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REVIEW ARTICLE

Clinical Implications of Novel Genomic Discoveries in Chronic Lymphocytic Leukemia

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ABSTRACT

Chronic lymphocytic leukemia (CLL) is a common B-cell malignancy with a remarkably heterogeneous course, ranging from indolent disease with no need for immediate therapy to rapidly progressive disease associated with therapeutic resistance. The recent US Food and Drug Administration approvals of novel targeted therapies such as inhibitors of B-cell receptor signaling and B-cell lymphoma 2 have opened up new opportunities in the clinical management of patients with CLL and heralded a new era in the clinical treatment of this disease. In parallel, the implementation of novel sequencing technologies has provided new insights into CLL complexity, identifying a growing list of putative drivers that underlie inter- and intratumor heterogeneities in CLL affecting disease progression and resistance. The identification of these novel genomic features that can indicate future drug resistance or guide therapeutic management is now becoming a major goal in CLL so that patients can best benefit from the increasingly diverse available therapies, as discussed herein.

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Chronic lymphocytic leukemia (CLL) is characterized by the accumulation and proliferation of clonal B cells in the blood, marrow, and lymph nodes. The clinical course of CLL is remarkably variable among patients, with median overall survival (OS) ranging from less than 3 years, despite the use of effective combination chemotherapy regimens, to more than 10 years without need of therapeutic intervention.[1,2] Genetic features underlying this variability in clinical course have been long identified. Conventional karyotype banding and fluorescent in situ hybridization (FISH) analysis are the bases of the existing widely used hierarchic prognostic model.[3] The recent advent of transformative next-generation sequencing (NGS) has uncovered many novel putative disease-driving somatic alterations and accurately quantified intrasample heterogeneity. With the recent US Food and Drug Administration approval of inhibitors targeting CLL pathways,[4-6] the identification of the connections between each therapy and potentially vulnerable genomically defined disease subpopulations has become a priority in CLL.

UNCOVERING SOMATIC ALTERATIONS AND GENOMIC COMPLEXITY IN CLL

Over the last decade, new genome-wide sequencing approaches have identified numerous somatic alterations associated with cancer. These studies have provided a wealth of fresh insights into the underlying mechanisms driving cancer.[7] The study of CLL has particularly benefited from the availability of these transformative technologies. With its ease of tissue accessibility and indolent disease kinetics, enabling repeated sampling within the same individual, CLL has been an optimal setting for examining questions of tumor heterogeneity and clonal evolution.

Earlier genetic studies of CLL that focused on the detection of copy numbers confirmed the recurrence of key cytogenetic abnormalities previously identified by FISH.[3,8] When considering data from an aggregate of 1,590 cases of CLL worldwide[9-13] (Fig 1A), the most common alterations and their frequencies have been focal deletions of chromosomes 13q [del(13q); 55% to 60%], 17p (3.5% to 10%), and 11q (6% to 27%) and trisomy 12 [tri(12); 10% to 16%]. Of note, the minimal deleted regions of these deletions were identified to encompass important putative CLL drivers: *ATM* and *BIRC3* within del (11q), *TP53* within del(17p), and the microRNA 15a/16-1 encoded within an intron of *DLEU2* in 13q23.[14] Other cytogenetic alterations were found at lower incidence, including chromosome 2p gains [amp(2p); 2% to 7%] containing *MYCN*, *REL*, and *BCL11A*,[15,16] amp(8q) (2% to 4%) responsible for *MYC* amplification,[9] and del(8p) [2% to 5%] and del(15q) encompassing *TNFRSF10A/B*[17] and *MGA*,[11] respectively.

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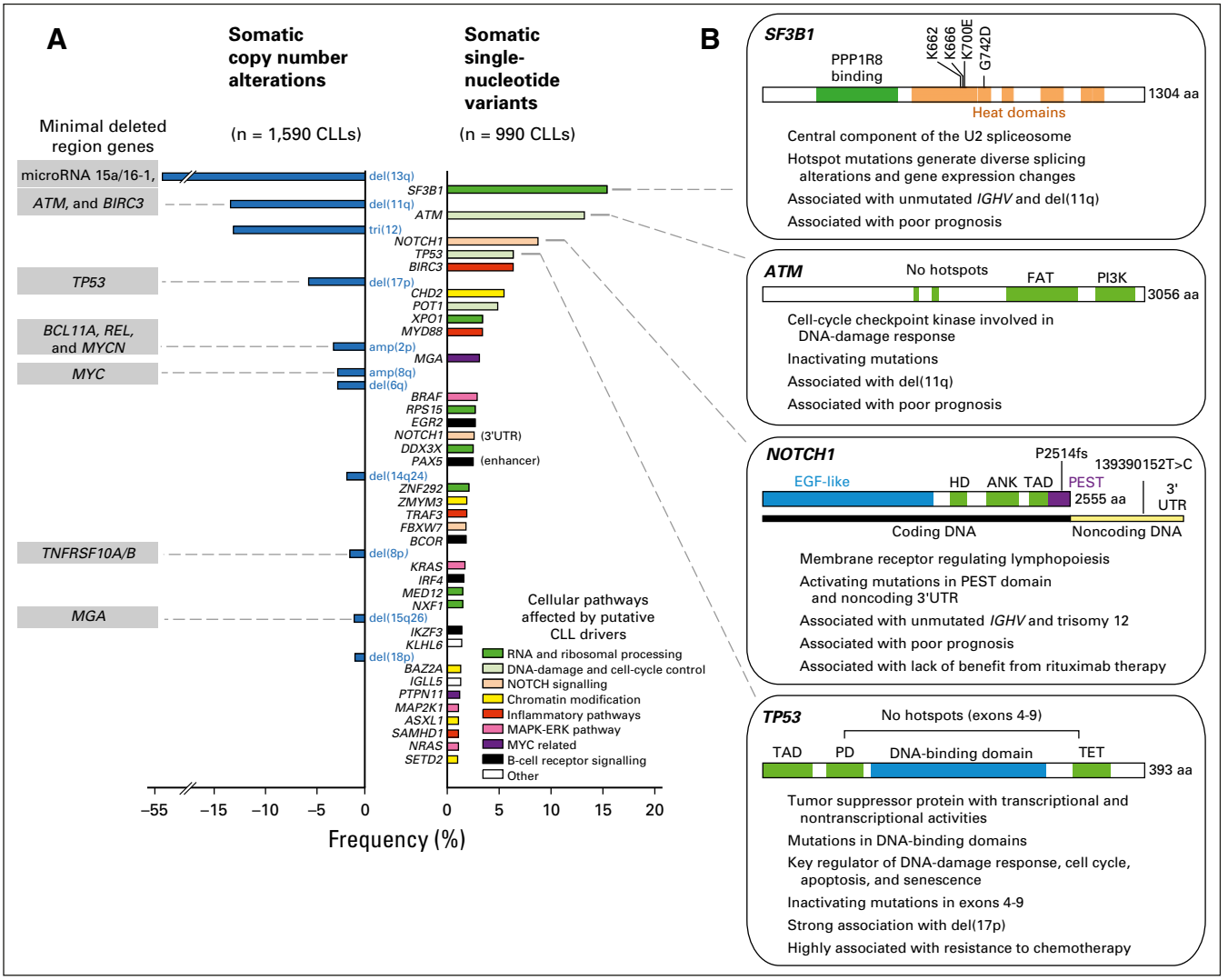

Fig 1. Putative driver gene mutations and recurrent somatic copy number variations in chronic lymphocytic leukemia (CLL). (A) Frequency of somatic copy number alterations when combining cohorts from the Spanish International Cancer Genome Consortium (ICGC) trial[12] (n = 452), German CLL Study Group CLL8 trial[11] (n = 353), Scandinavian SCALE trial[13] (n = 369), Ouillette et al[10] study (n = 255), and Dana-Farber Cancer Institute (DFCI)/Brown et al[9] study (n = 161), identified through single-nucleotide polymorphism array (except as identified by whole-exome sequencing [WES] for Spanish ICGC cohort). Also shown are the frequencies of somatic single-nucleotide variants (from DFCI/Broad Institute[14] [n = 548] and from Spanish ICGC[12] [n = 452]) identified through WES. Only the events over 1% in frequency in the combined cohorts are reported. *IGLL5*, *MAP2K1*, and *SAMHD1* have been reported in DFCI cohort only, whereas *ZNF292*, *KLH6*, *SETD2*, and *PAX5* enhancer have been reported in Spanish ICGC cohort only. All remaining genes were consistent among cohorts. Mutations in the 3′UTR of *NOTCH1* were detected in four of 150 patient cases with whole-genome sequencing. (B) Summaries of the characteristics of frequently mutated genes in CLL. amp, amplification; ANK, ankyrin repeat; del, deletion; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; FAT, FRAP-ATM-transformation/transcription domain associated protein; HD, heterodimerization domain; MAPK, mitogen-activated protein kinase; PD, programmed death; PEST, proline, glutamic acid, serine, and threonine; PI3K, phosphatidylinositol 3-kinase; SCALE, Scandinavian Lymphoma Etiology; TAD, transaction activation domain; TET, ten-eleven translocation.

Even more recently, NGS technologies such as whole-genome (WGS) and whole-exome sequencing (WES) have provided a more granular investigation of the genomic landscape of CLL.[18-24] Rather than revealing any universal genetic event accounting for all CLL cases, these technologies have demonstrated the diverse recurrent gene mutations associated with CLL and the high level of genetic heterogeneity among samples, consistent with the high degree of clinical variability characteristic of CLL. The development of robust algorithms and statistical modeling of these sequencing data have led to the uncovering of gene mutations likely positively selected and hence identified as putative CLL drivers.[25,26]

Two recent efforts using WES and WGS have together reported on approximately 1,000 patient cases of CLL, identifying recurrent gene mutations even of low frequency by highlighting a total of 75 significantly mutated genes.[12,27] In aggregate, mutations in 28 genes were found, common to both studies (Fig 1A). Conversely, the frequencies of gene mutations were dependent on the composition of the investigated cohort; 16 genes were unique to the Dana-Farber Cancer Institute (DFCI)/Broad Institute study, with *SF3B1* as the most frequently mutated gene, whereas 31 were only detected within the Spanish International Cancer Genome Consortium cohort, in which NOTCH1 was the top mutated gene. This latter study also showed that relevant mutations can affect the

noncoding genome (ie, *NOTCH1* 3′UTR and *PAX5* enhancer). Although these two studies are the largest cohorts characterized by NGS to date, it has been estimated that at least 2,000 patient cases would be required to achieve saturation in driver discovery.[26]

Examination of the growing list of putative CLL drivers has implicated the involvement of several key pathways in CLL biology (Fig 1A). *TP53* and *ATM* are well-known tumor suppressor genes that are commonly inactivated by gene mutations or chromosomal deletions in CLL [del(17p) and del(11q), respectively; Fig 1B]. Such observations have identified the DNA-damage response pathway as a crucial CLL node. The application of NGS to CLL has also unexpectedly uncovered an important role of RNA processing in CLL. As a striking example, *SF3B1* is a commonly mutated gene and encodes a component of the splicesome, which orchestrates the removal of introns from precursor mRNA.[19,21] Recently, *SF3B1* mutations were shown to cause alternative splicing (with preferential alteration in 3′ splice site selection)[28,29] and a complex of changes, including impairment of the DNA-damage response[30] and alteration in telomere biology.[31] Other frequently mutated genes involved in RNA processing and splicing have been identified (*XPO1*, *RPS15*, *DDX3X*, *ZNF292*, *MED12*, and *NXF1*), supporting the importance of this cellular process to CLL. NOTCH is another key pathway because it can be affected by either gain-of-function mutations of *NOTCH1* (2 basepair frameshift deletion),[23] mutation of *NOTCH1* 3′UTR,[12] alternative splicing (by mutated *SF3B1*) of a pathway regulator,[31] or *FBXW7* loss-of-function mutations.[32] B-cell receptor (BCR) signaling and the B-cell transcriptional program can also be impaired by mutations in *EGR2*, *BCOR*, *IRF4*, and *IKZF3*. Chromatin maintenance (*CHD2*, *BAZ2A*, *ZMYM3*, *ASXL1*, and *SETD2*), the inflammatory pathway (*BIRC3 MYD88*, *TRAF3*, and *SAMHD1*), mitogen-activated protein kinase (MAPK) –extracellular signal-regulated kinase (ERK; *BRAF*, *KRAS*, and *MAP2K1*), and MYC-related signaling (*MGA* and *PTPN11*) are other relevant pathways affected by mutations.

With the increase in the number of cases of CLL worldwide characterized by WES or WGS, it has been increasingly feasible to examine the likelihood that diverse somatic alterations cooperate to contribute to the oncogenic phenotype. Indeed, in the DFCI/Broad Institute study, most patients (approximately 60%) carried more than one driver.[27] Several studies have detected recurrent patterns of co-occurrence, highlighting likely preferred interactions between putative drivers. Del(17p) has been consistently associated with *TP53* mutation,[33] del(11q) with *ATM* and/or *SF3B1* mutation,[34] and *NOTCH1* mutation with tri(12).[35,36] Conversely, low co-occurrence between *SF3B1* mutation and tri(12) or *NOTCH1* mutation suggests redundancy in their functional activities, as recently suggested by the finding that a target of *SF3B1* mutation is a splice variant that dysregulates NOTCH signaling.[31]

In a reanalysis of our reported DFCI/Broad Institute data,[27] we found most CLL samples to display a unique combination of genetic alterations not occurring in any other patient sample, suggesting that each leukemia embarks on an independent evolutionary path (Fig 2). This analysis also revealed that for approximately 5% of patients, del(13q) is the sole detectable genetic abnormality. This event alone seems to be sufficient to drive CLL, because deletion in mice of the region corresponding to the human 13q14 led to the development of CLL-like disease, although at low penetrance and latency. Tri(12) and mutations of *CHD2* and *SF3B1* are also found as sole abnormalities in patients and could be sufficient drivers for CLL as well. Two thirds of recurrent combinations involved at least two of the following drivers: del(13q), del(11q), and *SF3B1* and/or *ATM* mutations. Finally, 8% of CLLs in this cohort did not carry any known driver.[27] In these cases, there may have been putative genetic drivers not yet discovered; other factors such as epigenetic deregulation or microenvironmental factors could have played a role in driving disease.

Such genomic complexity defies single gene–based approaches for understanding cancer biology. Definitive understanding of the exact function of novel drivers and how they cooperate will require studies in in vitro and in vivo models.[37,38] Emerging single-cell technologies will likely transform our ability to decipher genomic complexity in CLL and highlight new drivers that could be relevant to individual patients. An expectation, however, is that these private drivers will nonetheless affect common core CLL pathways.

UNDERSTANDING CLL LEUKEMOGENESIS THROUGH ITS PHYLOGENETIC RECONSTRUCTION

Identifying founding genomic lesions and establishing when they were acquired may facilitate better understanding of the natural history of a case of CLL and suggest points of intervention. Because each mutation essentially supplies a molecular barcode for NGS reads, clustering of reads with similar variant allele frequencies (corrected for local ploidy and tumor purity) has feasibly allowed for accurate quantification of intratumor heterogeneity and investigation of clonal architecture and disease phylogeny. These types of studies have revealed events that are preferentially clonal, consistent with earlier events, and others that are preferentially subclonal, consistent with later events. On the basis of this principle, several studies have consistently categorized del(13q), del(11q), tri(12), and *MYD88* mutations as early lesions, suggesting their role as CLL initiators, and *ATM*, *SF3B1*, and *TP53* mutations as later lesions.[24,27] Likewise, the application of machine learning–based approaches has supported the idea that CLL-associated lesions are temporally ordered in a specific fashion rather than being randomly accumulated, again revealing del(13q) and tri(12) as early and potentially initiating events leading to preferred evolutionary trajectories.[39]

A further layer of complexity that likely contributes to CLL disease heterogeneity is the impact of cell of origin on acquisition of subsequent somatic alterations. The methylome of CLLs with *IGHV*-unmutated status is more consistent with that of naïve B cells, whereas the epigenetic state of CLLs with mutated *IGHV* is more similar to antigen-experienced B cells,[40,41] although recent work has suggested that CLL cells can become fixed across diverse stages of B-cell differentiation.[42] The state of B-cell differentiation seems to be associated with a preferential acquisition of certain somatic mutations, with mutated *IGHV* having a narrow spectrum of drivers (ie, *MYD88*, *CD79A/B*, and *TLR2*) and unmutated *IGHV* having a broad spectrum of events.[27] CLL-associated genomic abnormalities have been found even in the hematopoietic progenitor cells of patients with CLL. Deep sequencing of CD34+CD19- sorted cells of patients with CLL has revealed the detection of mutations in *NOTCH1*, *SF3B1*, and *BRAF*.[43] These findings are in

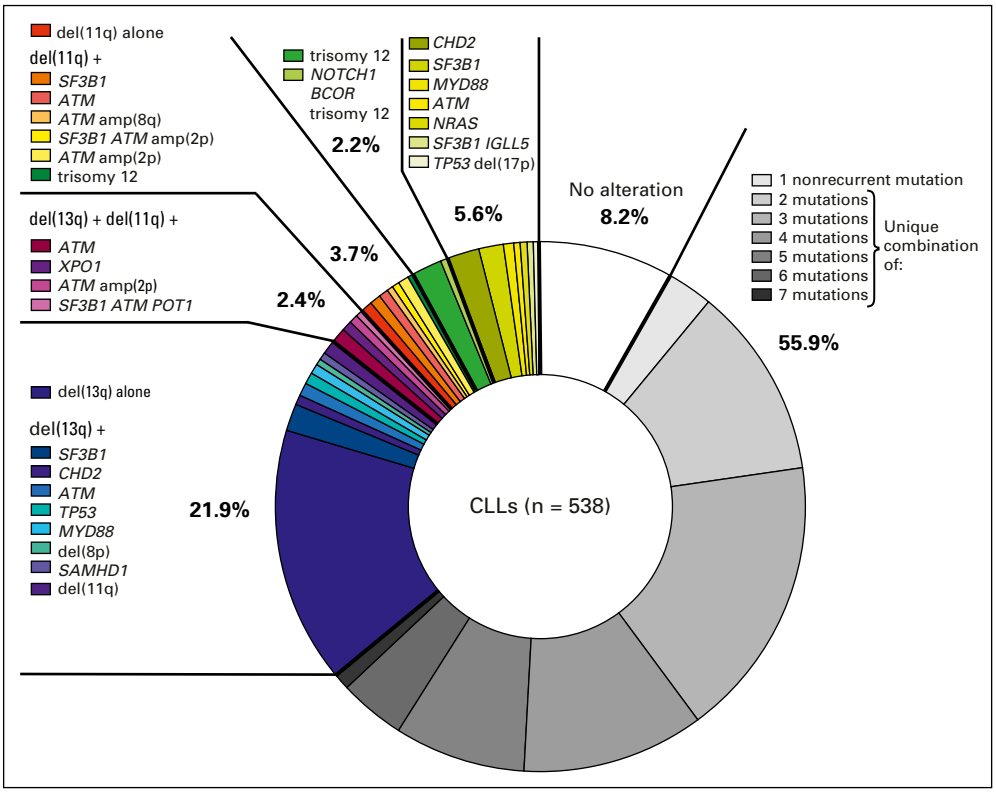

Fig 2. A summary of the frequencies and most common coassociations of putative chronic lymphocytic leukemia (CLL) drivers, per analysis of the Dana-Farber Cancer Institute/Broad Institute cohort of 538 CLLs.[27]

line with murine xenograft studies in which engrafted hematopoietic progenitor cells from patients with CLL could produce a mature CD5+-expressing clonal B-cell population with a phenotype of CLL.[44]

IMPACT OF SOMATIC EVENTS IN CLL ON RESPONSE TO THERAPY

The treatment landscape of CLL is currently evolving considerably. For more than a decade, the combination of fludarabine, cyclophosphamide, and rituximab has been the conventional first-line regimen for fit patients, providing a high response rate (90%) and prolonged progression-free survival (PFS; median, 57 months).[1] Although patients treated with this combination almost uniformly experience relapse, a subset of those with mutated *IGHV* can particularly benefit from this approach, with long-term PFS.[45-47] Other common chemoimmunotherapy-based monotherapy or combination regimens have included purine analogs (bendamustine, pentostatin, and cladribine),[48-50] alkylating agents (chlorambucil and cyclophosphamide),[51,52] and anti-CD20 (rituximab, ofatumumab, and obinutuzumab)[53-55] or anti-CD52 antibodies (alemtuzumab).[56] In addition to these chemotherapy-based regimens, recent US Food and Drug Administration approvals of novel therapies targeting BCR signaling (ie, idelalisib, an inhibitor of phosphatidylinositol 3-kinase,[5] and ibrutinib, the irreversible inhibitor of Bruton tyrosine kinase [BTK][4]) and the B-cell lymphoma 2 signaling pathway (ie, venetoclax[6]) have opened up the possibility of specifically targeting crucial CLL pathways, with less toxicity. Despite high response rates in patients for whom cytotoxic drugs have failed, resistance to these therapies is increasingly emerging.[57] In this setting, genomic characterization can identify the sources of resistance and help guide subsequent therapeutic decisions.

Intratumoral Heterogeneity Fuels CLL Resistance

It is increasingly evident that intratumoral heterogeneity not only fuels clonal evolution and leukemic progression but also provides the seeds for the development of therapeutic resistance.[24,27] Although therapy itself can potentially incite mutational events and increase genomic diversity,[58] multiple longitudinal studies using WES characterization in CLL have supported a scenario in which the genetic capacity for resistance is already present in the pretreatment sample as pre-existing subclones (Fig 3).[24,47,59-62] Alternatively, leukemic tumor cells at relapse could also be progeny of dormant parental cells. Recent findings showing the presence of somatic alterations in early hematopoietic progenitors are in line with this idea.[43]

Characterizing the Treatment-Specific Genomic Landscape of CLL

In the setting of exposure to broadly cytotoxic agents, *TP53* disruption has been clearly identified as the most crucial and independent factor of resistance. Indeed, multiple studies have shown that patients with *TP53* loss experience poor response and worse outcome after chemotherapy treatment.[63] In NGS studies tracking the fate of subclonal populations in patient samples before and after chemoimmunotherapy, subclones with disrupted *TP53* clearly undergo clonal expansion by the time of relapse (Fig 3).[24,27,64] Importantly, small subclones with *TP53* mutations

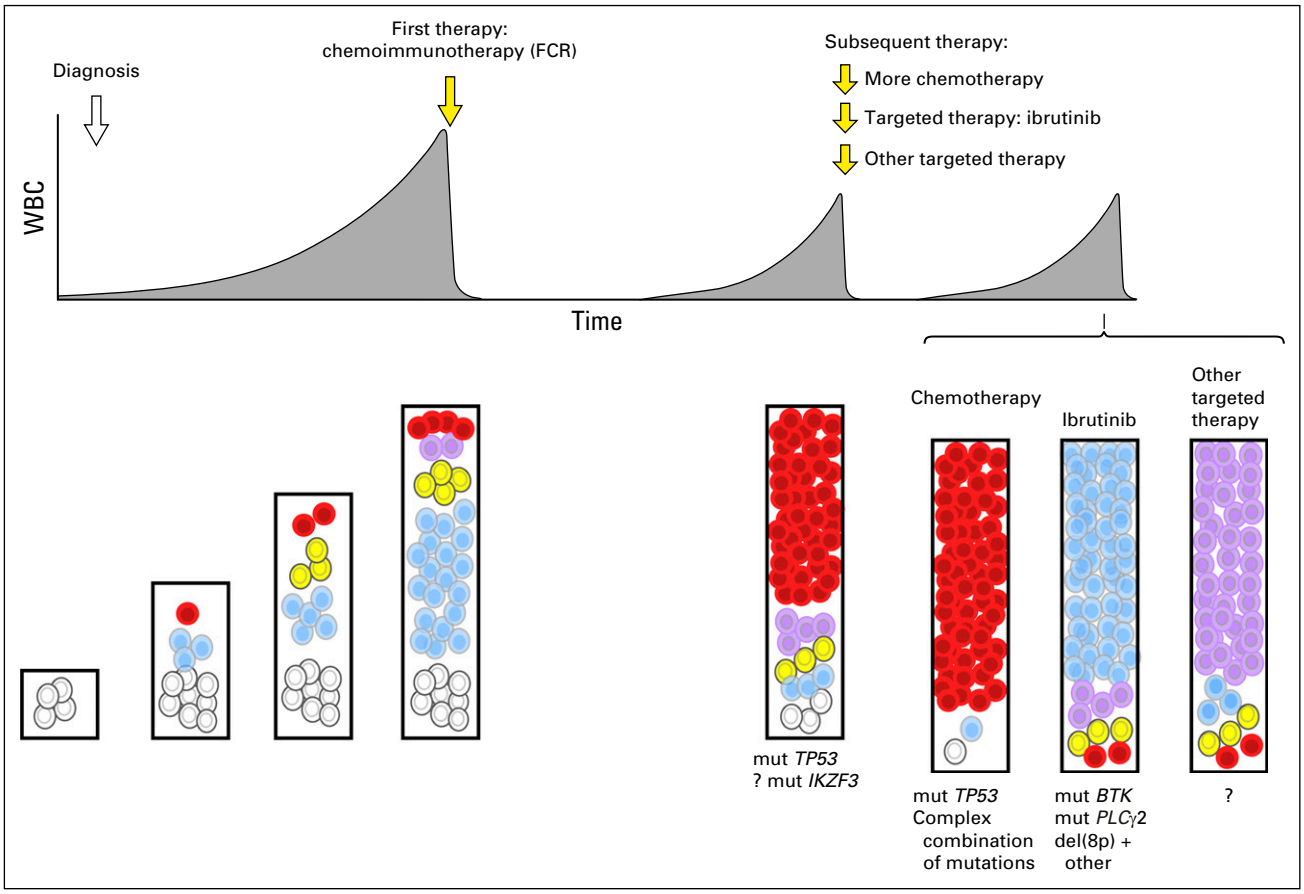

Fig 3. Schema of clonal evolution of chronic lymphocytic leukemia (CLL) in relation to exposure to therapy. From the start, sequential acquisition of somatic alterations in CLL leads to its genomic diversification. Exposure to chemoimmunotherapy as first-line treatment commonly selects *TP53*-disrupted subclones. Each therapy proposed at relapse may differentially shape the CLL architecture and would be associated with preferential alterations: *TP53* loss with chemotherapy-based treatment and *BTK* or *PLCG2* mutation (mut) or 8p deletion (del) with ibrutinib therapy. ? indicates other mutations. FCR, fludarabine, cyclophosphamide, and rituximab.

detected at diagnosis only by sensitive techniques are associated with the same adverse prognosis as macroscopic subclones and can indicate future fludarabine refractoriness.[65,66] Subclones with *TP53* mutations are also associated with lower death rates during therapy compared with subclones with *TP53* wild type, suggesting diminished sensitivity to therapy and higher growth rates during repopulation.[67] These mechanisms underlying clonal dynamics have not been yet observed with other drivers and highlight once again the leading role of *TP53* mutations in providing fitness advantage to the clone.

SF3B1 and *ATM* mutations seem to have variable evolution, with distinct clones rising or falling over time, suggesting that they likely do not bring the same advantage as *TP53* when considered individually. By contrast, targeted characterization of the relapsing CLL genome has shown that combinations of mutations involving *TP53*, *ATM*, and *SF3B1* could act synergistically to provide resistance to immunochemotherapy.[68] Thus, rather than sole abnormalities, combinations of somatic alterations could drive chemotherapy resistance in CLL.

By contrast, resistance to the targeted pathway inhibitor ibrutinib has been attributed to mutations directly affecting its target (BTK) or its downstream effector (PLCγ2).[57] BTK mutations (at the C481S site) are located in the ibrutinib binding site, resulting in a protein that is only reversibly inhibited by ibrutinib.[69] *PLCG2* mutations likewise provide gain of function and lead to activation of BCR signaling in a BTK-independent manner.[70] Resistance to ibrutinib has been also associated with marked clonal evolution.[71] Aside from *BTK* or *PLCG2* mutations, relapsing subclones have been shown to be progeny of parental del(8p) leukemic cells, present before therapy. Del(8p) was found to generate haploinsufficiency of the tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) receptor, resulting in TRAIL insensitivity that could contribute to ibrutinib resistance (Fig 3). Although ibrutinib represents a major advance in the treatment of patients with *TP53* disruption,[72] those treated with ibrutinib in this group of patients could nonetheless still have an adverse prognosis.[73] However, complex karyotype has been shown to be an even stronger predictor than del(17p) in this setting.[74] Thus, distinct modes of therapy may shape the CLL architecture differently.

Novel Approaches to the Detection and Monitoring of Subclonal Populations

Given that the bulk of recurrent putative CLL drivers have been discovered in WES or WGS studies (down to 3% frequency within the population), targeted NGS approaches are increasingly

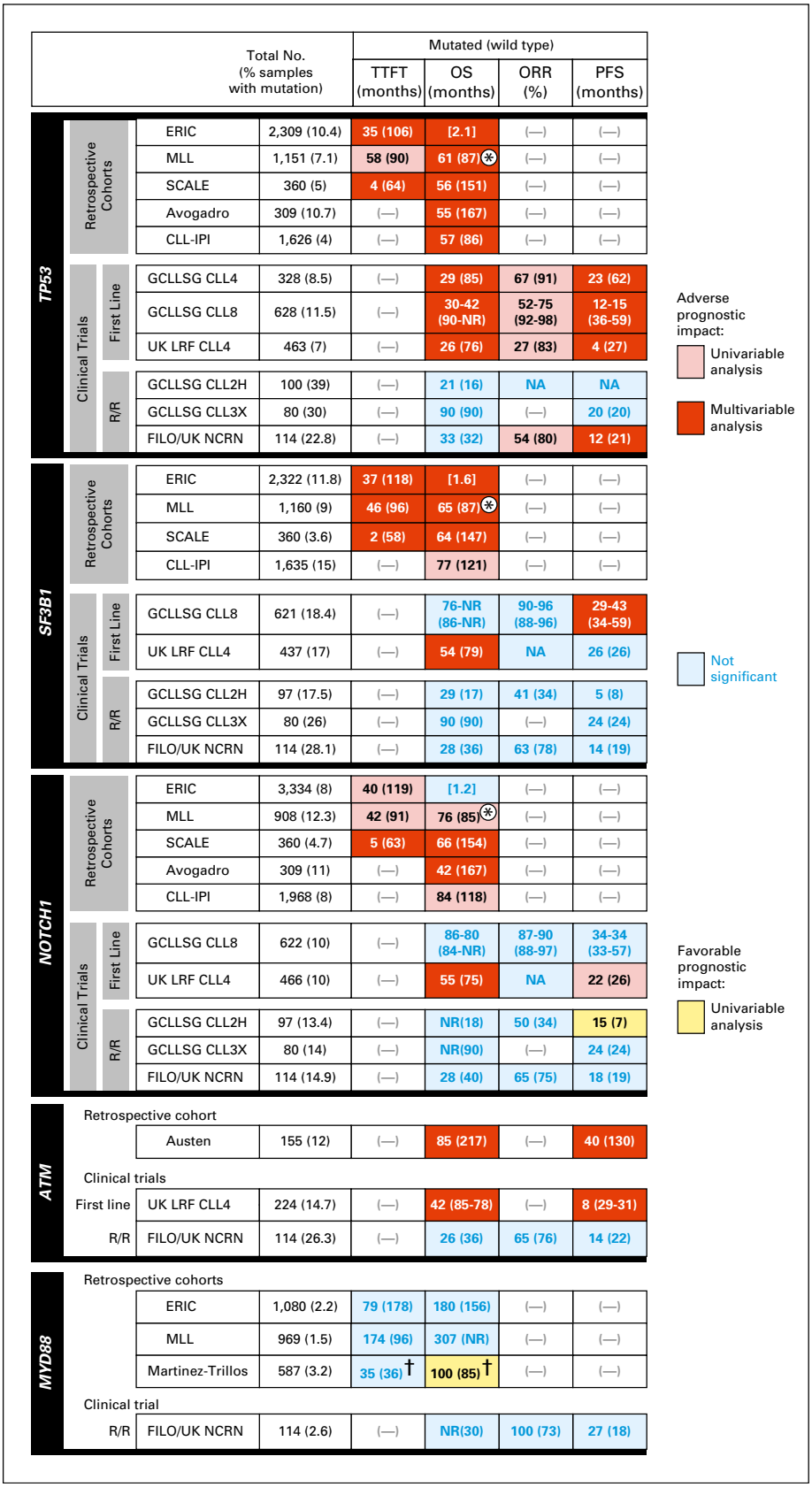

Fig 4. Summary of prognosis impact of somatic mutations evaluated in retrospective studies or clinical trials in chronic lymphocytic leukemia (CLL). The medians of time to first treatment (TTFT), overall survival (OS), and progression-free survival (PFS) in subgroups of patients with CLL with mutations are indicated in months and compared with subgroups of patients with wild type (in parentheses). When medians were not available in studies, the value of the hazard ratio in multivariable analyses is reported in square brackets. The UK Leukaemia Research Fund (LRF) CLL4 trial[83] compared median months for the *ATM* biallelic inactivation subgroup with *ATM* wild type and *ATM* mutation subgroup. Data from the German CLL Study Group (GCLLSG) CLL3X trial[84] were inferred from Kaplan-Meier curves. ERIC, European Research Initiative on CLL; FILO, French Intergroup on CLL; IPI, International Prognostic Index; MLL, Munich Leukemia Laboratory; NA, not available; NCRN, National Cancer Research Network; NR, not reached; ORR, overall response rate; R/R, relapsed/refractory; SCALE, Scandinavian Lymphoma Etiology. *5-year OS rate (%). †10-year rate (%).

attractive as a means of efficiently and more routinely sampling the mutational landscape of CLL in a cost-effective fashion.[68,75] Deep-targeted sequencing displays high sensitivity, with the ability to detect mutated alleles in down to one in 100 or 1,000 cells, but it is still prohibitively expensive for the detection of rarer events.

Single-cell technologies are emerging as another powerful tool to probe the genomic composition in heterogeneous cellular populations.[76] Single-cell WGS approaches can potentially provide a comprehensive snapshot of the subclonal composition of a population,[77,78] but this is not high throughput nor cost effective at the present time. Droplet digital polymerase chain reaction technology is an alternative method for analyzing single cells by compartmentalizing them using water-oil emulsion at high throughputs. Using this strategy, the presence of ibrutinib-resistant subclones was recently been quantified before treatment initiation, demonstrating that drug-resistant populations were present in small quantities even in advance of ibrutinib exposure[71] and that capacity for drug resistance was already inherent within the patient sample.

DEVELOPING GENOMICS-BASED SCHEMA FOR PROGNOSTICATION

Over the past decade, clinical prognostication in CLL has relied on the Rai and Binet clinical staging systems,[2,79] knowledge of the mutational status of immunoglobulin variable region (which separates CLL into either mutated or unmutated *IGHV* groups, the latter with worse outcome[80,81]), and FISH assessment of the most recurrent chromosomal aberrations in CLL. In a hierarchic classification scheme, del(17p) was found to confer the poorest survival, followed by del(11q), tri(12), normal karyotype, and then del (13q) as the sole abnormality.[3]

A key question in the field is whether the increasing array of discovered putative drivers holds prognostic relevance and could improve the accuracy of prognostication. A growing body of validation studies has evaluated the associations between genotype and parameters such as time to first treatment (TTFT), OS, therapeutic response, and PFS (Fig 4).

Despite variability in the design and size of these studies, several consistent findings can be gleaned. First, *TP53* disruption has clearly emerged as a reliable factor conferring adverse TTFT, PFS, and OS after first-line therapy.[33,63,82-84] In the recently reported CLL International Prognostic Index study, which evaluated 3,472 patients treated in prospective first-line trials, mutation in *TP53* contributed the greatest weight to the score.[85]

Second, the impact of *SF3B1* and *NOTCH1* mutations has been variably reported, which likely points to the importance of therapeutic context and patient status in evaluating this effect. In aggregate, several clinical trial studies have highlighted the independent poor prognostic influence of *SF3B1* mutations on PFS or OS.[33,82,84,86-88] Although *NOTCH1* mutations have independently conferred adverse OS in multiple studies, their impact on PFS has been inconsistent.[87-90] Interestingly, an analysis of the international CLL8 study reported a lack of benefit from the addition of rituximab in patients with *NOTCH1* mutations.[91]

Fewer studies have evaluated the role of *ATM* mutations; those that have been conducted have suggested an association with shorter OS.[92] More recent studies, however, have argued for a notable role of biallelic rather than monoallelic inactivation in significantly shorter PFS and OS in the first-line setting.[93]

Impact of other recurrent mutations remains under investigation. *KRAS* and *POT1* mutations have been associated with refractoriness and adverse outcome.[94] *EGR2* mutations were observed to be associated with shorter OS.[43] Although linked with poor prognostic features, *MED12* mutations do not affect OS.[97] WES of large CLL cohorts recently identified additional novel prognostic factors associated with shorter TTFT (*BRAF, ZMYM3*, and *IRF4*), OS (*ASXL1*), and PFS (*RPS15* and *SETD2*).[12,27,96]

Given the genomic complexity and co-occurrence in drivers in CLL, sophisticated models and external validation are now required to understand the relative prognostic value of the host of diverse genomic events. Hierarchic models have been widely used for classifying cytogenetic lesions.[3] By focusing on OS, a scoring system integrating both recurrent chromosomal aberrations and gene mutations was proposed to define four CLL risk groups: high (*TP53* and/or *BIRC3* abnormalities), intermediate [*NOTCH1* ± *SF3B1* mutations and ± del(11q)], low [tri(12) or normal karyotype], and very low risk [del(13q) as sole alteration], where survival is similar to the general population.[83] Prognostic scoring integrating a larger number of somatic alterations and focusing on both PFS and OS remains to be performed for accurately determining prognosis. The respective impact of somatic alterations is also expected to drastically change with the use of targeted agents, the resistance mechanisms of which may differ from those of conventional chemotherapies. An alternative approach would be to assign patients to subgroups based on distinct nonoverlapping molecular features, as recently proposed for acute myeloid leukemia, although this is challenging for a disease as genetically heterogeneous as CLL.[97]

Given the consistent poor prognosis associated with inactivating TP53 lesions, clinical *TP53* mutation testing [by FISH for the chromosome 17p locus and *TP53* sequencing in cases without del(17p)][98] is now highly recommended before each therapeutic line. Indeed, chemotherapy-based approaches have proven unsatisfactory in the presence of *TP53* mutations and are now no longer the therapy of choice for these patients. Conversely, allogeneic transplantation remains a valid strategy for patients with *TP53* disruption, as does the use of novel agents such as ibrutinib, idelalisib plus rituximab, and venetoclax.[6,89,99-101] Beyond *TP53*, sequencing of *SF3B1*, *NOTCH1*, and *ATM* may provide additional prognostic information, although therapeutic context will play a major role in assigning their relative importance. For analysis of clinical samples, a common panel of genes to integrate in patients with CLL could include *TP53*, *SF3B1*, *NOTCH1*, and *ATM*, given their frequency and potential prognostic impact. In the setting of ibrutinib treatment, the monitoring of gene mutations associated with ibrutinib resistance (ie, *BTK* and *PLCG2)* can define the potential need for alternative therapies. Larger panels of genes may be informative, but their prognostic impact remains to be determined (*MYD88*, *BIRC3*, *KRAS*, *POT1*, *EGR2*, *MED12*, *BRAF*, *ZMYM3*, *IRF4*, *ASXL1*, *RPS15*, *SAMHD1*, and *SETD2)*.

In conclusion, the application of advanced genomic technologies has rapidly uncovered new mechanisms underlying CLL biology, disease progression, and therapeutic resistance. To fulfill the promise of precision oncology, future studies will need to address the challenge of translating these findings into the routine

management of CLL so that patients can maximally benefit from recent therapeutic advances.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: All authors
Data analysis and interpretation: Romain Guièze, Catherine J. Wu
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

REFERENCES

1. Hallek M, Fischer K, Fingerle-Rowson G, et al: Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: A randomised, open-label, phase 3 trial. Lancet 376:1164-1174, 2010
2. Binet JL, Auquier A, Dighiero G, et al: A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 48:198-206, 1981
3. Döhner H, Stilgenbauer S, Benner A, et al: Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 343:1910-1916, 2000
4. Byrd JC, Furman RR, Coutre SE, et al: Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med 369:32-42, 2013
5. Furman RR, Sharman JP, Coutre SE, et al: Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med 370:997-1007, 2014
6. Roberts AW, Davids MS, Pagel JM, et al: Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. N Engl J Med 374:311-322, 2016
7. Garraway LA, Lander ES: Lessons from the cancer genome. Cell 153:17-37, 2013
8. Malek SN: The biology and clinical significance of acquired genomic copy number aberrations and recurrent gene mutations in chronic lymphocytic leukemia. Oncogene 32:2805-2817, 2013
9. Brown JR, Hanna M, Tesar B, et al: Integrative genomic analysis implicates gain of PIK3CA at 3q26 and MYC at 8q24 in chronic lymphocytic leukemia. Clin Cancer Res 18:3791-3802, 2012
10. Ouillette P, Collins R, Shakhan S, et al: Acquired genomic copy number aberrations and survival in chronic lymphocytic leukemia. Blood 118: 3051-3061, 2011
11. Edelmann J, Holzmann K, Miller F, et al: High-resolution genomic profiling of chronic lymphocytic leukemia reveals new recurrent genomic alterations. Blood 120:4783-4794, 2012
12. Puente XS, Beà S, Valdés-Mas R, et al: Non-coding recurrent mutations in chronic lymphocytic leukaemia. Nature 526:519-524, 2015
13. Gunnarsson R, Mansouri L, Isaksson A, et al: Array-based genomic screening at diagnosis and during follow-up in chronic lymphocytic leukemia. Haematologica 96:1161-1169, 2011
14. Calin GA, Dumitru CD, Shimizu M, et al: Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 99: 15524-15529, 2002
15. Chapiro E, Leporrier N, Radford-Weiss I, et al: Gain of the short arm of chromosome 2 (2p) is a frequent recurring chromosome aberration in untreated chronic lymphocytic leukemia (CLL) at advanced stages. Leuk Res 34:63-68, 2010
16. Pfeifer D, Pantic M, Skatulla I, et al: Genome-wide analysis of DNA copy number changes and LOH in CLL using high-density SNP arrays. Blood 109: 1202-1210, 2007
17. Rubio-Moscardo F, Blesa D, Mestre C, et al: Characterization of 8p21.3 chromosomal deletions in B-cell lymphoma: TRAIL-R1 and TRAIL-R2 as candidate dosage-dependent tumor suppressor genes. Blood 106:3214-3222, 2005
18. Puente XS, Pinyol M, Quesada V, et al: Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature 475:101-105, 2011
19. Wang L, Lawrence MS, Wan Y, et al: SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. N Engl J Med 365:2497-2506, 2011
20. Ramsay AJ, Quesada V, Foronda M, et al: POT1 mutations cause telomere dysfunction in chronic lymphocytic leukemia. Nat Genet 45: 526-530, 2013
21. Quesada V, Conde L, Villamor N, et al: Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. Nat Genet 44:47-52, 2011
22. Rossi D, Fangazio M, Rasi S, et al: Disruption of BIRC3 associates with fludarabine chemorefractoriness in TP53 wild-type chronic lymphocytic leukemia. Blood 119:2854-2862, 2012
23. Fabbri G, Rasi S, Rossi D, et al: Analysis of the chronic lymphocytic leukemia coding genome: Role of NOTCH1 mutational activation. J Exp Med 208: 1389-1401, 2011
24. Landau DA, Carter SL, Stojanov P, et al: Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. Cell 152:714-726, 2013
25. Cibulskis K, Lawrence MS, Carter SL, et al: Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol 31:213-219, 2013
26. Lawrence MS, Stojanov P, Polak P, et al: Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 499:214-218, 2013
27. Landau DA, Tausch E, Taylor-Weiner AN, et al: Mutations driving CLL and their evolution in progression and relapse. Nature 526:525-530, 2015
28. DeBoever C, Ghia EM, Shepard PJ, et al: Transcriptome sequencing reveals potential mechanism of cryptic 3′ splice site selection in SF3B1-mutated cancers. PLOS Comput Biol 11:e1004105, 2015
29. Darman RB, Seiler M, Agrawal AA, et al: Cancer-associated SF3B1 hotspot mutations induce cryptic 3′ splice site selection through use of a different branch point. Cell Reports 13:1033-1045, 2015
30. Te Raa GD, Derks IA, Navrkalova V, et al: The impact of SF3B1 mutations in CLL on the DNA-damage response. Leukemia 29:1133-1142, 2015
31. Wang L, Brooks AN, Fan J, et al: Transcriptomic characterization of SF3B1 mutation reveals its pleiotropic effects in chronic lymphocytic leukemia. Cancer Cell 30:750-763, 2016
32. O'Neil J, Grim J, Strack P, et al: FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. J Exp Med 204:1813-1824, 2007
33. Stilgenbauer S, Schnaiter A, Paschka P, et al: Gene mutations and treatment outcome in chronic lymphocytic leukemia: Results from the CLL8 trial. Blood 123:3247-3254, 2014
34. Austen B, Skowronska A, Baker C, et al: Mutation status of the residual ATM allele is an important determinant of the cellular response to chemotherapy and survival in patients with chronic lymphocytic leukemia containing an 11q deletion. J Clin Oncol 25:5448-5457, 2007
35. Balatti V, Bottoni A, Palamarchuk A, et al: NOTCH1 mutations in CLL associated with trisomy 12. Blood 119:329-331, 2012
36. Riches JC, O'Donovan CJ, Kingdon SJ, et al: Trisomy 12 chronic lymphocytic leukemia cells exhibit upregulation of integrin signaling that is modulated by NOTCH1 mutations. Blood 123:4101-4110, 2014
37. Platt RJ, Chen S, Zhou Y, et al: CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell 159:440-455, 2014
38. Heckl D, Kowalczyk MS, Yudovich D, et al: Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing. Nat Biotechnol 32:941-946, 2014
39. Wang J, Khiabanian H, Rossi D, et al: Tumor evolutionary directed graphs and the history of chronic lymphocytic leukemia. Elife 3:e02869, 2014
40. Kulis M, Heath S, Bibikova M, et al: Epigenomic analysis detects widespread gene-body DNA hypomethylation in chronic lymphocytic leukemia. Nat Genet 44:1236-1242, 2012
41. Kulis M, Merkel A, Heath S, et al: Whole-genome fingerprint of the DNA methylome during human B cell differentiation. Nat Genet 47:746-756, 2015
42. Oakes CC, Seifert M, Assenov Y, et al: DNA methylation dynamics during B cell maturation underlie a continuum of disease phenotypes in chronic lymphocytic leukemia. Nat Genet 48:253-264, 2016
43. Damm F, Mylonas E, Cosson A, et al: Acquired initiating mutations in early hematopoietic cells of CLL patients. Cancer Discov 4:1088-1101, 2014
44. Kikushige Y, Ishikawa F, Miyamoto T, et al: Self-renewing hematopoietic stem cell is the primary target in pathogenesis of human chronic lymphocytic leukemia. Cancer Cell 20:246-259, 2011
45. Thompson PA, Tam CS, O'Brien SM, et al: Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in

IGHV-mutated chronic lymphocytic leukemia. Blood 127:303-309, 2016

46. Fischer K, Bahlo J, Fink AM, et al: Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: Updated results of the CLL8 trial. Blood 127:208-215, 2016

47. Rossi D, Terzi-di-Bergamo L, De Paoli L, et al: Molecular prediction of durable remission after first-line fludarabine-cyclophosphamide-rituximab in chronic lymphocytic leukemia. Blood 126:1921-1924, 2015

48. Knauf WU, Lissichkov T, Aldaoud A, et al: Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. J Clin Oncol 27:4378-4384, 2009

49. Kay NE, Geyer SM, Call TG, et al: Combination chemoimmunotherapy with pentostatin, cyclophosphamide, and rituximab shows significant clinical activity with low accompanying toxicity in previously untreated B chronic lymphocytic leukemia. Blood 109:405-411, 2007

50. Robak T, Blonski JZ, Gora-Tybor J, et al: Cladribine alone and in combination with cyclophosphamide or cyclophosphamide plus mitoxantrone in the treatment of progressive chronic lymphocytic leukemia: Report of a prospective, multicenter, randomized trial of the Polish Adult Leukemia Group (PALG CLL2). Blood 108:473-479, 2006

51. Montserrat E, Alcalá A, Parody R, et al: Treatment of chronic lymphocytic leukemia in advanced stages: A randomized trial comparing chlorambucil plus prednisone versus cyclophosphamide, vincristine, and prednisone. Cancer 56:2369-2375, 1985

52. Rai KR, Peterson BL, Appelbaum FR, et al: Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med 343:1750-1757, 2000

53. Byrd JC, Murphy T, Howard RS, et al: Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. J Clin Oncol 19: 2153-2164, 2001

54. Coiffier B, Lepretre S, Pedersen LM, et al: Safety and efficacy of ofatumumab, a fully human monoclonal anti-CD20 antibody, in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: A phase 1-2 study. Blood 111:1094-1100, 2008

55. Cartron G, de Guibert S, Dilhuydy M-S, et al: Obinutuzumab (GA101) in relapsed/refractory chronic lymphocytic leukemia: Final data from the phase 1/2 GAUGUIN study. Blood 124:2196-2202, 2014

56. Hillmen P, Skotnicki AB, Robak T, et al: Alemtuzumab compared with chlorambucil as first-line therapy for chronic lymphocytic leukemia. J Clin Oncol 25:5616-5623, 2007

57. Woyach JA, Furman RR, Liu T-M, et al: Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. N Engl J Med 370:2286-2294, 2014

58. Landau DA, Carter SL, Getz G, et al: Clonal evolution in hematological malignancies and therapeutic implications. Leukemia 28:34-43, 2014

59. Schuh A, Becq J, Humphray S, et al: Monitoring chronic lymphocytic leukemia progression by whole genome sequencing reveals heterogeneous clonal evolution patterns. Blood 120:4191-4196, 2012

60. Amin NA, Seymour E, Saiya-Cork K, et al: A quantitative analysis of subclonal and clonal gene mutations before and after therapy in chronic lymphocytic leukemia. Clin Cancer Res 22:4525-4535, 2016

61. Rasi S, Khiabanian H, Ciardullo C, et al: Clinical impact of small subclones harboring NOTCH1, SF3B1 or BIRC3 mutations in chronic lymphocytic leukemia. Haematologica 101:e135-e138, 2016

62. Malcikova J, Stano-Kozubik K, Tichy B, et al: Detailed analysis of therapy-driven clonal evolution of TP53 mutations in chronic lymphocytic leukemia. Leukemia 29:877-885, 2015

63. Zenz T, Eichhorst B, Busch R, et al: *TP53* mutation and survival in chronic lymphocytic leukemia. J Clin Oncol 28:4473-4479, 2010

64. Zenz T, Kröber A, Scherer K, et al: Monoallelic TP53 inactivation is associated with poor prognosis in chronic lymphocytic leukemia: Results from a detailed genetic characterization with long-term follow-up. Blood 112:3322-3329, 2008

65. Rossi D, Khiabanian H, Spina V, et al: Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukemia. Blood 123:2139-2147, 2014

66. Lazarian G, Tausch E, Eclache V, et al: TP53 mutations are early events in chronic lymphocytic leukemia disease progression and precede evolution to complex karyotypes. Int J Cancer 139:1759-1763, 2016

67. Landau DA, Tausch E, Böttcher S, et al: Quantitative clonal dynamics define mechanisms of CLL evolution in response to combination chemotherapy. Blood 126:362, 2015 (abstr)

68. Guièze R, Robbe P, Clifford R, et al: Presence of multiple recurrent mutations confers poor trial outcome of relapsed/refractory CLL. Blood 126:2110-2117, 2015

69. Cheng S, Guo A, Lu P, et al: Functional characterization of BTK(C481S) mutation that confers ibrutinib resistance: Exploration of alternative kinase inhibitors. Leukemia 29:895-900, 2015

70. Liu TM, Woyach JA, Zhong Y, et al: Hypermorphic mutation of phospholipase C, γ2 acquired in ibrutinib-resistant CLL confers BTK independency upon B-cell receptor activation. Blood 126:61-68, 2015

71. Burger JA, Landau DA, Taylor-Weiner A, et al: Clonal evolution in patients with chronic lymphocytic leukaemia developing resistance to BTK inhibition. Nat Commun 7:11589, 2016

72. Byrd JC, Furman RR, Coutre SE, et al: Three-year follow-up of treatment-naïve and previously treated patients with CLL and SLL receiving single-agent ibrutinib. Blood 125:2497-2506, 2015

73. Winqvist M, Asklid A, Andersson PO, et al: Real-world results of ibrutinib in patients with relapsed or refractory chronic lymphocytic leukemia: Data from 95 consecutive patients treated in a compassionate use program—A study from the Swedish Chronic Lymphocytic Leukemia Group. Haematologica 101:1573-1580, 2016

74. Thompson PA, O'Brien SM, Wierda WG, et al: Complex karyotype is a stronger predictor than del (17p) for an inferior outcome in relapsed or refractory chronic lymphocytic leukemia patients treated with ibrutinib-based regimens. Cancer 121:3612-3621, 2015

75. Sutton LA, Ljungström V, Mansouri L, et al: Targeted next-generation sequencing in chronic lymphocytic leukemia: A high-throughput yet tailored approach will facilitate implementation in a clinical setting. Haematologica 100:370-376, 2015

76. Gawad C, Koh W, Quake SR: Single-cell genome sequencing: Current state of the science. Nat Rev Genet 17:175-188, 2016

77. Hughes AEO, Magrini V, Demeter R, et al: Clonal architecture of secondary acute myeloid leukemia defined by single-cell sequencing. PLoS Genet 10:e1004462, 2014

78. Saadatpour A, Lai S, Guo G, et al: Single-cell analysis in cancer genomics. Trends Genet 31:576-586, 2015

79. Rai KR, Sawitsky A, Cronkite EP, et al: Clinical staging of chronic lymphocytic leukemia. Blood 46: 219-234, 1975

80. Damle RN, Wasil T, Fais F, et al: Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 94:1840-1847, 1999

81. Hamblin TJ, Davis Z, Gardiner A, et al: Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 94:1848-1854, 1999

82. Baliakas P, Hadzidimitriou A, Sutton LA, et al: Recurrent mutations refine prognosis in chronic lymphocytic leukemia. Leukemia 29:329-336, 2015

83. Rossi D, Rasi S, Spina V, et al: Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia. Blood 121:1403-1412, 2013

84. Oscier DG, Rose-Zerilli MJ, Winkelmann N, et al: The clinical significance of NOTCH1 and SF3B1 mutations in the UK LRF CLL4 trial. Blood 121: 468-475, 2013

85. International CLL-IPI Working Group: An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): A meta-analysis of individual patient data. Lancet Oncol 17:779-790, 2016

86. Jeromin S, Weissmann S, Haferlach C, et al: SF3B1 mutations correlated to cytogenetics and mutations in NOTCH1, FBXW7, MYD88, XPO1 and TP53 in 1160 untreated CLL patients. Leukemia 28: 108-117, 2014

87. Mansouri L, Cahill N, Gunnarsson R, et al: NOTCH1 and SF3B1 mutations can be added to the hierarchical prognostic classification in chronic lymphocytic leukemia. Leukemia 27:512-514, 2013

88. Cortese D, Sutton LA, Cahill N, et al: On the way towards a "CLL prognostic index": Focus on TP53, BIRC3, SF3B1, NOTCH1 and MYD88 in a population-based cohort. Leukemia 28:710-713, 2014

89. Dreger P, Schnaiter A, Zenz T, et al: TP53, SF3B1, and NOTCH1 mutations and outcome of allotransplantation for chronic lymphocytic leukemia: Six-year follow-up of the GCLLSG CLL3X trial. Blood 121:3284-3288, 2013

90. Rossi D, Rasi S, Fabbri G, et al: Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. Blood 119:521-529, 2012

91. Pozzo F, Bittolo T, Arruga F, et al: NOTCH1 mutations associate with low CD20 level in chronic lymphocytic leukemia: Evidence for a NOTCH1 mutation-driven epigenetic dysregulation. Leukemia 30:182-189, 2016

92. Austen B, Powell JE, Alvi A, et al: Mutations in the ATM gene lead to impaired overall and treatment-free survival that is independent of IGVH mutation status in patients with B-CLL. Blood 106:3175-3182, 2005

93. Skowronska A, Parker A, Ahmed G, et al: Biallelic ATM inactivation significantly reduces survival in patients treated on the United Kingdom

Leukemia Research Fund Chronic Lymphocytic Leukemia 4 trial. J Clin Oncol 30:4524-4532, 2012

94. Herling CD, Klaumünzer M, Rocha CK, et al: Complex karyotypes and KRAS and POT1 mutations impact outcome in CLL after chlorambucil-based chemotherapy or chemoimmunotherapy. Blood 128:395-404, 2016

95. Kämpjärvi K, Järvinen TM, Heikkinen T, et al: Somatic MED12 mutations are associated with poor prognosis markers in chronic lymphocytic leukemia. Oncotarget 6:1884-1888, 2015

96. Parker H, Rose-Zerilli MJ, Larrayoz M, et al: Genomic disruption of the histone methyltransferase SETD2 in chronic lymphocytic leukaemia. Leukemia 30:2179-2186, 2016

97. Papaemmanuil E, Gerstung M, Bullinger L, et al: Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med 374:2209-2221, 2016

98. Pospisilova S, Gonzalez D, Malcikova J, et al: ERIC recommendations on TP53 mutation analysis in chronic lymphocytic leukemia. Leukemia 26: 1458-1461, 2012

99. Hallek M: Chronic lymphocytic leukemia: 2015 Update on diagnosis, risk stratification, and treatment. Am J Hematol 90:446-460, 2015

100. Dreger P, Corradini P, Kimby E, et al: Indications for allogeneic stem cell transplantation in chronic lymphocytic leukemia: the EBMT transplant consensus. Leukemia 21:12-17, 2007

101. Stilgenbauer S, Eichhorst B, Schetelig J, et al: Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: A multicentre, open-label, phase 2 study. Lancet Oncol 17:768-778, 2016

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Clinical Implications of Novel Genomic Discoveries in Chronic Lymphocytic Leukemia

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