic Lymphocytic Leukemia

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Abstract

Chronic lymphocytic leukemia is a common disease in Western countries and has heterogeneous clinical behavior. The relevance of the genetic basis of the disease has come to the forefront recently, with genome-wide studies that have provided a comprehensive view of structural variants, somatic mutations, and different layers of epigenetic changes. The mutational landscape is characterized by relatively common copy number alterations, a few mutated genes occurring in 10–15% of cases, and a large number of genes mutated in a small number of cases. The epigenomic profile has revealed a marked reprogramming of regulatory regions in tumor cells compared with

normal B cells. All of these alterations are differentially distributed in clinical and biological subsets of the disease, indicating that they may underlie the heterogeneous evolution of the disease. These global studies are revealing the molecular complexity of chronic lymphocytic leukemia and provide new perspectives that have helped to understand its pathogenic mechanisms and improve the clinical management of patients.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a common lymphoid neoplasm characterized by the proliferation and accumulation of mature, small $CD5^+$ B cells and that may involve bone marrow, blood, lymphoid tissues, and extranodal sites (1). The genetic basis of the disease has been long recognized, with the observation of familial aggregations of cases and the identification of a large number of genetic loci associated with increasing susceptibility (2, 3). The biological and clinical evolution of the disease is heterogeneous. The earliest clinically recognized step is monoclonal B cell lymphocytosis (MBL), a clonal expansion of mature B cells with the CLL phenotype. Some patients with MBL will progress to an overt leukemia that is distinguished from MBL only by the threshold of atypical cells in the peripheral blood. The disease may have a stable or indolent course, but in some patients, it progresses and becomes aggressive, with frequent relapses after treatment, and in approximately 5–10% of cases, it transforms into diffuse large B cell lymphoma (DLBCL) (1).

Genomic studies using next-generation sequencing (NGS) have expanded the landscape of genetic alterations identified in CLL, uncovering a large number of novel drivers that are starting to provide clues for understanding the heterogeneous behavior of the disease (4, 5). In addition to genomic alterations, the development of CLL is associated with marked modifications in different layers of the tumor cell epigenome. Genome-wide methylation studies combined with chromatin analyses of the tumor cells and their comparison with the modulation of the epigenomic landscape during the normal B cell differentiation process have revealed novel epigenetic subtypes of the disease that have clinical relevance and a major reprogramming of regulatory regions (6–8). In this review, we provide an overview of recent genomic and epigenomic studies that together offer a comprehensive framework for understanding the stepwise development of the disease and open new perspectives to improving the clinical management of patients.

GENETIC PREDISPOSITION: INTEGRATING GENETIC AND EPIGENETIC ALTERATIONS

Familial studies have shown the relevance of genetic factors in the development of CLL. First-degree relatives of CLL patients have a 2.4- to 8.5-fold increased risk of developing the disease, and up to 9% of patients have a family member who has CLL (2, 9). These patients also have an increased risk of developing other lymphoid neoplasms (2, 10). Initial studies identified a number of single nucleotide polymorphisms (SNPs) in candidate genes associated with the disease, but only a handful were seen in replication studies in large cohorts of patients (11, 12). More recent genome-wide association studies have identified up to 45 susceptibility loci associated with CLL (3, 13, 14). Most of these signals map to noncoding regions of the genome, with only a few loci located within the coding or untranslated regions of genes, thus challenging the interpretation of the mechanisms influencing the development of the disease.

The candidate genes associated with familial CLL segregation have been elusive. A *DAPK1* haplotype associated with downregulated expression of the gene was identified in the affected members of a large family (15). Whole-exome sequencing (WES) in familial CLL has identified germline mutations in *POT1* and other elements of the telomere shelterin complex that cosegregate with the disease (13). Similar WES studies have also identified a significant increase in rare variants in *ATM* coding regions in the germline of patients with sporadic CLL that may influence the development of the disease because the normal allele is frequently lost in the tumor cells (16). Intriguingly, the comparison between the candidate genes associated with CLL susceptibility loci and the somatically mutated genes discovered by whole-genome sequencing (WGS) and WES in overt CLL has revealed limited overlap. In addition to *ATM* and *POT1*, only *BCL2* and *IRF4* share somatic mutations and germline susceptibility variants (17). This difference suggests that susceptibility and somatically mutated CLL genes may influence different steps of the development of the disease, or, alternatively, they may target similar pathways.

The mechanisms linking the genetic susceptibility variants and the development of the disease are being elucidated thanks to the integration of genome-wide sequencing and transcriptome studies with multilayer epigenomic analyses of large series of CLL cases (4, 8, 18). Overlaying CLL susceptibility variants and near SNPs that were in linked disequilibrium with histone marks related to active regulatory genomic elements (H3K27ac, H3K4me3, and H3K4me1) has recently shown that 93% of the risk loci map onto an active promoter or enhancer in CLL cells (19). Interestingly, most of these regulatory regions are modulated in the normal B cell differentiation process, but a subset of them are disease specific, that is, inactive in all B cell subtypes but de novo active in CLL (8). The risk SNPs modify the binding sites of different transcription factors (TFs), with disruption of sites for SPI1 or nuclear factor (NF)-κB, and increase the binding affinity for members of the FOX, NFAT, and TCF/LEF families. Interestingly, these TFs are also associated with disease-specific active regulatory regions in CLL (8). The combined analysis of gene expression and looping interactions of the risk loci with promoters of candidate genes has identified more than 30 genes modulated by the risk variants. These genes are involved in critical pathways such as immune response (*SP140*, *IRF8*), cell survival (*BCL2*, *BMF*, *CASP8*, *BCL2L11*), or Wnt signaling (*UBR5*, *LEF1*) (18, 19).

CELL-OF-ORIGIN AND EPIGENETIC AND IMMUNOGENETIC CLL SUBTYPES

The initial steps in the development of CLL are not well known, but the earliest changes may occur in hematopoietic stem cells (HSCs). Xenotransplantation experiments have suggested that HSCs from CLL patients may be primed to develop clonal or oligoclonal expansions of CLL-like cells (20). These CLL HSCs expressed higher levels of the early lymphoid and B lineage TFs IKZF1, TCF3, and IRF8 than did HSCs from healthy individuals. The possible early involvement of these TFs in CLL is intriguing, following the recent observation that some CLL susceptibility loci increase the binding of TCF3 or the expression of IRF8, suggesting that genetic and epigenetic modifications in these regulatory regions may play an initiating role in CLL (19). Common CLL genetic alterations, such as trisomy of chromosome 12 (tri12) and deletion of chromosome 13q [del(13q)], have been found in the hematopoietic progenitors of some patients (21). Mutations in driver genes, such as *SF3B1*, *NOTCH1*, and *XPO1*, may be acquired in HSCs in some patients and also at more advanced stages after the B cell lineage commitment of precursor cells (22, 23). These observations suggest that the epigenetic and genetic changes leading to CLL appear in the hematopoietic progenitors or early B cell differentiation steps, although the development of their full oncogenic potential appears at different stages of mature B cells.

Immunogenetic Subtypes

Immunogenetic studies of the B cell receptor (BCR) in CLL have shown the crucial role of antigen interactions in driving the clonal selection of tumor cells (24, 25). The two major molecular subtypes of the disease are derived from different cells of origin that determine, at least in part, the subsequent acquisition of genomic and epigenomic alterations and the behavior of the disease (26, 27). CLL with unmutated immunoglobulin heavy-chain variable region (IGHV) status (known as U-CLL) originates from B cells that have not passed through the germinal center, and it has a more aggressive behavior than CLL with mutated IGHV (known as M-CLL), which derives from post–germinal center B cells (28). The role of antigen selection in the clonal expansion of CLL is supported by the striking bias in the use of certain IGHV genes, particularly IGHV1-69, IGHV3-21, IGHV3-7, and IGHV4-34, among others. In addition, some cases have an identical or quasi-identical amino acid sequence in complementarity-determining region 3 of immunoglobulin (IG) genes, supporting the role of antigens in the selection and promotion of these clones. These highly homologous IG rearrangements in unrelated CLLs have been called stereotypes, and they are detected in approximately 30% of cases (24, 25). Several hundred different stereotypes have been defined, but 19 are considered to be the major subsets, with 20 or more cases in each subgroup. Most of the cases correspond to U-CLL, but they can also be found in M-CLL. CLLs with some of these stereotypes have particular clinical and biological features (**Table 1**). The use of the light-chain IGLV3-21 gene has been recently associated with more aggressive disease, particularly in those cases carrying IGHV mutations (29). The marked bias in BCR use seems related to clonal selection by certain auto- and external antigens in the initial steps of disease development (30). Interestingly, the Ig of the CLL BCR may also recognize homotypic epitopes, thus generating interactions between Ig molecules that may trigger downstream signaling of different intensities (31, 32). A particular mutation present in *IGLV3-21* seems to facilitate homotypic interactions (29, 32).

Table 1 Clinical and biological characteristics of the major stereotype subsets in chronic lymphocytic leukemia

Subset (frequency)	IG genes	Epigenetic subtype	IGHV somatic hypermutation	Mutated drivers	Clinical outcome
Subset 1 (~2.4%)	IGHV1, -5, -7 IGHD6-19 IGHJ4 IGKV1-39	Naive-like	U-CLL	*NOTCH1* *NFKBIE* *TP53*	Very aggressive; median TTFT = 1.6 years
Subset 2 (~2.8%)	IGHV3-21 IGHJ6 IGLV3-21	Intermediate	U-CLL and M-CLL	*SF3B1* del(11q22–23) Rarely *TP53*	Very aggressive; median TTFT = 1.9 years
Subset 4 (~1%)	IGHV4-34 IGHD5-18 IGHJ6 IGKV2-30	Memory-like	M-CLL Ongoing somatic hypermutation	ND	Very indolent; median TTFT = 11 years
Subset 6 (~0.9%)	IGHV1-69 IGHJ3	ND	U-CLL	*NOTCH1*	Very aggressive; median TTFT = 1.6 years
Subset 8 (~0.5%)	IGHV4-39 IGHD6-13 IGHJ5 IGKV1-39	ND	U-CLL	*NOTCH1* Trisomy 12 Rarely *TP53*	Very aggressive; median TTFT = 1.5 years; Richter transformation

Data from References 25, 96, and 157.

Abbreviations: del, deletion; IG, immunoglobulin; IGHV, immunoglobulin heavy-chain variable region; M-CLL, mutated chronic lymphocytic leukemia; ND, no data; U-CLL, unmutated chronic lymphocytic leukemia; TTFT, time to first treatment.

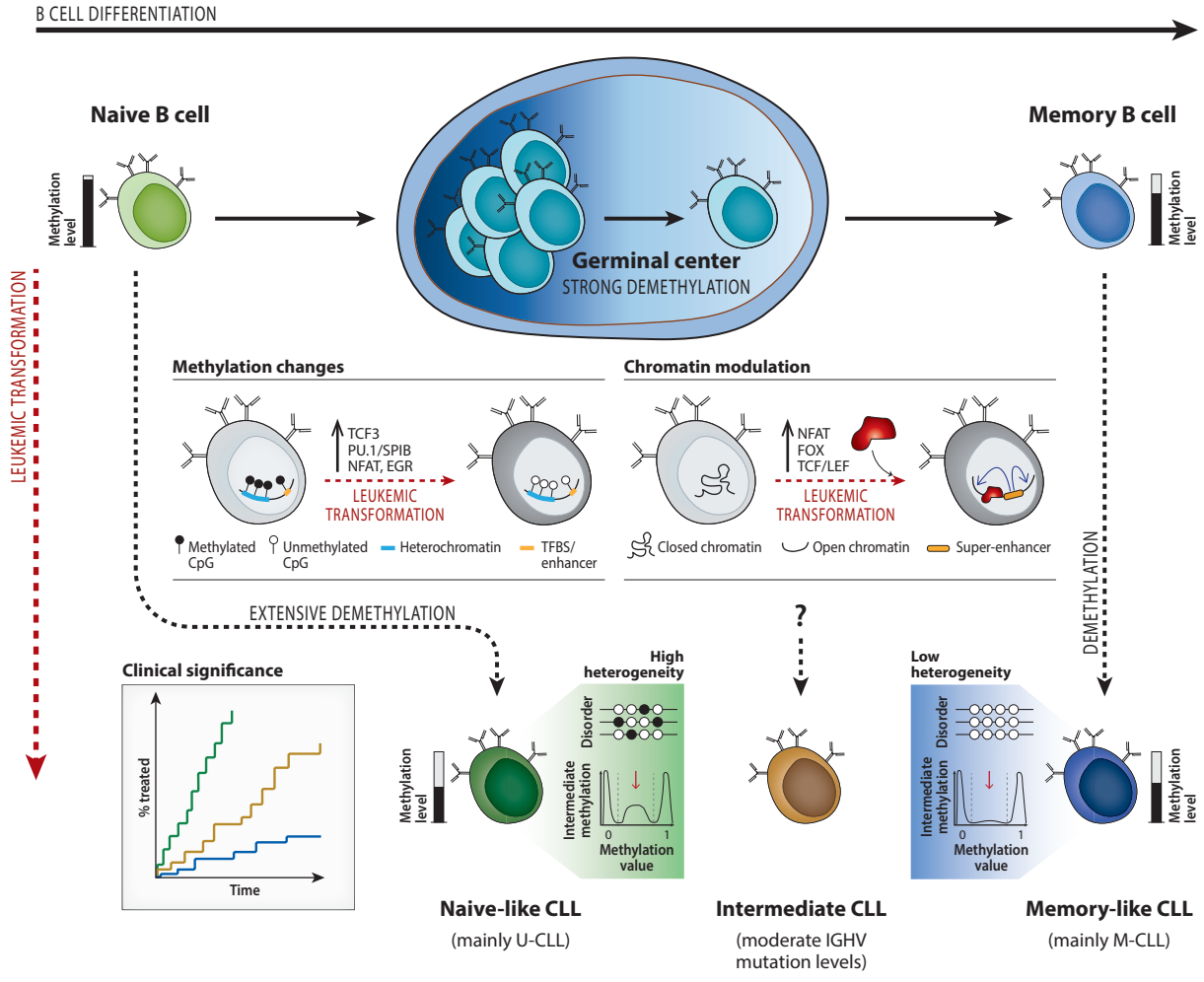

Figure 1

Epigenetic changes seen in the light of the B cell maturation process. Extensive demethylation occurs both during normal B cell maturation through the germinal center and through the process of development from normal to leukemic cells. This leukemic demethylation mostly occurs in heterochromatin, but it also occurs in specific transcription factor binding sites and enhancers. The expression of specific transcription factors has been linked with de novo activation of super-enhancers in CLL cells. Three epigenetic subtypes of CLL have been identified: naive-like, memory-like, and intermediate. Naive-like CLLs have higher methylation heterogeneity than memory-like CLLs. These three epigenetic subtypes have different clinical outcomes. The illustrated curves of time to first treatment are representative of different studies. Methylation level refers to the global methylation level. For methylation values, 0 = unmethylated and 1 = methylated. Abbreviations: CLL, chronic lymphocytic leukemia; IGHV, immunoglobulin heavy-chain variable region; M-CLL, mutated CLL; U-CLL, unmutated CLL; TFBS, transcription factor binding site.

Epigenetic Subtypes

Recent epigenetic studies have found that M-CLL maintains the DNA methylation signature of a normal post–germinal center cell (i.e., memory-like), whereas U-CLL retains a naive-like methylation signature (6, 7) (**Figure 1**). Interestingly, these studies have also identified a third epigenetic CLL subtype with an intermediate methylation profile, that is, between naive-like and memory-like, suggesting that it could originate in a not-yet-identified normal B cell. The three epigenetic

CLL subtypes, naive-like, intermediate, and memory-like, differ in their profile of somatic mutations, use of IGHV genes, and clinical outcomes (4, 6, 33) (**Table 1**). Naive-like CLLs usually have unmutated IGHV genes, with frequent use of IGHV1-69, mutations in *NOTCH1*, deletions in chromosome 11q, and gains in chromosome 2p16. The intermediate CLL group carries moderate IGHV mutation levels, with increased use of IGHV3-21, IGHV1-18, and BCR stereotype 2, and higher frequencies of mutations in *SF3B1* and *MYD88*. The memory-like cases carry mutated IGHV genes, with frequent use of IGHV4-34 and IGHV3-7 (4). These three epigenetic subtypes also differed in the time to first treatment and overall survival, and the subtypes retained their prognostic value in multivariate analyses that included other classical parameters, such as IGHV mutational status (33) (**Figure 1**). This prognostic value has been confirmed in four independent studies (7, 33–35). All of these observations suggest that the cell of origin of the disease, defined by its immunogenetic or epigenetic profile, is an important determinant of the biology of the tumor.

CHROMOSOMAL ABNORMALITIES: FROM SINGLE ALTERATIONS TO COMPLEX KARYOTYPES

Chromosome Banding and Fluorescence in Situ Hybridization

Initial chromosome banding analyses (CBAs) revealed the presence of numerical and structural alterations in CLLs (36, 37). These studies provided the first insights into the genetic heterogeneity of this disease, despite the relatively low number of chromosomal alterations, with an average of one per tumor. However, it was the use of fluorescence in situ hybridization (FISH) that allowed Döhner and colleagues (38) to identify genomic aberrations in more than 80% of patients. The finding of four recurrent cytogenetic alterations—del(13q)/miR-15a/16–1, del(11q)/*ATM*, del(17p)/*TP53*, and tri12—that strongly correlated with patients' outcomes brought this four-alteration FISH panel into routine clinical use (38). The introduction of more effective mitogens has expanded the use of CBA in CLL, showing that approximately 20–35% of the abnormalities are not assessed by the FISH panel. CBA also identifies balanced and unbalanced translocations in up to 35% of cases (**Figure 2**). Although most of these translocations are nonrecurrent, the involved break points occur in regions frequently deleted in CLL [e.g., del(13q)] (39–43). The most common translocations involve IGH and different oncogenes. The IG heavy chain *BCL2* translocation [t(14;18)] is found in 2% of cases, predominantly M-CLL, and is associated with increased expression of *BCL2*. IGH *BCL3* [t(14;19)] and *BCL11A* [t(2;14)] occur in less than 1% of cases and are enriched in U-CLL, with atypical morphological features (44–46). Some studies have questioned whether all cases described as CLL with these translocations fulfill the current criteria for this entity or correspond to a different category (47).

Microarrays

The introduction of chromosomal microarray analysis (CMA) has expanded the identified landscape of chromosomal alterations in CLL with the identification of a larger number of regions with copy number alterations (CNAs) and copy number neutral loss of heterozygosity (CNN LOH), some of which potentially have clinical relevance (4, 48, 49) (**Figure 2**). These novel CNAs encompass, among others, gains of 2p16 (7–30%), with minimal gained regions including *BCL11A*, *REL*, *MYCN*, and *XPO1* (4, 48, 50, 51); losses of 2q37 (1%), involving *SP140* and *SP110* (4); del(3p21) (2%), affecting *SMARCC1* and *SETD2*, among others (4, 52); del(6q15) (2.5%), involving *ZNF292* (4, 53); and del(15q15) (4%), with the smallest common region, including *MGA* (48). Regions with CNN LOH have been detected in 5% of patients, and this affects frequently deleted loci, such as

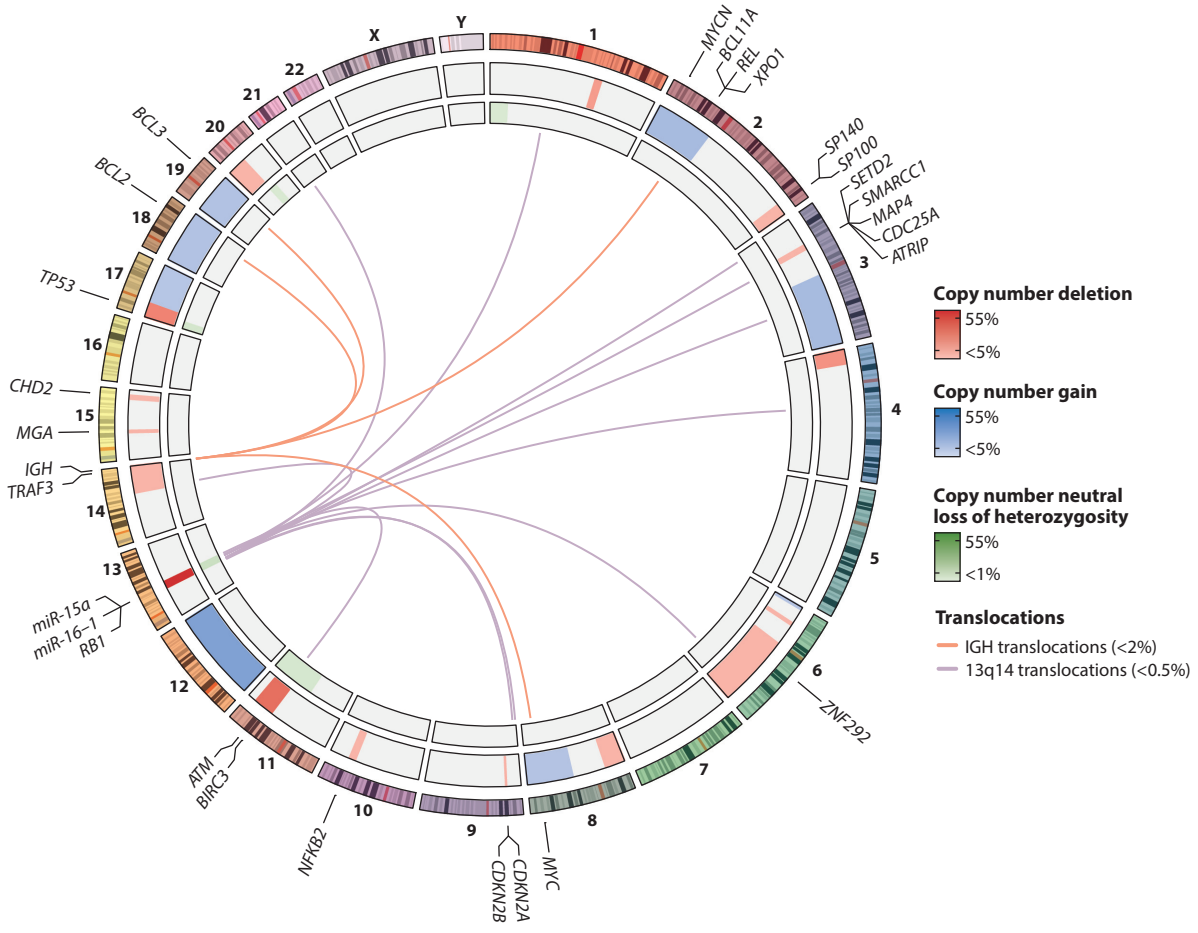

Figure 2

Recurrent chromosomal alterations and target genes. Circular plot showing the chromosomes (*outer ring*), recurrent copy number deletions (*red*), gains (*blue*; *middle ring*), and copy number neutral loss of heterozygosity (*green*; *inner ring*) in patients with chronic lymphocytic leukemia. Colors for the chromosomes were selected arbitrarily for illustrative purposes. Intensity-scaled color represents the fraction of patients with chronic lymphocytic leukemia carrying each alteration. The frequency of each aberration was extracted from References 4 and 49. Translocations are represented using links connecting the two break points. Most chromosomal alterations are present in <10% of cases. The main genes located in the minimal region of these alterations are depicted. Figure adapted from image created with circlize R (158).

11q, 13q, and 17p, and it is associated with inactivating mutations of the target gene, particularly *TP53* (4, 48, 50).

Complex Karyotypes

Early genetic studies using CBA identified an increased number of cytogenetic alterations (i.e., genomic complexity) in up to 20% of CLLs. Complex karyotypes, defined by the presence of three or more numerical or structural abnormalities, or both, were predictive of disease progression, worse outcome, and refractoriness (36). Recent studies in large cohorts of patients and in the context of clinical trials have confirmed and refined the clinical impact of complex karyotypes in CLL

(43, 54–56). The prognostic value of a complex karyotype is independent of *TP53* aberrations, and in some studies it has been associated with the presence of unbalanced translocations (54, 55). Interestingly, an increasing genomic complexity gradually worsens the clinical outcome since patients with more than five alterations showed shorter overall survival than patients with low or medium complex karyotypes (i.e., three or four alterations, respectively) (56). The relationship between increased genomic complexity and poor clinical outcome has also been observed both by CBA and CMA (42, 57). However, a subset of patients carrying complex karyotypes that include tri12, tri19, and additional trisomies or structural abnormalities seems to correspond to a particular genetic subgroup of CLL that has distinctive clinicobiological features (e.g., IgG expression, younger age) and longer overall survival than patients without complex karyotypes (56, 58). Complex karyotypes have been observed in 8% of patients with MBL, suggesting that they may appear early in the development of the disease (56).

Next-Generation Sequencing

NGS allows for the identification of genome-wide large and focal CNAs, CNN LOH, and balanced and unbalanced structural variants (5, 59) (**Figure 2**). WGS has also identified chaotic rearrangements in CLL, such as chromothripsis and chromoplexy (4). Chromothripsis is a massive, localized chromosome fragmentation and repair rearrangement that seems to occur as a one-off catastrophic event (60). Chromoplexy is also a complex rearrangement phenomenon that is characterized by a lengthy series of rearrangements (from 3 to more than 40) between chromosomes, often occurring as closed chains, frequently associated with large DNA deletions at their junctions (61). These complex rearrangements may also be detected by CMA and have been associated with U-CLL, *TP53* and *SETD2* alterations, a high number of chromosomal alterations, and worse clinical outcome (4, 52, 57). The advantage of using WGS to assess the complete catalogue of genomic alterations through a single technique may support its introduction in future clinical studies (59).

MUTATIONAL LANDSCAPE

Impact of Genome Sequencing on CLL

The development of NGS technologies provided the opportunity to characterize the genomic alterations present in CLL tumors at an unprecedented resolution. In 2011, the first glimpse of the mutational landscape of CLL was obtained by sequencing the genome of four CLL tumors (62). Despite the presence of more than 1,000 mutations per tumor, none of them were shared between the four analyzed tumors, but the use of a validation cohort of more than 200 samples allowed for the identification of four CLL driver genes: *NOTCH1*, *MYD88*, *XPO1*, and *KLHL6*. Since this initial study, hundreds of samples have been analyzed by exome sequencing, as well as more than 200 tumors by WGS (4, 5, 62–67). These studies, which together comprise more than 1,000 tumors, have provided the most comprehensive characterization of genetic and genomic alterations in CLL.

On average, each CLL tumor accumulates 2,500 somatic mutations, but the mutation burden correlates with IGHV mutational status. Thus, M-CLL tumors accumulate more mutations than U-CLL ones (3,000 versus 2,000). While most mutations in a tumor are a consequence of cytosine deamination caused by ageing, the higher burden of mutations in M-CLL tumors is mainly caused by the action of a mutational process that appears to be specific for germinal center–experienced neoplasms, including CLL and DLBCL (4, 68). This mutational signature, which can be detected genome-wide in M-CLL tumors, is different from the one introduced by activation-induced cytidine deaminase, which is highly specific for Ig loci as well as some off-target genes. Therefore, this

signature appears to reflect the transit of cells through the germinal center, and its contribution to the transformation process appears to be limited as M-CLL tumors have a lower number of mutated drivers and a better prognosis than U-CLL tumors, despite the overall increase in mutational burden.

The global picture that emerges from these studies confirms that CLL is a heterogeneous disease, with more than 60 driver genes and 12 recurrent structural variants, none of them mutated at diagnosis in more than 15% of tumors, depending on the characteristics of the cohort, with the exception of del(13q) and tri12 (**Figure 3**). Only a few genes are mutated in more than 5% of tumors at diagnosis, including *NOTCH1* (8–12%), *SF3B1* (9–11%), *TP53* (5–8%), or *ATM* (5–7%). This list of driver genes is followed by a long tail of genes mutated at low frequencies, most of them in less than 2% of tumors, highlighting the challenges facing the molecular diagnosis of CLL in the clinic. Many of these genes appear to have a higher frequency of mutation in U-CLL tumors, although a few genes, such as *CHD2* or *MYD88*, are highly specific for M-CLL (69–71) (**Figure 3**). Furthermore, the frequency at which these genes appear to be mutated varies considerably depending on whether samples were obtained at diagnosis or during progression, reflecting the impact of these driver alterations in the clinical evolution of CLL (5, 72, 73).

Mutated Pathways in CLL

Despite the diverse number of mutated genes in CLL, most of them cluster in a small number of cellular pathways, including DNA damage response (*ATM*, *TP53*, and *POT1*), NOTCH1 signaling (*NOTCH1* and *FBXW7*), RNA splicing and metabolism (*SF3B1*, U1, *XPO1*, *DDX3X*, and *RPS15*), NF-κB signaling (*BIRC3*, *NFKB2*, *NFKBIE*, *TRAF2*, and *TRAF3*), B cell receptor and Toll-like receptor signaling (*EGR2*, *BCOR*, *MYD88*, *TLR2*, *IKZF3*, and *KRAS* or *NRAS*), and chromatin modifiers (*CHD2*, *SETD2*, *KMT2D*, *ASXL1*) (**Figure 4**). With the few exceptions mentioned in the previous section, most of these genes are mutated at low frequencies, and, therefore, their impact on prognosis or response to treatment is still poorly characterized. The fact that many of these genes belong to specific signaling pathways raises the possibility that their clinical impact might be similar, which may benefit the interpretation of this large amount of information. Thus, tumors with mutations in the RAS/BRAF/MAPK/ERK pathway appear to define a subgroup of patients with adverse clinical features, and in vitro, these tumors are characterized by a poor response to BRAF inhibitors, but they may respond to a pan-ERK inhibitor (74). Current efforts aimed at increasing the number of sequenced tumors and improving follow up of patients might improve our understanding of this information, as it relates to either individual genes or specific pathways.

Genomic Stability and DNA Damage Response

Mutations in the tumor suppressor genes *TP53* and *ATM* and deletion of their respective loci (17p and 11q) constitute two of the most frequent alterations in CLL. They are usually associated with poor prognosis and have been classically used for patient stratification, although this is mainly limited to the detection of chromosomal deletions by FISH. These two genes are key elements in the DNA damage response pathway, and their aberrations are associated with increased levels of genomic complexity (75–77). The use of NGS at deep coverage has shown that approximately 60% of tumors with *TP53* or *ATM* abnormalities carry both mutations and deletions in these genes. Only approximately 10% of tumors have chromosomal deletions without mutations (78). Interestingly, the presence of a mutated gene without a deletion is detected in 30% of tumors in which *TP53* is mutated and in 20% of tumors in which *ATM* is mutated, indicating the limitations

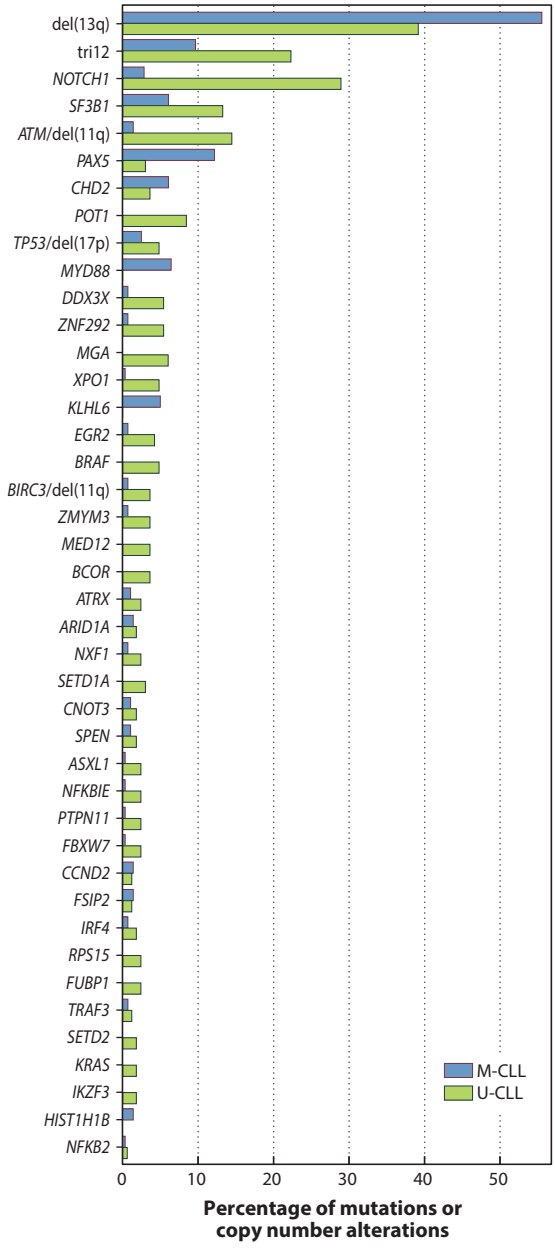

Figure 3

CLL driver genes. Mutation frequency of CLL drivers according to the IGHV mutational status of the tumor. Most driver genes are mutated in both subtypes but at different frequencies, with only a small subset of genes being subtype specific. Abbreviations: CLL, chronic lymphocytic leukemia; del, deletion; IGHV, immunoglobulin heavy-chain variable region; M-CLL, mutated CLL; tri, trisomy; U-CLL, unmutated CLL.

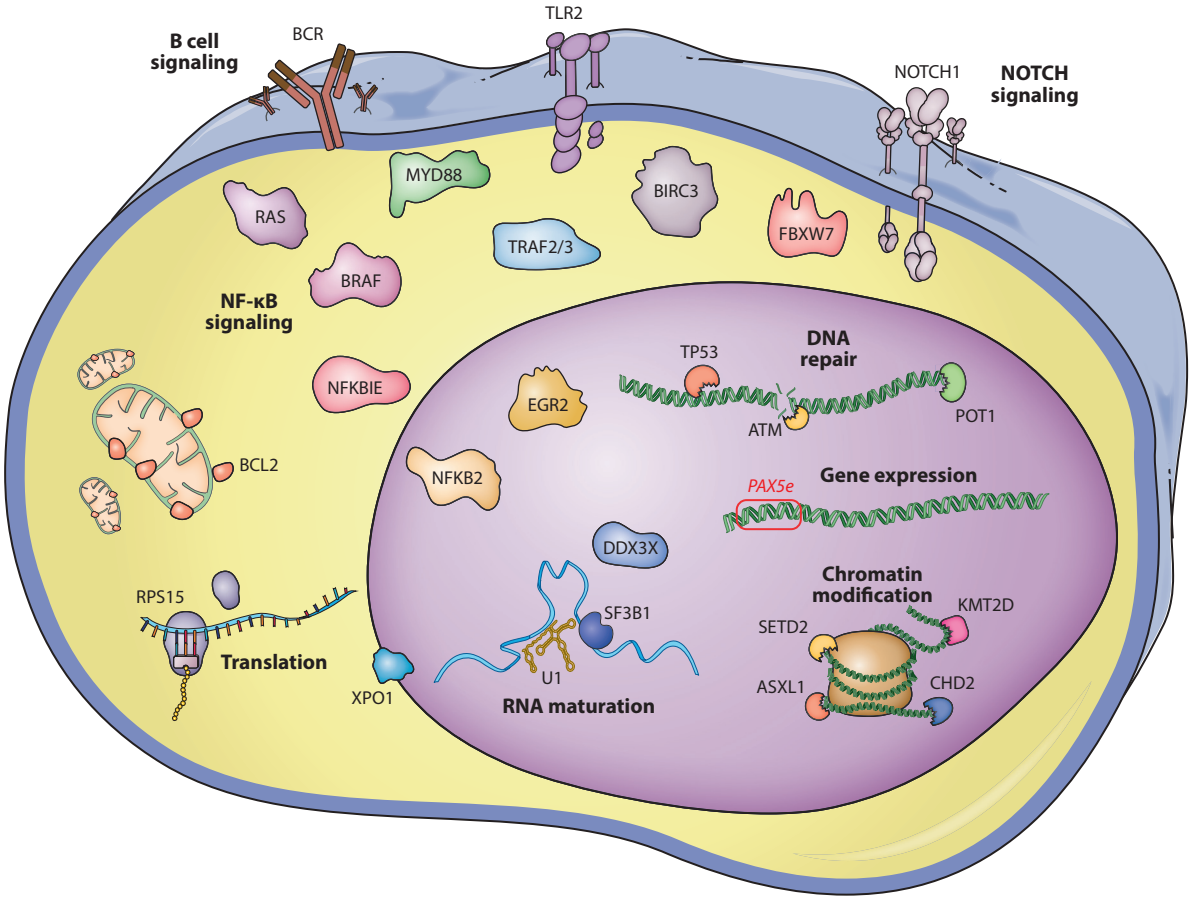

Figure 4

Main molecular pathways affected by mutations in chronic lymphocytic leukemia (CLL). Recurrently mutated genes in CLL affect different signaling pathways, including DNA repair; B cell, NOTCH1, and NF-κB signaling; RNA maturation and export; translation; gene expression; and chromatin modification. PAX5e refers to the enhancer of PAX5. The figure was adapted from an image created using Servier Medical Art and licensed under a Creative Commons Attribution 3.0 Unported License.

of performing only FISH to detect these aberrations in clinical practice (76, 78). *TP53* and *ATM* mutations also play a role in chemoresistance in CLL that seems to be overcome, at least in part, by novel agents (79). Mutations in these genes in even small subclonal populations influence the evolution of the disease (78, 80). The development of novel therapeutic agents aimed at patients with aberrations in these genes highlights the need to evaluate the potential use of deep coverage gene sequencing approaches in clinical practice (81–83).

POT1 is one of the novel cancer genes revealed by NGS studies, being mutated in 4–8% of CLLs (43, 63, 84). It encodes a component of the shelterin complex of the telomeres, and virtually all somatic mutations are missense and occur in the domains required to bind telomeric DNA. Three of the four *POT1* germline mutations identified in CLL families occur in the ACD binding domain and two are truncating (13). CLL cells carrying somatic *POT1* mutations have numerous telomeric and chromosomal abnormalities that suggest these mutations favor the acquisition of the malignant features of CLL cells (84). *POT1* mutations confer an adverse prognosis that is independent of IGHV mutational status (84).

NOTCH1 Pathway

Apart from *TP53* and *ATM*, *NOTCH1* has emerged as the most recurrently mutated gene in CLL, with more than 12% of patients harboring mutations in this known oncogene (4, 85). Most of these tumors contain a recurrent 2-bp deletion, causing a frame shift (fs) in the protein (p.P2514Rfs*4) and leading to the disruption of the PEST sequence required for degradation of the NOTCH1 intracellular domain (ICN1). Furthermore, the integration of WGS information with RNA sequencing data has revealed that approximately 20% of tumors with mutations in *NOTCH1* are not caused by mutations in the coding region but are due to the presence of a few recurrent mutations in the 3′ untranslated region of the gene (4, 86). When transcribed, these mutations create aberrant splicing that removes 530 bases of the canonical *NOTCH1* mRNA, including the last 158 coding bases. This event results in the loss of the PEST domain and the accumulation of INC1, highlighting the relevance of noncoding mutations in cancer and the need to properly address them to achieve an accurate diagnosis.

The relevance of *NOTCH1* in CLL is further supported by the finding that approximately 50% of CLLs without mutations accumulate ICN1 within the nucleus and show a NOTCH1 expression signature similar to that detected in cases with mutations in *NOTCH1* (85, 87). The molecular mechanisms by which peripheral blood CLL cells are able to activate NOTCH1 signaling in the absence of mutations as well as its clinical relevance are still unknown. In this regard, while *NOTCH1* is more frequently mutated in patients with U-CLL than in those with M-CLL (29% versus 2.9%, respectively) (4, 85, 86), ICN1-positive CLL cells in *NOTCH1* wild-type cases occur at similar frequencies in both IGHV CLL subtypes (85). This suggests that NOTCH1 signaling in these tumors may be activated by additional extrinsic factors, such as the microenvironment. The fact that *NOTCH1*-mutated cases have a worse prognosis than unmutated cases raises the possibility that despite the general accumulation of ICN1 in CLL cells, a PEST-lacking ICN1 might have additional biological effects, contributing to the worse prognosis in these patients. The E3 ubiquitin ligase FBXW7, a negative regulator of *NOTCH1*, is mutated in 1–4% of CLLs. A recent study has demonstrated that these mutations stabilize ICN1 and are associated with increasing levels of genes regulated downstream of NOTCH1 (88).

Splicing Machinery and RNA Metabolism

The introduction of NGS has uncovered the splicing machinery as a target of numerous mutations, both in hematological malignancies and in solid tumors (63, 64, 89–92). In CLL, *SF3B1*, encoding a subunit of splicing factor 3B, is mutated in up to 10% of cases. These mutations lead to mis-splicing near the 3′ splicing sites in multiple genes, having an impact on processes such as DNA damage response, telomere maintenance, and NOTCH1 signaling (93–95). Although *SF3B1* mutations are more frequent in U-CLL, they are especially enriched in cases carrying stereotyped BCR subset 2 (44%) (4, 96). Recent studies in a murine model have shown that *SF3B1* mutations in B cells induce senescence and require the concomitant inactivation of *ATM* to generate a neoplastic transformation of the cells. This experimental model is reminiscent of the human situation in which *SF3B1* mutations are significantly associated with *ATM* mutations and del(11q) (72). *SF3B1* mutations seem to decrease BCR signaling and render tumor cells more sensitive to BTK inhibitors (97). In addition to *SF3B1* mutations, CLL carries mutations in other genes regulating splicing and RNA transport, such as *XPO1* and *DDX3X*, but their particular functions in the disease are not well known (63, 64). We have recently identified a recurrent mutation in the U1 spliceosomal RNA in 4% of CLL cases, and this is associated with U-CLL and poor outcome (97a).

The discovery of RPS15, a component of the ribosome involved in translational machinery, as a novel driver in CLL has expanded knowledge of the role of RNA processing in the pathogenesis of

the disease. *RPS15* is mutated in approximately 1–12% of CLLs, depending on the cohort studied (98, 99). These mutations interfere with the translational fidelity of the proteins, leading to a major change in the proteome of the cells, with modulation of different pathways, particularly metabolism and RNA biology. Similar to *SF3B1*, mutation in a single gene triggers a cascade of downstream alterations in different mRNA transcripts and proteins that may influence the pathogenesis of the disease.

NF-κB Signaling

NF-κB activation plays an important role in the pathogenesis of CLL, but only a few genes in this pathway are recurrently mutated in the disease (100). BIRC3 is an E3 ubiquitin ligase that acts as an inhibitor of the noncanonical NF-κB pathway. The *BIRC3* gene is mutated in less than 1% of CLLs at diagnosis, but the frequency increases as the disease progresses and reaches more than 25% of cases who are refractory to fludarabine (4, 101). Mutations are usually truncating and frequently associated with deletions of the 11q region, where it is mapped close to *ATM*. NFKBIE is a negative regulator of the canonical NF-κB pathway, and its associated gene, *NFKBIE*, is inactivated by truncating mutations in 1–7% of CLLs. These mutations are associated with poor prognosis and co-occur with other adverse genetic alterations (72, 102). Other genes mutated are *NFKB2* and *TRAF3*, although these mutations occur in less than 2% of patients (5).

B Cell Receptor and Toll-Like Receptor Signaling

BCR signaling is a major determinant in CLL biology (9). However, contrary to other lymphoid neoplasms, activating mutations in this pathway are uncommon in CLL. EGR2 is a TF that seems to act downstream of the BCR pathway and carries activating mutations in 2–8% of CLLs (22). Patients with mutations in *EGR2* are usually diagnosed at a younger age but have an aggressive disease, presenting at an advanced clinical stage; *EGR2* mutations are associated with adverse prognostic factors and having other mutated genes (79, 103).

MYD88 is an adaptor element in the Toll-like receptor signaling pathway; *MYD88* carries activating mutations in around 3% of patients. Tumor cells with the *MYD88* p.L265P mutation release high levels of CCL2, CCL3, CCL4, interleukin (IL)-6, and IL1RA in response to Toll-like receptor stimulation (62). Similarly, *TLR2* mutations are occasionally present in patients, are activating, and also increase the secretion of IL6 and IL1RA (104), suggesting that these mutations may promote a favorable microenvironment for the survival of tumor cells. *MYD88* and *TLR2* mutations are almost exclusively seen in M-CLL. Patients with *MYD88* mutations are usually younger than those with the wild-type gene. The impact on outcome is controversial, being identified as favorable in one study but not in others (70, 71). Interestingly, in preclinical studies, novel inhibitors of IRAK4, an element of this pathway, seemed to be effective (71, 105).

GENOMIC COMPLEXITY, SUBCLONAL HETEROGENEITY, AND CLONAL EVOLUTION

Genomic Complexity

The high number of low-frequency driver alterations described in the previous section highlights the complex interpatient heterogeneity of CLL. Along this line, the genomic landscape strongly differs among patients, with some carrying multiple driver alterations (i.e., more than four), whereas, intriguingly, approximately 15% of the patients have disease in which a known driver aberration may not be identified (4, 5, 72). This interpatient divergence can be partially explained by three major factors. (*a*) The cell of origin: M-CLLs have a lower number of driver alterations

and account for most cases carrying either no apparent diver mutation or a single driver aberration [mainly isolated del(13q)] (4, 5, 72). (*b*) Age of the patient: Some driver alterations, such as those in *MYD88*, seem to be enriched in young patients (64, 70). (*c*) Clinical phase of the disease: Mutations in *SF3B1*, *POT1*, *ATM*/del(11q), *RPS15*, and *TP53*, among others, are more frequent in patients enrolled in clinical trials, while mutations in *TP53*, *BIRC3*, *MAP2K1*, and *DDX3X* and deletions of 17p and 11q seem enriched in tumors after treatment with immunochemotherapy (5, 73).

The high genomic complexity observed in more than half of patients results from the co-occurrence of multiple driver alterations within the same tumor, which may distort their clinical significance if analyzed independently (4, 72). For instance, mutations in *SF3B1*, *ATM*, *POT1*, and *XPO1*, all of which are associated with adverse prognosis, tend to co-occur, raising the question of their individual contribution to the evolution of the tumor (72). Along this line, a multi-hit profile of concurrent driver alterations affecting *TP53*, *ATM*, and *SF3B1*, or some combination of these, has been associated with a poorer response to conventional regimens and shorter overall survival after relapse compared with patients with one or no mutations in these genes (106). Interestingly, the increasing accumulation of driver alterations from zero to more than four has been associated with gradual impairment of the time to first treatment and overall survival, a situation similar to the relationship between complex karyotypes and outcome (4). The overall survival of patients with no mutated drivers identified is similar to that of the general population, further reinforcing the role of these mutations in the prognosis of the disease. Most (90%) of these driverless cases with good prognosis are M-CLL, raising the possibility that as-yet-undescribed mutations are responsible for their transformation or that specific BCR determinants might contribute to the growth and expansion of this CLL cell population (82).

Subclonal Composition

The study of subclonal architecture gained clinical attention when Landau and colleagues (5, 65) showed that the presence of subclonal driver alterations was an independent risk factor for rapid disease progression. When clonal alterations are defined as those present in virtually all tumor cells and subclonal alterations are those found in only a fraction, half of the tumor samples analyzed by WES harbored subclonal driver alterations. Targeted deep NGS approaches have identified subclonal mutations, even at low levels (<1% of cells), for all driver genes. Some of these minor subclones carrying mutations in key driver genes, such as *TP53*, *SF3B1*, or *NOTCH1*, have clinical relevance because they appear to influence the evolution of the disease (72, 78, 80, 107). The increasing number of subclonal driver alterations, rather than just their presence or absence, correlates with the overall survival of patients treated with conventional strategies. In contrast, the accumulation of driver alterations independently of their clonal or subclonal representation, seems to be a marker of a shorter time to first treatment (72). Larger and homogeneously treated cohorts of patients are needed to clarify the relevance of subclonal architecture in future predictive and prognostic models specifically designed for each decision point (i.e., diagnosis, treatment initiation, and initiation of subsequent treatment) (108).

Clonal Evolution

The analysis of clonal and subclonal alterations also allows for the reconstruction of the phylogeny of the tumors (109). Thus, clonal mutations correspond to earlier driving events (or passenger events present at the time of transformation), while subclonal mutations correspond to those acquired at a later phase. Initiating events in CLL are more commonly CNAs, mainly del(13q), tri12, and del(11q), followed by the late acquisition of mutations in driver genes such as *SF3B1*, *POT1*,

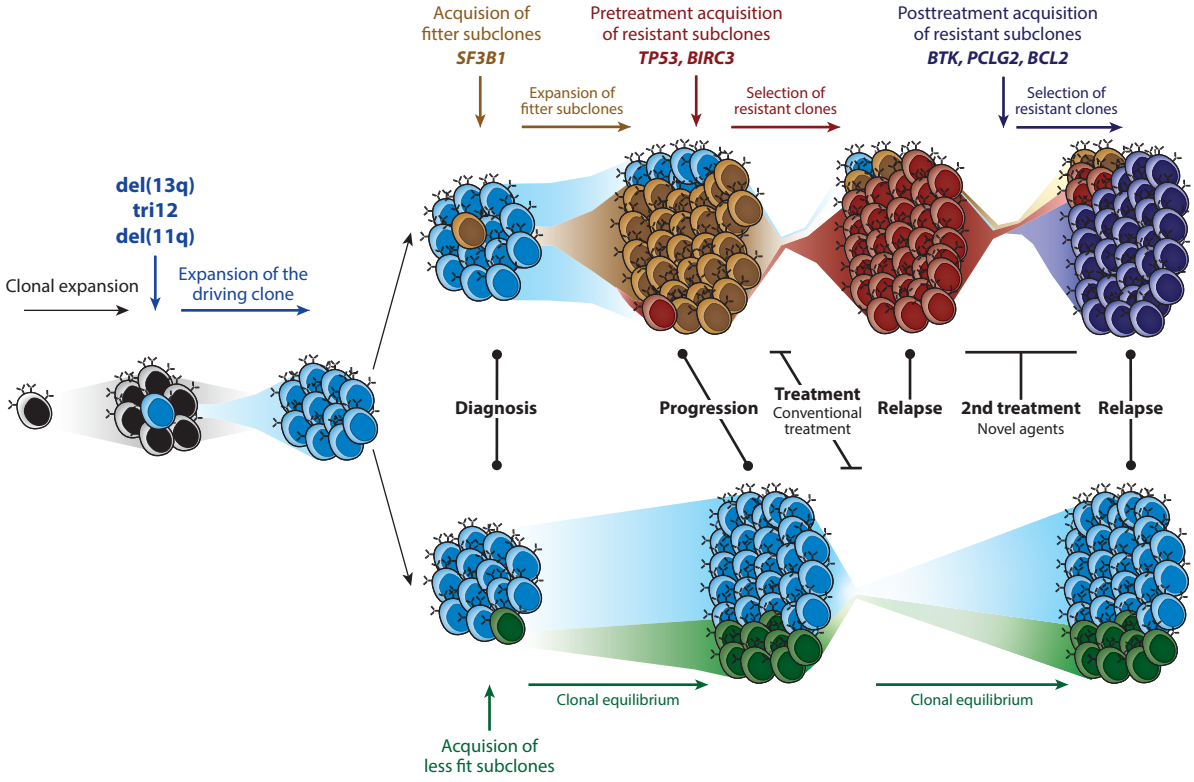

Figure 5

Clonal evolution in CLL. Representation of the main evolutionary trajectories and temporal acquisition of driver alterations throughout the course of the disease. CLL starts with the expansion of a subclone carrying an early driver alteration (or alterations). (*Top*) Next, the acquisition of mutated subclones with a proliferative advantage may expand before treatment, shaping the clonal structure of the tumor. Similarly, the presence or acquisition of resistant subclones may cause a rapid relapse. (*Bottom*) However, stable tumors with less fit subclones may evolve, maintaining equilibrium between subclones both before and after treatment pressure. Abbreviations: CLL, chronic lymphocytic leukemia; del, deletion; tri, trisomy.

TP53, *ATM*, *NOTCH1*, or *BIRC3*, among others (5, 72). This model of the temporal acquisition of driver alterations has been confirmed in sequential samples (72). Intriguingly, strong hierarchic relationships have not been observed among gene mutations thus far. The low incidence of driver gene mutations in CLL suggests that larger data sets may be required to uncover novel evolutionary paths.

Although early events may provide a proliferative advantage to normal B cells (110), the acquisition of secondary alterations seems to determine the subsequent clonal evolution and clinical progression of CLL. In this sense, the study of longitudinal samples using WGS or WES has shown three recurrent patterns of evolution: stable equilibrium (i.e., the clonal or subclonal populations are maintained in relatively similar proportion over time), linear evolution (i.e., mutations are sequentially acquired in a single clone), or a branched evolution (i.e., there is coexistence and evolution of distinct genetic subclones that maintain a common ancestor) (5, 65, 111–113) (**Figure 5**). Although cell populations are relatively stable before treatment, the presence of certain mutations may confer competitive advantages, thus generating subclonal evolution even before any treatment. Thus, subclonal *SF3B1* mutations have been associated with accelerated progression of the disease before treatment (78, 114). This finding correlates with the fact that

an increasing *SF3B1*-mutated subclonal population gradually shortened the time to first treatment (72). After chemotherapy, the balanced equilibrium among populations may be disrupted, and in most cases, the populations undergo major changes, with expansions of fitter or resistant subclones, the reduction or disappearance of sensitive populations, or an increase in previously undetectable subclones. However, in 5–30% of cases, during relapse there is a stable composition of tumor cells that is similar to the population before treatment (5, 65, 112, 113) (**Figure 5**).

Few genes have been identified as being recurrently selected at relapse. The most common is *TP53*, in which minor subclones present before treatment are expanded after immunochemotherapy, thus dominating the relapse, a finding concordant with the poor prognosis of patients carrying small subclonal *TP53* mutations (72, 78, 80, 115). Other genes identified as recurrently selected at relapse in a smaller number of cases are *IKZF3* and *SAMHD1* (5, 112, 116). The possible clinical relevance of these different patterns of clonal evolution is not well understood. However, the observation that patients with clonal evolution, either before or after treatment, have shorter overall survival suggests that subclonal complexity may favor more aggressive behavior of the tumors (5, 78).

Resistance to Novel Agents

Clonal evolution also has an important role in the development of resistance to novel agents (**Figure 5**). Global clonal shifts after these treatments are associated with worse outcome (117). The acquisition of mutations in *BTK* or *PLCG2* and *BCL2* have been identified in cases that have developed resistance to BTK inhibitors (*BTK* and *PLGC2*) and the BCL2 inhibitor venetoclax (118–123). *BTK* or *PLCG2* mutations are not usually detected before treatment, or they are detected only at very low frequencies (less than 2 in 1 million) (124). However, they emerge progressively after treatment and can be detected 3–15 months before clinical progression (118–120). In some cases, different mutations in these genes may be detected in multiple subclones and in different topographical locations, such as in blood and lymph nodes, emphasizing the relevance of subclonal plasticity in the evolution of the disease (120, 125). In addition to mutations in specific drug targets, resistance to these treatments may involve more complex mechanisms, including mutations and CNAs in other genes [e.g., del(8p) targeting TRAIL-receptor and *ITPKB* mutations], transcriptional reprogramming, and, less commonly, transformation to DLBCL or transdifferentiation to nonlymphoid-cell tumors (e.g., histiocytic sarcoma clonally related to the precedent CLL), that may represent a pathway to escape from dependence on BCR signaling (120, 121, 124).

Transformation to Diffuse Large B Cell Lymphoma (Richter Syndrome)

An extreme situation in the clonal evolution of CLL is its transformation to DLBCL, known as Richter syndrome (RS). In most patients, RS represents a histological transformation to DLBCL, whereas in others, it corresponds to transformation to Hodgkin's lymphoma (126). Due to its low incidence, the biology of the Hodgkin's lymphoma transformation is less understood, but most cases seem to correspond to a second lymphoma associated with Epstein–Barr virus infection. In contrast, approximately 80% of DLBCL RS tumors are clonally related to the previous CLL, and, mostly, they evolve through linear evolution from the predominant CLL clone observed at diagnosis (127, 128). Only a minority of DLBCL RS tumors evolve by following a branching pattern. CLLs carrying stereotyped BCR subset 8 (**Table 1**), *NOTCH1*, or *TP53* mutations have an increased risk of transformation after chemoimmunotherapy (i.e., administration of a combination of conventional chemotherapy and a monoclonal antibody such as rituximab or obinutuzumab). DLBCL RS has also been observed in patients treated with ibrutinib in which the CLL lacked

the canonical *BTK* and *PLCG2* mutations (129). In this context, near-tetraploidy and complex karyotype seem to be independent risk factors for discontinuing ibrutinib due to transformation (130).

Different studies have analyzed the genomic landscape of DLBCL RS tumors compared with their pre-CLL phase and with de novo DLBCL. In this regard, WES and CNA analyses identified a mean of 22 genetic lesions (range, 0–133) acquired from the CLL phase through to DLBCL RS, with the deletion of *CDKN2A* [del(9p21)] being one of the most recurrent alterations (30%) acquired at transformation (128, 131). Other common alterations found in DLBCL RS that may be present in the CLL phase are mutations and deletions of *TP53* (50%) and *MYC* translocations (16%) or amplification (10%) (128, 131, 132). Overall, approximately 90% of DLBCL RS tumors carry alterations in tumor suppression, cell proliferation, or cell cycle pathways, or some combination of these, and have a genomic complexity (with 8.5 CNAs) that is intermediate between CLL (with 3 CNAs) and DLBCL (with 16 CNAs). DLBCL RS tumors differ from de novo DLBCL because they lack common mutations in *CREBBP/EP300*, *B2M*, *TNFAIP3*, *PRDM1*, or *BCL2* and *BCL6*, whereas *TP53*, *CDKN2A*, and *MYC* alterations occur at similar frequencies in both types of DLBCL (128, 131).

EPIGENOMIC ALTERATIONS

Methylation Landscape

Normal naive B cells undergo strong demethylation when they go through the germinal center and differentiate into memory B cells (**Figure 1**). The CLL genomes of both U- and M-CLL also have marked global hypomethylation and lower hypermethylation when compared with their normal naive and memory B cell counterparts. Interestingly, in U-CLL, the difference in the demethylation process from that of the normal counterparts is stronger than it is in M-CLL, and these differences lead to a confluence of the methylation profiles of both subtypes of CLL (6), which is concordant with the relatively small differences in the transcriptome profiles of these two CLL subtypes (93, 133, 134). Hypomethylation mainly occurs in heterochromatin, but it also occurs at TF binding sites and enhancers that modulate genes with relevant function in B cell biology, such as BCR signaling, the NF-κB pathway, and the interaction between cytokines and their receptors, among others (6, 7). Gains in hypomethylation also occur at binding sites for TFs such as TCF3 and PU.1/SPIB, which are related to B cell development, and NFAT and EGR, known to be downstream effectors of BCR activation (7). Hypermethylation in CLL cells is targeted at Polycomb-related repressing marks, certain promoters, transcribed regions associated with H3K36me3, and the binding sites of some TFs (e.g., EBF1 and FOS) involved in B cell differentiation, suggesting that a reduced maturation of B cells may contribute to leukemogenesis (6, 7).

The global methylation profile of CLL is already acquired at the MBL stage (4), and it seems relatively stable over time, including during posttreatment evolution (7, 135). Minor changes in the methylation of regions targeted by PRC2 have been observed in approximately 25% of progressive CLLs after therapy, with a pattern that resembles the evolution from naive to memory B cells (136). In spite of this global stability of the methylome, some CLLs have intratumoral variability in certain regions that may have an impact on the levels of expression of different genes, thus facilitating cell plasticity and tumor cell evolution (137). Interestingly, increased methylation heterogeneity is higher in U-CLL than M-CLL and is also associated with the presence of subclonal populations, suggesting that both phenomena may be linked in more unstable and aggressive tumors (137, 138). These observations suggest that the methylation profile may be more dynamic than initially thought. However, most studies have been performed at single time points during tumor evolution or in small cohorts of patients. Longitudinal studies using sequential

samples may provide insights to improve understanding of the role of epigenomic modulation in the evolution of the disease.

Regulatory Chromatin Landscape

The epigenomic profile of CLL has been recently expanded with the analysis of the full reference epigenome of seven representative CLLs, including a genome-wide map of several histone marks that identify nonoverlapping functional regions, chromatin accessibility, and three-dimensional chromatin architecture, combined with transcriptomic information and WGS information (8). Expanded information about regulatory regions measured by the chromatin accessibility ATAC-seq (assay for transposase-accessible chromatin with high-throughput sequencing) assay and mapping of H3K27 acetylation has been generated in large cohorts of patients (8, 139, 140). A remarkable finding is the variability of active regulatory regions between individual cases, with approximately 30% of these sites present only in very few cases. Nonetheless, approximately 10% of these regions are common to virtually all tumors, and 60% are present in 5–95% of cases (140). The variability of active regions is larger in U- than M-CLL, and U-CLL carries a significantly larger number of active sites than M-CLL, two features that may be related to the more aggressive behavior of this subtype (8, 140). Comparison of the functional regions in CLL with those that are dynamically changing during normal B cell differentiation has revealed that most regions modulated in CLL (approximately 80%) are already active in normal, naive, germinal center, memory, or plasma cells (8). The significance of these changes is not fully understood, but they may reflect common functions, as suggested by the shared active genomic regions between U-CLL, but not M-CLL, and germinal center cells that control genes related to proliferation (8). The chromatin accessibility regions shared between U-CLL and other hematopoietic cell subtypes suggests that these tumors maintain a less differentiated state, whereas active regions in M-CLL are enriched in more mature and memory B cells (140). Similar to the cell-of-origin methylation signature, U-CLL and M-CLL share a number of accessible sites for the ATAC assay with their respective normal naive and memory B cell counterparts. However, the active regulatory regions recognized by H3K27ac in U-CLL and M-CLL do not show a significant overlap with those of naive or memory B cells. These findings indicate that the methylation and chromatin accessibility signatures related to the cell of origin in U- and M-CLL reflect the functional past history of the cell but not the active disease status (8).

The active genomic regions present in all CLLs and not seen in any normal B cell subtype may play specific roles in the pathogenesis of the disease. The majority of these regions are active super-enhancers (8, 139). These newly active regions are enriched in the transcription binding motifs of members of the NFAT, FOX, and TCL/LEF families (**Figure 1**). These findings are concordant with the tumor-specific hypomethylation sites also enriched in binding sites for these TFs (6, 7). Functional studies have demonstrated the role of some of these TFs in the pathogenesis of the disease (7, 73). Only the super-enhancer related to *EBF1*, active in normal B cells, appears to lose activity in CLL (8, 139).

Three-dimensional chromatin configuration studies have shown that de novo active regions in CLL have a high number of genomic interactions with actively transcribed genes that regulate functions relevant for CLL biology, such as surface receptor signaling, cell adhesion, and activation (8). Functional studies have highlighted the crucial role of the *PAX5* super-enhancer as an upstream master regulator of expression networks in CLL and its requirement for tumor cell survival (139). These findings are intriguing, given that somatic mutations in this super-enhancer downregulate *PAX5* expression and occur exclusively in the less aggressive M-CLL subtype (4). The role of super-enhancer activation in CLL is relevant because its function can be targeted by pharmacological agents, thus opening new perspectives on treatment of the disease (139).

Integrating Genomic and Epigenomic Alterations

The extensive genomic and epigenomic information about CLL generated recently highlights the relevance of these alterations in modulating the heterogeneous biological behavior of the disease. However, the possible interactions between these layers are not well understood. Some observations suggest that epigenomic–genomic cross talk occurs during the evolution of this disease. The methylation heterogeneity of some CLLs is higher in tumors with subclonal mutations in driver genes, and the degree of changes in methylation and subclonal genetic changes in the progression of the disease seem to evolve in parallel (137, 138). These changes occur in tumors with more aggressive behavior, suggesting that they are linked to the mechanisms driving the evolution of the disease, but how these interactions occur is unclear.

Somatic mutations in chromatin remodeler genes may modify the epigenomic landscape of the tumors, but they are uncommon in CLL compared with other lymphoid neoplasms. CHD2 binds to histone marks involved in transcriptional regulation, particularly H3K4me3. *CHD2* is mutated in 5% of CLLs and 7% of MBLs, particularly in M-CLL. The mutations are truncating or affect functional domains interfering with the normal nuclear distribution of the CHD2 protein; they are also associated with changes in the transcriptomic profile (69). SETD2 is a histone methyltransferase responsible for the trimethylation of the histone H3K36me3, which is related to active transcription. SETD2 has also been related to the maintenance of genomic stability. Somatic mutations and gene deletions have been identified in 2–5% of CLLs, particularly in U-CLL (4, 52). These alterations have been associated with *TP53* mutations, genomic complexity, chromothripsis, and poor outcome for patients. ARID1A is part of the large ATP-dependent chromatin remodeling complex SNF–SWI, which is required for transcriptional activation. *ARID1A* has truncating mutations in approximately 2% of CLLs. The H2K27 methyltransferase EZH2 is not mutated in CLL, but it is overexpressed predominantly in U-CLL and seems to promote cell survival (141). Altogether, these findings suggest that somatic mutations in epigenetic regulators are involved in a subset of CLLs, but it is not known how they influence the epigenomic profile.

In a recent study we explored the possible reconfiguration of the tumor epigenome in relation to common driver alterations (8). Only *MYD88* mutations and tri12 are associated with specific remodeling of chromatin activation and accessibility regions. Concordant with the functional effect of *MYD88* mutations in CLL, the particular epigenomic profile targets regulatory regions related to the overexpression of genes activated by NF-κB signaling. Intriguingly, the tri12 epigenomic profile was closer to that of normal B cells than it was to CLL without tri12. These findings suggest that CLL tumors carrying these drivers are distinct epigenetic subtypes that may underlie the particular clinical and biological characteristics of individual patients.

CLINICAL AND PROGNOSTIC IMPLICATIONS OF NOVEL GENOMIC DISCOVERIES

For many years, the prognosis of patients has been established using purely clinical parameters, such as those included in the Rai and Binet Staging Systems (142, 143), IGHV mutational status (26, 27), and the FISH cytogenetic hierarchical model (38). The discovery of new CLL genomic drivers has prompted several groups to refine these prognostic classifications. Rossi et al. (144) proposed a new prognostic model incorporating both mutations (*NOTCH1*, *SF3B1*, *TP53*, *BIRC3*) and Döhner's cytogenetic aberrations (38), while Baliakas et al. (145) proposed that the same genetic alterations should be assessed separately in U- and M-CLL. A recent international collaborative effort incorporated both clinical and genomic data and cytogenetic data into one prognostic model, known as the CLL International Prognostic Index (CLL-IPI) (146). This model has been

Table 2 Recommended first-line therapy for patients with symptomatic chronic lymphocytic leukemia, according to genomic and clinical subgroups

TP53 aberrations (deletion or mutation)	IGHV mutations	Comorbidites	Age (years)	Treatment
Absent	Present	Absent	<65–70	FCR or I
			>65–70	BR or I
		Present	Irrelevant	Clb-O, I, IO[a] or VO[b]
	Absent	Absent	Irrelevant	I
		Present	Irrelevant	I, IO[a] or VO[b]
Present	Irrelevant	Irrelevant	Irrelevant	I, V,[c] idelalisib + R[c]

Abbreviations: BR, bendamustine + rituximab; Clb-O, chlorambucil + obinutuzumab; FCR, fludarabine + cyclophosphamide + rituximab; I, ibrutinib; IGHV, immunoglobulin heavy-chain variable region; IO, ibrutinib + obinutuzumab; R, rituximab; V, venetoclax; VO, venetoclax + obinutuzumab.

[a]IO has been approved by the US Food and Drug Administration for patients with comorbidities but not yet by the European Medicines Agency.

[b]VO has not been approved yet, but positive Phase III data are expected during 2019.

[c]This treatment is used for patients who are ineligible for ibrutinib therapy.

extensively validated and includes two genomic and cytogenetic factors [*TP53* aberrations (either mutation or deletion) and IGHV mutational status] together with age, clinical staging, and β_2-microglobulin result. Of note, both *TP53* aberrations and IGHV mutations had the strongest impact on a patient's outcome (146). Although the CLL-IPI is not yet universally used, assessment of both *TP53* aberrations and IGHV mutations is systematically recommended for all patients with CLL (83). Not surprisingly, simplified versions of the CLL-IPI including only these two factors have been proposed (147); the reason for this is that both factors are not merely prognostic but they are also predictive (i.e., they are able to predict the patient's response to specific therapeutic agents).

Consequently, modern CLL therapy is starting to be tailored to each tumor's genomic features (**Table 2**). Chemoimmunotherapy has been the cornerstone of CLL therapy for decades. Chemoimmunotherapy is effective in patients with M-CLL and CLL with wild-type *TP53* (148–150), although it must be adapted to the patient's age and comorbidities. Recently, a number of independent clinical trials have confirmed that the outcome of patients treated with ibrutinib (with or without rituximab or obinutuzumab) is comparable to that of patients treated with chemoimmunotherapy (151, 152). As a result, ibrutinib could be offered to these patients if they prefer a less intensive but indefinite-length oral therapy (ibrutinib) instead of an intensive time-limited approach (chemoimmunotherapy). In contrast, results in patients with either U-CLL or *TP53* aberrations are significantly inferior when they are treated with chemoimmunotherapy, and these patients should be offered treatment with ibrutinib (151, 152). Moreover, and specifically for patients with *TP53* aberrations who are ineligible for ibrutinib, other targeted agents, such as the BCL2 antagonist venetoclax (153) or the PI3K inhibitor idelalisib plus rituximab (154), have also been approved (**Table 2**).

Unfortunately, a proportion of patients treated with older or novel agents will eventually develop progressive disease or DLBCL RS transformation. In some of these patients, allogeneic hematopoietic cell transplantation and other cell therapies are recommended. Both *TP53* aberrations or complex karyotype as determined by CBA, or both, have been used to identify patients suitable for these potentially curative approaches, although later they may become toxic, even if patients initially respond to therapy (155). Other genomic aberrations have been evaluated as predictors of poor response to novel agents, but none has been unequivocally identified (79).

Since *TP53* mutations and IGHV mutation status are used to inform clinical decisions, it is important to establish the most appropriate techniques for determining these. This is particularly relevant for *TP53* mutations because small subclones that can be detected only by NGS have prognostic impact (72, 80). However, this remains a controversial issue, and a recent European guideline still recommends using conventional Sanger sequencing for *TP53* molecular testing (76, 156). Further efforts to standardize NGS techniques and analytical pipelines are needed to incorporate this information into clinical practice.

CONCLUSIONS AND PERSPECTIVES

Recent genomic and epigenomic studies have provided a comprehensive overview of the alterations that may drive the development and progression of CLL. A large number of novel mutated genes clustering in functional pathways has been uncovered. Three epigenetic subtypes of the disease have been identified that only partially overlap with the two subtypes defined by IGHV mutational status. The CLL epigenome is reprogrammed through the modulation of regulatory regions that appear de novo in the disease, whereas other regions capture functions already present in different stages of the B cell differentiation process. One of the major challenges that has emerged from recent studies is the discovery of the tremendous molecular heterogeneity of the disease, which partially underlies the clinical diversity seen in patients. Most studies have been performed at diagnosis or in pretreated patients, and it is not well understood how the disease modulates the genome and epigenome throughout the evolution of the disease. Additionally, the clinical and functional implications of a large number of novel mutated genes and modulated regulatory regions are still unknown. All of this information is essential to understand the interactions between genomic and epigenomic modifications and the cross talk between tumor cells and the microenvironment that influence the evolution of the disease. Translating these new perspectives into clinical practice will require an integrated effort to better understand these basic mechanisms in the context of the complex evolution of the disease.

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