Predicting Prognosis in Chronic Lymphocytic Leukemia in the Contemporary Era

Chadi Nabhan, MD; Gordana Raca, MD, PhD; Y. Lynn Wang, MD, PhD

**IMPORTANCE** Next-generation sequencing has identified new genetic markers that have altered prognosis for patients with chronic lymphocytic leukemia (CLL) at diagnosis. Understanding the significance of these prognostic indicators and recognizing their potential impact on treatment selection and patients' outcomes is critical for clinicians and investigators.

**OBJECTIVE** To review novel prognostic factors at CLL diagnosis that have shown an impact on the prognosis and outcomes of this disease.

**EVIDENCE REVIEW** Literature from January 2004 through December 2014 was searched in PubMed, Cochrane Central Register of Controlled Trials, and Scopus to identify English-language, peer-reviewed articles on clinical and prognostic factors for CLL (TP53, ATM, NOTCH1, SF3B1, BIRC3, and MYD88). Reference lists were subsequently reviewed for additional articles. A total of 450 articles was identified, and 48 articles meeting inclusion criteria were reviewed.

**FINDINGS** Among prognostic markers reviewed, chromosomal aberrations have been validated and are currently used clinically to predict prognosis. Patients with 17p13.1 deletion have poor response to chemoimmunotherapy and are treated differently, with some undergoing allogeneic transplantation in first remission. CD38 and ZAP-70 status of malignant cells and unmutated immunoglobulin variable heavy chain gene have similarly been validated to predict adverse prognosis, but their implications on treatment selection have not been proven. The presence of TP53 and ATM mutations predicts worse prognosis, which has been corroborated in various studies. Patients with TP53 mutations have lower responses to chemoimmunotherapy. Furthermore, patients with TP53 and ATM mutations have inferior progression-free survival and overall survival, independent of other factors. Patients carrying the NOTCH1 and SF3B1 mutations have worse prognosis; patients with the NOTCH1 mutation have lower response rates to standard chemoimmunotherapy. The impact of BIRC3 on prognosis and survival requires further confirmation.

**CONCLUSIONS AND RELEVANCE** The heterogeneous clinical course of CLL is likely explained by underlying molecular prognostic factors. Moving forward, analyzing these factors at diagnosis is recommended for better prognostication.
Chronic lymphocytic leukemia (CLL) is diagnosed in more than 15,000 cases annually, contributing to 5000 deaths yearly. Notable advances in molecular understanding of CLL have led to better prognostic risk stratification and have largely explained the heterogeneous clinical course of this disease. In this review, we discuss the various prognostic parameters in CLL, focusing on cytogenetics, cellular-based factors, and gene mutations. We comprehensively analyze these factors in light of the historical as well as modern approach to CLL therapy. Furthermore, we provide a framework for the future of CLL therapeutic investigations, especially as the newly approved B-cell receptor (BCR) pathway targeted agents have become widely used.

Data Sources and Literature Review

PubMed, Cochrane Central Register of Controlled Trials, and Scopus were searched to find literature on clinical and prognostic factors for chronic lymphocytic leukemia (Table). Searches were limited to literature published from January 2004 through December 2014. English language limits were applied. The search used subject heading and keyword variations based on the following concepts: CLL, chronic lymphocytic leukemia AND NOTCH1, SF3B1, ATM, TP53, BIRC3, MYD88 AND progression-free survival, disease-free survival, and survival. Additional references were identified by searching relevant articles’ reference lists. The results of the searches were combined, then deduplicated, and the final number of articles that met the selection criteria was 48 out of 450 identified articles.

Historical Background

Despite recent advances in treating CLL, watchful waiting remains the recommended approach for asymptomatic patients. When treatment is indicated and initiated, chemiomunotherapy combinations are the preferred front-line treatment. The activity noted with the fludarabine, cyclophosphamide, and rituximab (FCR) regimen led to the conduct of a prospective phase 3 randomized clinical trial comparing FCR to fludarabine and cyclophosphamide (FC) and demonstrating that FCR had better responses and superior survival. Another phase 3 study, designed for frail patients with comorbidities, randomized 781 patients to chlorambucil alone or chlorambucil plus either rituximab or obinutuzumab, a glycoengineered type 2 anti-CD20 antibody. The combination of chlorambucil with either antibody improved response rates and prolonged progression-free survival (PFS). Furthermore, the combination of chlorambucil and obinutuzumab improved overall survival (OS) compared with chlorambucil alone (hazard ratio [HR], 0.41; P < .001). These studies, as well as a recent evidence-based comprehensive review and systematic analysis, provided additional evidence that chemiomunotherapy is the logical front-line therapy for patients with CLL (CLL patients).

Risk Stratification

Over the years, clinicians have used traditional prognostic factors that are easily obtained using staging, serum testing, and other accessible parameters to predict progression risk of CLL and disease-related mortality. As newer prognostic factors became available, better ability to predict responses to therapy, duration of responses, time to first treatment, OS, and selections of therapy ensued. Understanding these prognostic indicators allows for better design of future clinical trials that are tailored to the basic mechanism of the disease.

Chromosomal Aberrations

In addition to normal cytogenetics, recurrent chromosomal abnormalities in CLL include 17p deletion, 11q deletion, trisomy 12, and 13q deletion (Figure 1). Over a decade ago, Döhner et al reported a pivotal work showing that 82% of CLL patients carry at least 1 genomic abnormality when fluorescence in situ hybridization (FISH) testing was used. Furthermore, they demonstrated that patients with the 17p13.1 or 11q22.3 deletions have the worst prognosis, with a median survival of 32 and 79 months, respectively, whereas patients with a normal cytogenetic status and those who carry the 13q deletion have a median survival of 111 and 133 months, respectively. This investigation and others led to the development of the FISH-based hierarchical prognostic model based on the presence of 17p deletion, 11q deletion, trisomy 12, normal cytogenetics, and 13q deletion. The prognostic significance in the 17p13.1 deletion is related to the TP53 gene that is mapped to this chromosomal location. The 17p13.1 deletion can occur with or without TP53 mutations on the second allele and TP53 mutations can also be present without deletions.

Both deletions and mutations lead to impaired TP53 functions. In patients with 17p deletion and TP53 mutations of the second allele, p53 function is completely lost. In addition to atypical immunophenotypes (where some patients have brighter expression of CD20 and surface immunoglobulin) that have been occasionally observed in these patients, other adverse prognostic factors, such as high expressions of CD38, unmutated immunoglobulin heavy chain gene (IGHV), and zeta-associated protein-70 (ZAP-70) expression have been seen in higher frequency in patients with 17p deletions. It is important to note that 17p13.1 occurs in less than 10% of CLL patients but carries the worst prognosis as it has been observed in 30% to 50% of patients who develop resistance and refractoriness to chemotherapy. Patients who carry this deletion have historically had inferior response rates, PFS, and OS. However, in later investigations, Tam et al showed that there is a clinical heterogeneity in patients with 17p deletions in which those who acquire the
### Table. Prognostic Factors at the Time of Diagnosis in Patients With CLL

<table>
<thead>
<tr>
<th>Category</th>
<th>Prognostic Factor</th>
<th>Description</th>
<th>Biologic Function</th>
<th>Prognostic Significance</th>
<th>Inclusion in Guidelines Recommendations</th>
<th>Evidence for Prognostic Value</th>
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</thead>
<tbody>
<tr>
<td><strong>Select clinical and laboratory parameters</strong></td>
<td>Rai stage</td>
<td>Uses the presence or absence of lymphadenopathy, organomegaly (spleen and liver), and cytopenias</td>
<td>Separates patients into groups with different anticipated survival times based on disease burden</td>
<td>Higher Rai stage predicts shorter survival</td>
<td>Yes (NCCN, IWCLL, ESMO)</td>
<td>Strong</td>
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<td></td>
<td>Lymphocyte doubling time</td>
<td>Measures the number of months it takes the absolute lymphocyte count to double</td>
<td>Estimates the pace of disease</td>
<td>Short doubling time (&lt;12 mo) predicts higher risk</td>
<td>Yes (NCCN, IWCLL, ESMO)</td>
<td>Strong</td>
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<td></td>
<td>β-2 microglobulin and LDH</td>
<td>Measures serum β-2 microglobulin and LDH</td>
<td>Estimates tumor burden</td>
<td>Increasing levels indicate a poorer prognosis</td>
<td>Yes (NCCN, ESMO)</td>
<td>Strong</td>
</tr>
<tr>
<td><strong>Immunoglobulin heavy chain gene mutational status</strong></td>
<td>IGHV</td>
<td>Mutation status of the immunoglobulin gene variable region</td>
<td>Determines the origin from a pregerminal (unmutated IGHV) or postgerminal (mutated IGHV) center B-cell</td>
<td>Unmutated IGHV indicates shorter survival and a higher risk of relapse</td>
<td>Yes (NCCN, IWCLL, ESMO)</td>
<td>Strong</td>
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<td></td>
<td>Flow cytometry markers</td>
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<td></td>
<td>CD38</td>
<td>Transmembrane glycoprotein</td>
<td>Possible role in apoptosis</td>
<td>CD38 expression (&gt;30%) predicts inferior survival</td>
<td>Yes (NCCN, IWCLL, ESMO)</td>
<td>Strong</td>
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<tr>
<td></td>
<td>ZAP-70</td>
<td>Zeta chain associated protein 70</td>
<td>A tyrosine kinase expressed by NK and T cells and not expressed by B cells</td>
<td>&gt;20% ZAP70-positive cells indicate higher risk of progression, short time from diagnosis to treatment, and poorer survival</td>
<td>Yes (NCCN, IWCLL, ESMO)</td>
<td>Strong</td>
</tr>
<tr>
<td><strong>FISH abnormalities</strong></td>
<td>17p</td>
<td>Deletion of the q13 region of the short arm of chromosome 17</td>
<td>Results in a loss of 1 copy of the TP53 gene</td>
<td>Associated with inferior response, shorter time to progression, and worse survival when chemotherapy is used</td>
<td>Yes (NCCN, IWCLL, ESMO)</td>
<td>Strong</td>
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<tr>
<td></td>
<td>11q</td>
<td>Deletion of the q22 region of the long arm of chromosome 11</td>
<td>Results in a loss of 1 copy of the ATM gene</td>
<td>Indicates high risk</td>
<td>Yes (NCCN, IWCLL, ESMO)</td>
<td>Strong</td>
</tr>
<tr>
<td></td>
<td>Trisomy 12</td>
<td>Gain of chromosome 12</td>
<td>Molecular consequences of trisomy 12 unknown</td>
<td>Indicates intermediate risk unless NOTCH1 mutation is present</td>
<td>Yes (NCCN, IWCLL)</td>
<td>Strong</td>
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<td></td>
<td>13q</td>
<td>Deletion of the q14 region of the long arm of chromosome 13 and loss of chromosome 13</td>
<td>Results in a loss of miR-15a/16-1 micro RNA cluster: miR-15a and miR-16-1 regulate cell cycle genes and control cell proliferation</td>
<td>Indicates low risk</td>
<td>Yes (NCCN, IWCLL)</td>
<td>Strong</td>
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<tr>
<td></td>
<td>6q</td>
<td>Deletion of the q2 region of the long arm of chromosome 6</td>
<td>Molecular consequences of the 6q deletion are unknown</td>
<td>Indicates intermediate risk</td>
<td>IWCLL only</td>
<td>Weak</td>
</tr>
<tr>
<td><strong>Gene mutations</strong></td>
<td>TP53</td>
<td>Tumor protein p53</td>
<td>Tumor suppressor protein, mediates cell cycle arrest, apoptosis, senescence and DNA repair</td>
<td>TP53 mutations are independent predictors of poor survival when standard therapy is used</td>
<td>Yes (NCCN, ESMO)</td>
<td>Strong</td>
</tr>
<tr>
<td></td>
<td>ATM</td>
<td>Ataxia telangiectasia mutated</td>
<td>Member of the PI3/PI4-kinase family, important cell cycle checkpoint kinase</td>
<td>ATM mutations indicate high risk for progression, in particular when associated with 11q loss</td>
<td>No</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>BIRC3</td>
<td>Baculoviral IAP repeat containing 3</td>
<td>A member of the IAP family of proteins that inhibits apoptosis</td>
<td>BIRC3 disruption indicates poor outcome</td>
<td>No</td>
<td>Weak</td>
</tr>
<tr>
<td></td>
<td>NOTCH1</td>
<td>Notch homologues</td>
<td>A ligand-activated transmembrane protein that regulates downstream pathways important for cell growth</td>
<td>NOTCH1 mutations are associated with adverse prognosis</td>
<td>No</td>
<td>Strong</td>
</tr>
<tr>
<td></td>
<td>SF3B1</td>
<td>Splicing factor 3b, subunit 1</td>
<td>Role in mRNA splicing</td>
<td>SF3B1 mutations are associated with adverse prognosis</td>
<td>No</td>
<td>Strong</td>
</tr>
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<td></td>
<td>MYD88</td>
<td>Myeloid differentiation primary response 88</td>
<td>Cytosolic adapter protein with a role in the innate and adaptive immune response</td>
<td>MYD88 mutations are associated with favorable outcome</td>
<td>No</td>
<td>Intermediate</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>XPO1</td>
<td>Exportin 1</td>
<td>Implicated in nuclear export signal-dependent protein transport</td>
<td>XPO1 mutations may be associated with high risk of progression</td>
<td>No</td>
<td>Weak</td>
</tr>
<tr>
<td></td>
<td>Genetic complexity</td>
<td>Multiple chromosomal abnormalities by karyotyping or array analysis, or subclonal driver mutations by molecular testing</td>
<td>Indicators of genomic instability and clonal evolution</td>
<td>Associated with adverse prognosis</td>
<td>No</td>
<td>Intermediate</td>
</tr>
</tbody>
</table>

Abbreviations: CLL, chronic lymphocytic leukemia; ESMO, European Society of Clinical Oncology; FISH, fluorescence in situ hybridization; IGHV, immunoglobulin heavy chain gene; IWCLL, International Workshop on chronic lymphocytic leukemia; LDH, lactate dehydrogenase; mRNA, messenger RNA; NCCN, National Comprehensive Cancer Network; NF-kB, nuclear factor-kB; TP53, tumor protein p53; ZAP-70, zeta-associated protein-70.

Summary of recommendations by the National Comprehensive Cancer Network, version 4.2014. International Workshop on Chronic Lymphocytic Leukemia, and European Society of Clinical Oncology. Binet staging system is used in Europe. Recommended only in the context of well-designed clinical trials but not in general practice.
Figure 1. Array Findings in a Case of High-Risk Chronic Lymphocytic Leukemia (CLL)

A. Screenshot from the array analysis software, summarizing all detected abnormalities. Blue bars indicate copy number gains (duplications), and red bars indicate copy number losses (deletions). Duplications were detected on chromosomes 4, 7, and 9, and deletions were detected on chromosomes 7 and 17. None of the abnormalities were detected by karyotyping owing to low propensity of CLL cells to grow in culture, or by the fluorescence in situ hybridization (FISH) panel owing to localization of the abnormalities outside of the regions targeted by the FISH probes. B. The complexity of the copy number abnormalities detected on chromosome 7. The “copy number state (segments)” plot depicts chromosomal segments with a copy number gain (blue) and copy number loss (red). The “weighted Log2 ratio” plot represents per marker log2 ratio of normalized signal intensity for the leukemia sample with respect to a reference. The “allele peaks” plot represents allelic ratio values for individual single nucleotide polymorphism (SNP)-genotyping markers, allowing to depict genotypes (AA, AB or BB) for SNP sites along the length of the chromosome. The “smooth signal” plot depicts calibrated copy number estimate for different regions of the chromosome. C. Enlarged view of the deletion (red bar) on chromosome 17, which involves the 5′ region of the TP53 gene resulting in its disruption. The deletion was not detected by the TP53 FISH probe because it only partially overlaps with the probe target region.
17p deletion as part of their clonal evolution during therapy fare an inferior survival when compared with those who carry a 17p deletion at the time of diagnosis.

The other adverse genomic aberration involves deletions in the long arm of chromosome 11, which occur in up to 20% of CLL patients at diagnosis. These patients often present with bulky nodes and demonstrate other poor prognostic features. The prognostic significance of this deletion stems mainly from mutations in the ATM gene, located on the long arm of chromosome 11. While original studies suggested short time to first treatment, shorter remission duration, and poorer survival in these patients, recent chemoinmunotherapy programs have proven to overcome these adverse features.

Contrary to 17p and 11q deletions, the presence of trisomy 12 in CLL at initial diagnosis has traditionally carried intermediate prognostic significance. Landau et al proposed that trisomy 12 occurs early in the course of CLL, facilitating subsequent clonal aberrations. Further studies demonstrated that patients with trisomy 12 can acquire trisomy 19 and that these patients carry other adverse prognostic features. Similarly, but to a better extent, the presence of 13q deletion carries a favorable prognosis and is the most common genomic abnormality detected in CLL, seen in 50% of cases. Biallelic loss in 13q has been observed in 30% of CLL patients with 13q deletion, and this finding has been of controversial significance. Because microRNAs miR-15a and miR-16-1 are located within the commonly deleted region on chromosome 13, their deletions contribute to the pathogenesis of this subtype of CLL. Both of these microRNAs have been shown to accelerate the proliferation of human and mouse B cells by modulating the expression of genes controlling cell-cycle progression; their deletion might partially explain the good prognosis associated with 13q deletion. Moreover, investigations have demonstrated that miR-15a and miR-16-1 expression is inversely correlated with BCL-2 expression in CLL and that both microRNAs negatively regulate BCL-2 at a posttranscriptional level. The clinical significance of this observation remains uncertain.

While it is often reported as part of the FISH-CLL panel when this test is ordered, the impact of treatment selection when detecting trisomy 12 or 13q deletion abnormalities in CLL patients is uncertain.

**Mutational Status of the IGHV Gene**
As a B-cell malignant neoplasm, CLL can evolve either from pregerminal or postgerminal B cells. This divides CLL into 2 subsets: a disease whose cells have successfully passed through the germinal center, resulting in a mutated phenotype, and another subset that originates from B cells with the unmutated or germline sequence. Ghia et al reported on guidelines to analyze the IGHV rearrangement citing that sequences that are more than 2% nonhomologous to germline are considered to have undergone somatic hypermutation. While the molecular basis for this differential outcome in CLL based on IGHV mutational status remains debatable, investigators have recently proposed that the unmutated CLL overexpresses proteins associated with transcriptional and translational activity. Furthermore, when unmutated, CLL cells tend to be more adhesive and less migratory, which explains the higher prevalence of adenopathy and tumor burden in this subset. This biologic difference has translated into differential outcomes in which OS in unmutated CLL was inferior irrespective of other variables. In fact, median survival was 95 months in patients with unmutated CLL compared with 293 months in those with mutated CLL (P < .001). On a practical level, sequencing the IGHV is labor intensive, which led to research efforts to identify surrogates for the mutational status, specifically CD38 and ZAP-70.

**CD38 Expression**
While the function of CD38, a transmembrane glycoprotein that is expressed on normal B cells, continues to be investigated, some have suggested that this protein plays an important role in apoptosis. Furthermore, CD38 promotes survival and proliferation of B cells on their way to and after neoplastic transformation. Most agree that a greater than 30% proportion of CD38-expressing CLL cells portends an unfavorable prognosis because this threshold has predicted inferior response rates, shorter time from diagnosis to therapy, and worse OS. However, CD38 expression can change over time, challenging the prognostic significance of this antigen and arguing that it should be used as a complement to other prognostic features and not in isolation.

**ZAP-70 Expression**
Gene expression profiling identified ZAP-70, a cytoplasmic tyrosine kinase with distinct role in B-CLL pathobiology. While there has been near-universal agreement on the prognostic significance of ZAP-70, little consensus exists on the best method of detection and whether the test is reproducible when performed at various laboratories. Crespo et al studied ZAP-70 expression in CLL cells from 56 patients using immunohistochemical analysis, Western blotting, and flow cytometry. These investigators demonstrated that when more than 20% of the cells are ZAP-70-positive, patients have higher risk of disease progression and lower survival rates compared with those with less than 20% ZAP-70-positive cells. Furthermore, Rassenti et al established that patients with more than 20% ZAP-70-positive cells have shorter time from diagnosis to treatment, suggesting that ZAP-70 might be more indicative of worse prognosis compared with the mutational status of the IGHV. Despite these results, a multivariable Cox regression model performed in 1948 CLL patients treated prospectively in phase 3 studies did not identify ZAP-70 as an independent predictor of survival, whereas IGHV was identified as such.

**Gene Mutations**
**TP53**
Rossi et al performed DNA sequencing of TP53 exons 2 to 10 and FISH analysis for 17p in 309 patients with initial CLL diagnosis. In total, 14% of patients had TP53 disruption by either a mutation and/or deletion, and of these, 22% demonstrated a TP53 mutation without the presence of 17p deletion by FISH. Next-generation sequencing proved that low levels of subclonal mutations in TP53 were present in 9% of CLL at diagnosis and in a higher percentage of relapsing patients. Patterns of TP53 mutations include missense mutations, present in 74% of cases compared with small deletions and insertions (20%), and nonsense mutations (4%), with most mutations located within the DNA binding domain encoded by exons 5 to 8. In a prospective randomized clinical trial comparing FC with chlorambucil or fludarabine, mutations of TP53 were noted in 8.5% of patients, including 87.5% who carried the 17p deletion, and con-
ferred a poor prognosis independent of other adverse features. Several studies showed that TP53 mutations represent an independent predictor of survival in a multivariate analysis and that traditional chemoimmunotherapy programs provide unsustainable outcomes. To that end, exploring the presence of the TP53 mutation in a patient newly diagnosed as having CLL is recommended because it affects selection of therapy. Agents that do not require an intact p53 pathway are usually recommended in this setting.  

**ATM and BIRC3**

Patients with 11q deletion could have a mutation in the ATM or BIRC3 genes. ATM mutations occur in CLL regardless of the presence of 11q deletions. In an analysis of 155 CLL samples, 12% carried the ATM mutations, 4% had the TP53 mutations, and 2% contained mutations in both genes. Further analysis demonstrated that the ATM mutations were usually present at diagnosis. To assess the association between 11q deletion and ATM mutations, the same group sequenced the residual ATM allele in 72 CLL patients who had an 11q deletion. This investigation revealed that the residual ATM allele was mutated in 36% of cases. The inactivation of the second ATM allele was associated with a reduction in patients’ survival beyond that already dictated by the presence of an 11q deletion. Conceptually, this suggested that CLL with 11q deletion could be divided into two subgroups based on the integrity of the residual ATM allele, with patients with complete loss of ATM function, owing to biallelic ATM disruption, having the worst responses to chemotherapeutics and a poorer clinical outcome.

The BIRC3 gene is a negative regulator of alternative NF-kB signaling pathway. Its mutations or deletions, noted in less than 5% of cases, lead to the activation of alternative NF-kB pathway. Because both the ATM and the BIRC3 genes are located on 11q, their interrelation is critical to understand their impact on CLL pathobiology and prognosis. A cohort of 166 patients enriched for 11q deletions were screened for the presence of ATM and BIRC3 deletions and/or mutations. BIRC3 deletion occurred in 83% of 11q-deleted cases and always coexisted with the ATM deletion. Furthermore, 40% of the BIRC3-deleted cases had concomitant deletions and mutations of ATM. BIRC3 disruption identified patients with a poor outcome and exerted a prognostic role that was independent of the commonly described genetic risk factors. In patients with 11q deletion, the ATM mutation was associated with a reduced OS and PFS comparable with that seen with patients with TP53 abnormalities, whereas BIRC3 deletion and/or mutation had no impact on OS and PFS. This investigation might suggest that the ATM is more important prognostically than the BIRC3, but additional studies are needed for further clarification. Of note, in routine clinical practice, testing for the BIRC3 and/or ATM mutation is not currently recommended.

**NOTCH1**

Chronic lymphocytic leukemia cells constitutively express NOTCH1 receptors and their ligands, leading to resisting apoptosis. Approximately, 4% to 10% of newly diagnosed patients and 30% of CLL patients whose disease was relapsed and/or refractory carry the NOTCH1 mutations. NOTCH1 mutations have also been observed in 25% to 30% of patients who carried the trisomy 12 aberrations, negating the intermediate prognosis of this cytogenetic abnormality. High frequency of NOTCH1 mutations is associated with the unmutated IGHV variant of the disease that may contribute to early progression, chemotherapy refractoriness, and possible transformation. Furthermore, patients with mutated NOTCH1 have shorter survival irrespective of other adverse prognostic factors. The prognostic significance of the NOTCH1 sustains even when chemoimmunotherapy is administered because there is resistance to anti-CD20 antibodies when this mutation exists. A post hoc analysis of the CLL-8 study comparing FCR with FC demonstrated that NOTCH1 mutational status predicted failure of rituximab to improve response rates and survival in patients with NOTCH1. This might be partly explained by a recent investigation that showed that the presence of NOTCH1 mutation in CLL patients correlated with having lower levels of CD20 expression. This might, in part, explain the witnessed resistance to traditional therapies in patients with the NOTCH1 mutation. To date, routine testing for NOTCH1 mutation is not recommended but is often performed in clinical trials.

**SF3B1**

In an attempt to better identify the molecular landscape of CLL, DNA samples from 91 CLL patients were obtained. Parallel sequencing of 88 whole exomes and whole genomes, together with sequencing of matched germline DNA, was performed to characterize the spectrum of somatic mutations in this disease. SF3B1 was identified as the second most frequently mutated gene (15%), affecting primarily in tumors with 11q deletions. Others have suggested that the SF3B1 is somatically mutated in 9.7% of affected individuals. When CLL patients who showed the SF3B1 mutations were evaluated, they had lower responses to chemoimmunotherapy, faster disease progression, and poor OS. Routine testing for SF3B1 outside of clinical trials is not yet recommended.

**Clinical Implications**

To assess prognosis at diagnosis in CLL, testing the peripheral blood for abnormal cytogenetics using FISH is recommended. Patients with a 17p13.1 deletion and/or TP53 mutation are often treated differently by being offered participation in clinical trials or by using BCR-pathway inhibitors or other agents that exert their activity in a p53-independent manner. Thus, analyzing cytogenetics for CLL patients at diagnosis is recommended. Most clinical laboratories will report on ZAP-70 and CD38 status, but their implication on therapeutic selection is not confirmed and has not been validated. Testing the mutational status of the IGHV, while informative prognostically, is not routinely performed because it is labor intensive and is not available in all clinical settings.

The newly identified gene mutations and next-generation sequencing further refined the clinical heterogeneity and prognosis of CLL. Researchers evaluated 163 patients enrolled in either a CLL protocol for young patients receiving FC or fludarabine plus alemtuzumab or a protocol for elderly patients receiving chlorambucil plus rituximab. Interestingly, NOTCH1 and SF3B1 mutations did not have an impact on response rates, whereas patients with TP53 and BIRC3 mutations had inferior responses despite receiving similar therapies. Furthermore, young patients carrying the BIRC3 mutation had inferior PFS, which was not influenced by the other mutations. Stilgenbauer et al showed that TP53, NOTCH1, and SF3B1 were mut-
Figure 2. A Proposed Diagram on the Future of Testing and Treating Chronic Lymphocytic Leukemia (CLL)

A large international effort to better refine the prognosis of CLL analyzed 23 prognostic markers in 1948 CLL patients who were prospectively treated. A multivariable Cox regression model identified 8 independent predictors of OS: sex, age, performance status, 17p deletion, 11q deletion, IGHV mutation status, serum β2-microglobulin, and serum thymidine kinase. Using a weighted grading system, a prognostic index was derived that separated 4 risk categories with 5-year survival rates ranging from 18.7% to 95.2%. The validity of the index was externally confirmed in a series of 676 patients newly diagnosed as having CLL. Subsequently, a comprehensive prognostic index with high discriminatory power and prognostic significance on the individual patient level was developed and is ready to use in ongoing and newly designed clinical trials.

While these new prognostic factors are starting to have an impact on therapeutic selection, current guidelines do not recommend using these factors to select therapy except in cases in which patients carry 17p deletion or TP53 mutation. Recognizing that these newer tests are not universally available to the practicing oncologist, a practical and simplified algorithm will soon be needed. A proposed future approach is presented in Figure 2.

Emerging Therapies

The recognition of the integral role that BCR signaling plays in the pathogenesis of CLL has led to the development of various targeted therapies that inhibit downstream proteins implicated in the continued cellular proliferation and prevention of apoptosis. While 2 therapeutic agents targeting different survival pathways in the BCR activation cascade have been approved for treating CLL, namely, ibrutinib and idelalisib, several others are in various phases of development. Discussing all agents in development for CLL is beyond the scope of this review. We limit our discussion to ibrutinib, idelisib, and ABT-199 because these 3 agents have shown activity in high-risk CLL.
**Ibrutinib**

Ibrutinib is the first bruton tyrosine kinase (BTK) inhibitor to demonstrate activity in CLL, by irreversibly binding and inactivating the BTK through forming a covalent bond with a cysteine residue (C481) and leading to inhibition of cellular proliferation and induction of apoptosis. In the pivotal study that led to ibrutinib’s approval, Byrd et al treated 85 relapsed CLL patients with 2 different daily dosing schedules of ibrutinib (420 mg or 840 mg), showing a 26-month OS rate of 83% and PFS rate of 75%. These results were solidified when a prospective randomized clinical study showing that ibrutinib was superior to ofatumumab in relapsed CLL in all primary and secondary end points. In the treatment-naive setting, O’Brien et al reported on elderly CLL patients 65 years or older who received ibrutinib, 420 mg, until progression. The overall response rate was 71% with an OS rate of 96% at the 2-year follow-up. Notably, responses to ibrutinib did not vary based on cytogenetic risk profile, in which 68% of the 28 patients with 17p deletion responded. In contrast, and despite similar response rates, PFS was shorter in this high-risk cohort, implying that these responses might not be durable in high-risk disease. To better evaluate the efficacy of ibrutinib in CLL patients with 17p deletion, a large phase 2 study that exclusively enrolled 144 CLL patients with relapsed and/or refractory disease with 17p deletion reported an overall response rate of 82% and a median PFS that was not reached at a median follow-up of 13 months. These results were similar to previously reported outcomes in this subset of patients when treated with either FCR or alemtuzumab. An updated analysis confirmed that responses to ibrutinib are sustained even in patients with 17p deletion. In fact, median duration of response has not been reached for all patients, and it was 25 months for those with 17p deletion. Furthermore, a 3-year follow-up confirmed safety and that most initially observed adverse events resolve. These responses led to the US Food and Drug Administration approval of ibrutinib as a front-line therapy for symptomatic CLL patients who carry the 17p deletion.

**Idelalisib**

As a reversible inhibitor of PI3Kδ, idelalisib therapy leads to inhibition of cellular proliferation. After a phase 1 study showed an overall response rate in 72% of relapsed and/or refractory patients, a multicenter, randomized, double-blind, placebo-controlled phase 3 study was conducted comparing idelalisib plus rituximab to rituximab plus placebo in relapsed disease. The 220 enrolled patients possessed comorbidities, such as reduced renal function, major coexisting illnesses, or myelosuppression due to previous therapies. This study was closed prematurely after interim analysis demonstrated superiority in PFS in the idelalisib group (5 months vs not reached; \( P < .001 \)). Furthermore, OS was superior in the idelalisib treatment arm at 12 months (92% vs 80%; \( P = .02 \)). Responses were similar in high- vs low-risk disease, including patients with 17p deletion.

**ABT-199**

ABT-199 is a small, orally administered molecule that inhibits the BCL-2 proto-oncogene leading to promoting apoptosis and cell kill. ABT-199 is a modified version of the earlier BCL-2 targeted agent (ABT-263), which was associated with thrombocytopenia as a dose-limiting toxicity.

Seymour et al presented data on 84 patients with relapsed CLL (of whom 23 [27%] had 17p deletion) who were treated with single-agent ABT-199 in a dose-escalated fashion. The overall response rate was 79% with a median duration of response reaching 20.5 months. Because 91% of patients in complete remission were progression-free at 12 months, this novel agent might offer a difficult and traditionally refractory patient population a viable effective option. Interestingly, the overall response rate for patients with 17p deletion was 78%. Preliminary studies support that ABT-199 can be safely combined with rituximab or obinutuzumab.

**Conclusions**

With broader implementation of next-generation, sequencing-based testing in routine diagnostics, mutation profiling of a plethora of CLL-associated genes will be incorporated in standard risk stratification and therapeutic response prediction. Despite the improved understanding of the genomic landscape of CLL, critical clinically relevant questions remain unanswered and are the subject of rigorous clinical trials. Whether early therapy in asymptomatic but high-risk patients is beneficial is unknown. Historical trials that have failed to show such advantage were conducted before the availability of newer therapies that have proven efficacy in high-risk disease, such as ibrutinib, idelalisib, and ABT-199. Furthermore, how to treat CLL patients with refractory disease or those who attain short response duration is unknown. Finally, and because CLL is a disease of elderly individuals, optimizing treatment regimens for this patient population is critical, especially in elderly fit patients who have high-risk disease and are likely to die of their CLL. While several years separate us from answering these questions, the advances noted for CLL in the past decade are unprecedented and are bringing us a step closer to a possible cure.
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