



Recent advances in chronic lymphocytic leukemia therapy

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Abstract

Chronic lymphocytic leukemia is a genetically heterogeneous disease, and a complex set of genetic alterations is associated with its pathogenesis. CLL is the most common leukemia in the western countries, whereas it is rare in Asia, including Korea. The prognostic models integrate the traditional staging systems developed by Rai *et al.* and Binet *et al.* with biochemical and genetic markers. With the advent of molecular biology, a variety of targeted agents, including anti-CD20 antibodies, inhibitors of BCR signaling pathway, and BCL-2 inhibitors, have been introduced, which has changed the landscape of CLL treatment greatly. This review will focus on the risk stratification and the management of CLL in the era of novel small molecules.

Key Words Chronic lymphocytic leukemia, Prognostic models, Immunochemotherapy TP53

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is clinically a heterogeneous disease characterized by the clonal proliferation and accumulation of mature B-lymphocytes in the blood, bone marrow, lymph nodes, and spleen, proceeded by monoclonal B-cell lymphocytosis virtually in all cases of CLL [1-3]. CLL is the most common leukemia in the United States with approximately 15,000 new cases per year [4]. However, it is rare in Asia, including Korea, with 0.18 per 100,000 person-years of age-standardized incidence. Although the annual incidence has almost doubled over a decade, about 160 new cases were diagnosed in 2012 [5].

The traditional staging systems developed by Rai *et al.* [6] and Binet *et al.* [7] assess the extent of the disease in an individual patient and allow a treatment decision to be taken. However, more than half of patients with newly diagnosed CLL present with early stage of the disease [8]. Moreover, it is difficult to predict the heterogeneity of the CLL disease course using traditional staging systems, with some patients experiencing rapid progression of the disease despite maximal therapy, and others following a more indolent course that even requires no treatment [9-11].

The advancement in knowledge on the genetic and molecular biology of CLL through new technologies has identified several novel prognostic markers such as the mutation status of the variable region of immunoglobulin heavy chain (*IGHV*) genes, *TP53* mutation, del(17p), and del(11q) [12]. Many researchers have tried to integrate genetic and biological information with the clinical staging systems for more individualized management of CLL patients [12, 13].

With progression in the understanding of the biology of CLL, several novel agents, such as agents targeting B cell receptor (BCR) signaling and BCL-2 pathways, in addition to CD20 surface antigen, have been approved, which has considerably changed the treatment landscape of CLL for the last 2 decades, and led to improvement in clinical outcomes for patients subsequently [14].

This review will focus on the risk stratification and management of CLL in the era of novel small molecules, mainly in the setting of frontline therapy.

PATHOGENESIS AND CELL OF ORIGIN

Limitless replicative potential is one of the hallmarks of cancer [15]. As tumorigenesis is a multistep process of genetic

alterations, the precursor of malignant stem cells should have a long life span to accumulate such mutations [15, 16]. In human hematopoiesis, self-renewing hematopoietic stem cells (HSCs) can accumulate genetic alterations and produce progenitors with the same alterations, which are potential targets for additional mutations [17]. Such defective HSCs and downstream progenitors eventually transform into leukemia stem cells with self-renewal capability and without normal differentiation activity, and undergo clonal expansion [18, 19]. Recent data and evidence suggest that leukemogenesis also applies to CLL as to acute myeloid leukemia. The earliest genetic and epigenetic alterations to pluripotent HSCs eventually lead to CLL development [20].

The monoclonal B cells in CLL express CD19, CD5, and CD23, and have reduced level of membrane IgM, IgD, and CD79b, which is a phenotype of mature, activated B lymphocytes [9]. During the development of B cells, immunoglobulin variable (*V*) gene segments are rearranged, and encode an immunoglobulin molecule that engages antigen as the B cell receptor. When an antigen binds to the receptor, the cell moves into the germinal center (GC) in the lymphoid follicles and its *V* genes undergo somatic hypermutation. This process introduces mutations in the rearranged *V_HD_JH* and *V_LJ_L* gene segment. Cells with high antigen-binding affinity proliferate in the presence of the antigen, while those with no antigen bound or autoantigen bound are eliminated, which is known as affinity maturation. This stimulation and selection process usually requires the help of T lymphocytes. However, in the marginal zones around lymphoid follicles, outside GCs, this process can proceed in a T cell independent manner [21]. The proteins on the surface of B cells concomitantly change to support B cell interaction and differentiation during the process of activation. One of these surface proteins is CD38 that delivers signals to regulate the apoptosis of B cells [22, 23].

CLL with mutated immunoglobulin heavy chain variable

region (IGHV-M) gene indicates that antigen-exposed B cells undergo a T cell-dependent GC reaction and malignant B cells are derived from GC or post-GC B cells [24]. Transcriptome analyses revealed that CLLs with unmutated *IGHV* (IGHV-UM) gene are derived from unmutated mature CD5⁺ B cells (CD5⁺CD27⁺ IGHV-UM-naïve B cells), whereas CLLs with IGHV-M are from a previously unrecognized CD5⁺CD27⁺ post-GC B cell subset [25]. Although the cellular origin of CLL remains to be validated, it has been suggested that IGHV-M CLL is derived from GC-experienced B cells and IGHV-UM CLL arises from pre-GC-naïve B cells or GC-independent memory B cells [20].

The mutation status of the *IGHV* genes is an important prognostic factor of CLL. CLL with IGHV-M have a benign clinical course with longer progression free survival (PFS) and overall survival (OS) [26, 27]. Mutation rates of ≥2% difference from germline detected by comparing DNA sequences are considered mutated [28-30]. The analysis of *IGHV* mutation is laborious and not performed routinely in clinical laboratories [9]. Considering the clinical importance of the mutation status of *IGHV*, testing for the zeta-chain-associated protein kinase 70 (ZAP-70) can be considered a surrogate of mutation status of *IGHV* as a clinical test since ZAP-70 expression detected by flow-cytometric analysis is associated with *IGHV* mutational status [9, 31, 32].

The zeta-chain-associated protein kinase 70 (ZAP-70) is a crucial protein in T cell signaling [33]. It is rarely present in normal B cell as it is part of the T cell receptor [9]. When the expression of ZAP-70 is manipulated experimentally in B cells, it can facilitate signal transmission down the pathway initiated by antigen engagement with the B cell receptor [34]. About 70-80% of CLL with IGHV-UM gene express ZAP-70. ZAP-70 expression in CLL correlates with poor clinical outcomes [31, 32].

Table 1. Overview of prognostic models in CLL.

	Wierda <i>et al.</i> [35]	Rossi <i>et al.</i> [36]	CLL-IPI [13]
N of patients	930 with Rai 0-1 stage	637 (1,274 samples)	3,472
Clinical implication	TTFT	OS at 5 yr	OS at 5 yr
Clinical and biochemical markers	LDH Involved LN sites Diameter palpable LN	Age Rai stage	Age > 65 yr (1) Binet B-C or Rai I-IV (1) β2-microglobulin (3.5 mg/L) (2)
Genetic markers	Del(11q) by FISH Del(17p) by FISH IGHV unmutated	IGHV unmutated TP53/BIRC3 NOTCH1/SF3B1/del11q22-q23 Trisomy 12 or wild type Del13q14	TP53 or del(17p) (4) IGHV unmutated (2)
Risk groups	Weighted formula	High: 50.9% of OS Intermediate: 65.9% Low: 77.6% Very low: 86.9%	Very high (7-10): 23.3% High (4-6): 63.3% Intermediate (2-3): 79.3% Low (0-1): 93.0%

Abbreviations: ALC, absolute lymphocyte count; CLL-IPI, International CLL-Integral Prognostic Index; FISH, fluorescence in situ hybridization; IgA, immunoglobulin A; IGHV, immunoglobulin heavy chain variable region gene; LDH, lactate dehydrogenase; LN, lymph node; OS, overall survival; TTFT, time to first treatment.

PROGNOSTICATION OF CLL

Several scoring systems have been suggested to integrate the novel prognostic markers into the clinical staging systems to assess the aggressiveness as well as the extent and burden of the disease, and subsequently to guide more individualized therapy for CLL patients (Table 1).

A study of 930 previously untreated patients at the MD Anderson Cancer Center between 2004 and 2009 evaluated prognostic markers to predict the time to first treatment (TTFT) [35]. About 90% of patients analyzed were having early stage of the disease, Rai 0–I, and were a relatively young age group (median age of 59 yr). Univariate analysis identified traditional and new prognostic factors associated with shorter TTFT, including the following: higher absolute lymphocyte count (ALC); lower hemoglobin, platelet count, and IgA level; higher beta-2 microglobulin and lactate dehydrogenase (LDH); greater percentage of lymphocytes and number of involved lymph node sites; increased spleen, liver, and lymph node size; advanced Rai stage, presence of 11q deletion or 17p deletion using fluorescence in situ hybridization (FISH) analysis; IGHV-UM; expression of ZAP-70 using either flow cytometry or immunohistochemistry (IHC); expression of CD38 (>30%); and complex karyotype. As multivariate analysis confirmed, IGHV-UM, largest diameter of palpable lymph node, del(11q) or del(17p) using FISH, number of involved nodal sites, and LDH were included in a formula weighting the independent prognostic factors to predict TTFT [35].

Rossi *et al.* [36] included not only chromosomal abnormalities detected using FISH analysis but also single genetic abnormalities analyzed using polymerase-chain reaction (PCR) amplification and DNA sequencing of high-molecular-weight genomic DNA, and next-generation sequencing (NGS) into a prognostic model for OS from the initial diagnosis. In total, 637 newly diagnosed CLL patients and 1274 samples of peripheral blood mononuclear cells (PBMCs) were analyzed. Four CLL subgroups were classified based on the mutational and cytogenetic abnormalities: the high risk, *TP53* and/or *BIRC3* disruption (5-yr OS, 50.9%; 10-yr OS, 29.1%); the intermediate risk, *NOTCH1* and/or *SF3B1* mutations and/or del11q22-q23 in the absence of *TP53* and *BIRC3* abnormalities (5-yr OS, 65.9%; 10-yr OS, 37.1%); the low risk, +12 and wile-type for all genetic lesions (5-yr OS, 77.6%; 10-yr OS, 57.3%); the very low risk, del13q14 as the sole genetic lesion (5-yr OS, 86.9%; 10-yr OS, 69.3%). Multivariate analysis selected the genetic models as an independent risk factor of OS, along with age, Rai stage, and IGHV-UM. Survival analysis was validated by the genetic model using an independent external series of 370 newly diagnosed CLL patients [36].

The International CLL-Integral Prognostic Index (CLL-IPI) working group collected individual patient data from eight phase 3 trials to create a prognostic model combining genetic, biochemical, and clinical parameters [13]. The full analysis dataset included 3,472 treatment-naïve patients; 2,308 were

randomly segregated into the training dataset and 1,164 into the internal-validation dataset. In addition to an internal validation, two separate cohorts (838 patients from the Mayo Clinic and 416 from a Scandinavian population-based cohort) were used for external validation. The training dataset identified five independent prognostic factors: *TP53* status [no abnormalities vs. del(17p) or *TP53* mutation or both; hazard ratio (HR), 4.2; assigned score of 4], *IGHV* mutational status (mutated vs. unmutated; HR, 2.6; assigned score of 2), serum β_2 -microglobulin concentration (≤ 3.5 mg/L vs. > 3.5 mg/L; HR, 2.0; assigned score of 2), clinical stage (Binet A or Rai 0 vs. Binet B–C or Rai I–IV; HR, 1.6; assigned score of 1), and age (≤ 65 yr vs. > 65 yr; HR, 1.7; assigned score of 1). A prognostic index of summation of 5 scores was then categorized into four risk groups, which showed significant differences in OS at 5 years: low risk (0–1, 93.2%), intermediate (2–3, 79.3%), high (4–6, 63.3%), and very high (7–10, 23.3%) [13]. Additionally, CLL-IPI analyzed TTFT in watch-and-wait patients from the CLL1 trial, in which significant differences were recorded between the four risk groups [37]. Of note, recurrent genetic abnormalities such as *NOTCH1*, *SF3B1*, and cytogenetic aberrations [e.g., trisomy 12, del(13q) other than *TP53*, and del(17p)] failed to demonstrate independent prognostic information [13].

In summary, *TP53* and *IGHV* status are independent and relevant novel makers providing additive prognostic information to clinical and biological factors including clinical stage, age, and serum β_2 -microglobulin concentration. However, most of the prognostic scoring models in CLL were developed using data of patients treated with chemotherapy and/or chemoimmunotherapy before the introduction of novel small molecules for CLL treatment. Therefore, it is unclear whether the prognostic markers identified from the pre-novel agent era are still informative to the prognosis and the treatment outcomes of CLL patients in the current scheme of CLL treatment.

FRONTLINE CHEMOIMMUNOTHERAPY

As adding rituximab, a monoclonal antibody directed against the CD20 surface antigen, to conventional chemotherapy has become the standard of care for indolent and aggressive B cell non-Hodgkin's lymphoma (NHL), CLL8 trial, a randomized phase 3 trial from German Chronic Lymphocytic Leukaemia Study Group (GCLLSG), compared rituximab plus fludarabine and cyclophosphamide (FCR) with fludarabine and cyclophosphamide (FC) for previously untreated 817 CLL patients [38]. More patients in the FCR group had a complete remission compared to those in the FC group (44% vs. 22%, respectively; $P < 0.0001$), which translated into improvement in the 3-year PFS (65% vs. 45%, HR, 0.56; $P < 0.0001$) and 3-year OS (87% vs. 83%, HR, 0.67; $P = 0.001$). FCR was more frequently associated with grade 3 and 4 neutropenia (34% vs. 21%, $P < 0.0001$) and leukopenia (24% vs. 12%, $P < 0.0001$). FCR resulted in significantly higher PFS in most genetic subgroups, includ-

ing del(17p), del(11q), del(13q), and trisomy 12. However, the del(17p) subgroup had the shortest median PFS (FCR, 11.3 mo vs. FC 6.5 mo; HR, 0.47; $P=0.019$). CLL-UM also benefited as a subgroup from FCR in 3-year PFS (55% vs. 35%; HR, 0.62; $P=0.0003$) and 3-year OS (86% vs. 79%; HR, 0.62; $P=0.023$) [38]. After a median follow-up of 5.9 years, survival benefit of FCR over FC was maintained; median PFS (56.8 mo vs. 32.9 mo, respectively; $P<0.001$); median OS (not reached vs. 86 mo, respectively; $P=0.001$) [39]. However, FCR had a higher rate of prolonged neutropenia during the first year after treatment (16.6% vs. 8.8%; $P=0.007$) [39]. A significant risk of late infection was reported, with 10% and 4% for the first and second years of remission, from the MDACC group [40].

Since the combination of bendamustine and rituximab (BR) had shown promising results in a phase 2 study in frontline setting for CLL [41], CLL10 trial, a randomized, phase 3, non-inferiority study, was conducted to compare BR with FCR for fit, treatment-naïve CLL patients with concerns of safety of FCR [42]. Given the negative impact on survival of del(17p) from previous studies [38, 39], CLL with del(17p) detected by FISH was excluded [42]. After a median observation time of 37.1 months, median PFS was 41.7 months for BR and 55.2 months for FCR (HR, 1.643; $P=0.0003$), which failed to show non-inferiority of BR. For the safety analysis, grades 3 and 4 neutropenia, leukopenia, thrombocytopenia, and the severe infections were more frequent with FCR, which was more pronounced in patients older than 65 years. Severe infections occurred more frequently after termination of FCR than BR (39% vs. 25%, respectively). In the subgroup analysis, it seemed that elderly patients (>65 yr) and IGHV-M might have benefited from BR with no inferiority in PFS. Therefore, BR might be a considerable alternative for fit, elderly CLL patients without del(17p)/*TP53* mutation [42].

For medically unfit or elderly patients who cannot tolerate toxic chemoimmunotherapy such as FCR or BR, COMPLEMENT-1 trial evaluated the efficacy and safety of ofatumumab plus chlorambucil compared to chlorambucil alone in previously untreated CLL with advanced age or comorbidity [43]. About 70% of 447 patients were older than 65 years and had comorbidity ≥ 2 . Median cumulative illness rating scale for geriatrics (CIRS-G) was 9 (range, 4-21). Median PFS was 22.4 months for ofatumumab plus chlorambucil compared with 13.1 months for chlorambucil alone (HR, 0.57; $P<0.0001$). Although grade 3 or greater neutropenia was more frequent in the ofatumumab-chlorambucil group compared to the chlorambucil group (26% vs. 14%), severe infections were not different between the two groups [43].

Obinutuzumab has also been investigated for unfit, treatment-naïve CLL patients in combination with chlorambucil. CLL11 trial compared obinutuzumab-chlorambucil or rituximab-chlorambucil with chlorambucil monotherapy for CLL patients with high CIRS (>6) or renal insufficiency [44]. In total, 781 patients had a median age of 73 years, creatinine clearance of 62 mL/min, and CIRS of 8. Obinutuzumab-chlor-

ambucil showed prolonged PFS compared with rituximab-Chlorambucil (median, 26.7 mo vs. 16.3 mo; $P<0.001$) as well as with Chlorambucil only (vs. 11.1 mo; $P<0.001$). Obinutuzumab-chlorambucil also resulted in higher negative rate of minimal residual disease (MRD). Although infusion-related reaction and neutropenia were more common with obinutuzumab-chlorambucil than with rituximab-chlorambucil, the risk of infection did not increase [44]. Based on the result from CLL11 trial, obinutuzumab-chlorambucil has been approved for the upfront treatment of unfit patients [45].

To identify novel prognostic and predictive markers for chemoimmunotherapy, genomic aberrations and *IGHV* status were centrally analyzed in CLL8 cohort [46]. *TP53*, *NOTCH1*, and *SF3B1* were the most frequently observed mutations (11.5%, 10.0%, and 18.4%, respectively). CLL with IGHV-UM showed a higher frequency of the three mutations (42.9% vs. 24.1%), of *TP53* mutation (15.0% vs. 6.2%), and *NOTCH1* mutation (13.6% vs. 4.5%), whereas there was no significant increase in *SF3B1* mutation. Predictive marker analysis of treatment regimen showed that rituximab failed to improve response and survival in patients with *NOTCH1* mutation, therefore, a predictive marker for rituximab response. IGHV-UM, *TP53*, and *SF3B1* mutations along with del(11q) were identified as independent prognostic markers on PFS and IGHV-UM and *TP53* mutations on OS for FCR [46].

BRUTON'S TYROSINE KINASE INHIBITORS

Bruton's tyrosine kinase (BTK) is a non-receptor tyrosine kinase, that plays a crucial role in B cell development, differentiation, and signaling, which is critical for the proliferation and survival of leukemic cells in various B cell malignancies [47, 48]. BTK is not only involved in the signal transduction pathway downstream of the BCR, but also in other signaling pathways in B cells, including chemokine receptor, Toll-like receptor (TLR), and Fc receptor signaling, which is essential for interactions with the tumor microenvironment, and eventually chemokine-mediated homing and adhesion of B cells [48, 49].

Ibrutinib is a first-in-class oral covalent inhibitor of BTK. Preclinical studies have shown that ibrutinib inhibits extracellular signal-regulated kinase (ERK) signaling, nuclear factor kappa light-chain enhancer of activated B cells (NF- κ B) DNA binding, cytosine-phosphate-guanine (CpG)-mediated CLL-cell proliferation, and tumor-cell migration [49-52]. Ibrutinib was granted accelerated approval for CLL by the Food and Drug Administration (FDA) based on the results from the phase 1b-2 trial for previously treated CLL patients [49, 53]. In a multicenter, open-label, randomized, phase 3 trial, the study of ibrutinib versus ofatumumab in patients with relapsed or refractory CLL (RESONATE) reported that ibrutinib demonstrated 88% of PFS at 6 months (median PFS not reached), as compared with a median of 8.1 months in the ofatumumab group (HR, 0.22; $P<0.001$). At 12

months, OS was 90% in the ibrutinib group and 81% in the ofatumumab group (HR, 0.43; $P=0.005$) [53].

As early data from 31 elderly, treatment-naïve CLL patients showed promising outcomes with single-agent ibrutinib with 84% of overall response rate (ORR), 96% of PFS at 30 months, and 97% of OS at 3 months, RESONATE-2 trial was conducted to evaluate the efficacy and safety of single-agent ibrutinib as compared with chlorambucil in patients 65 years of age or older with previously untreated CLL [54, 55]. With median follow-up duration of 18.4 months, ibrutinib resulted in significantly longer PFS than chlorambucil (median, not reached vs. 18.9 mo; HR, 0.16; $P<0.001$). OS was also prolonged with ibrutinib than chlorambucil (estimated 24-mo OS, 98% vs. 85%, respectively; HR, 0.16; $P<0.001$). Adverse events of any grade occurred in at least 20% of patients in both groups. Diarrhea, fatigue, cough, and nausea were the most common adverse events of any grade in the ibrutinib group. Results of the RESONATE-2 trial supported the approval of ibrutinib for previously untreated patients with CLL [54]. Extended analysis of RESONATE-2 with a maximum of 36 months of follow-up sustained the superiority of ibrutinib monotherapy over chlorambucil with 89% of 24-month PFS and 92% of ORR. The rate of complete response (CR) increased substantially from 7% at 12 months to 18% with extended follow-up [56]. Great improvement in quality of life (QoL) occurred with ibrutinib versus chlorambucil in the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) ($P=0.0013$). Rate of discontinuation due to adverse events was 12% with neutropenia (12%), anemia (7%), and hypertension (95%) being most frequent grade ≥ 3 adverse events. There was considerable beneficial effect on survival outcomes with the group of CLL with IGHV-UM as no significant difference was observed in PFS of patients with IGHV-UM versus IGHV-M (24 mo PFS, 90% vs. 89%, respectively) [56].

Acalabrutinib is a second-generation, selective, oral, irreversible inhibitor of BTK that has improved pharmacologic features, including favorable plasma exposure, rapid oral absorption, a short half-life, and the greater selectivity to BTK than ibrutinib [57]. A phase 1-2, multicenter study of acalabrutinib for patients with relapsed CLL defined a dose of 100 mg twice daily as the recommended dose [57]. About a third of patients had high-risk feature of del(17p) and 75% of CLL with IGHV-UM. The efficacy and the safety profiles were promising with 95% of ORR, including 85% with a partial response with median follow-up of 14.3 months. There was no case of Richter's transformation, and only one case of CLL progression occurred. Most frequent adverse events of the grades were headache (43%), diarrhea (39%), and increased weight (26%), mostly being grades 1-2 [57].

For patients intolerant to ibrutinib, an open-label phase 2 dose expansion study was conducted to evaluate the efficacy and the tolerability of acalabrutinib [58]. Among 33 patients, median duration of prior ibrutinib treatment was 11.6 months. After a median of 19.0 months, 10 had discontinued

due to progressive disease or adverse events and 23 remained on acalabrutinib. The most frequent adverse events included diarrhea (58%), headache (39%), and cough (33%). Grade 3/4 hematologic toxicities occurred in 12% with neutropenia and 9% with thrombocytopenia. ORR was reported as 76%, including 1 complete and 19 partial responses. 1-year PFS was 83.4% [58].

PHOSPHOINOSITIDE 3-KINASE- δ -INHIBITOR

Idelalisib is an oral potent small molecule that selectively inhibits the phosphoinositide 3-kinase δ (PI3K δ), an enzyme that is expressed in normal and malignant cells [59-61]. Idelalisib inhibits several signaling pathways, including BCR signaling, CXCR4, and CXCR5 signaling, which are involved in trafficking and homing of B cells to the lymph nodes and to the bone marrow [62].

A multicenter, randomized, double-blind, placebo-controlled, phase 3 study of idelalisib in combination with rituximab was conducted for patients with relapsed or refractory CLL, who were unfit to receive chemotherapy due to renal insufficiency, previous therapy-induced myelosuppression, or coexisting illness (CIRS > 6) [63]. The comparator was rituximab alone group received rituximab plus oral placebo. The median PFS was 5.5 months in the placebo group whereas in the idelalisib group it was not reached (HR, 0.15; $P<0.001$). ORR was 81% in idelalisib group and 13% in placebo group ($P<0.001$). The OS at 12 months was 92% and 80%, respectively (HR, 0.28; $P=0.002$). As serious adverse events occurred in 40% of the idelalisib group and in 35% of the placebo group, the combination of idelalisib and rituximab improved survival and tumor response, and were tolerable compared with rituximab alone [63]. These results led to the approval of idelalisib in combination with rituximab for the treatment of patients with relapsed CLL [61].

Upon primary study termination and unblinding, patients could transition to the extension study to receive open-label idelalisib monotherapy [61]. The final results were reported in 2019, that idelalisib plus rituximab followed by idelalisib monotherapy showed median PFS of 20.3 months after a median follow-up time of 18 months [61]. The median OS was 40.6 and 34.6 months for patients initially randomly assigned to idelalisib/rituximab and placebo/rituximab groups, respectively. Prolonged exposure to idelalisib increased the incidence of diarrhea, colitis, and pneumonitis [61].

A randomized, phase 3 trial of idelalisib combined with other anti-CD20 antibody, ofatumumab, for the treatment of patients with relapsed CLL demonstrated improvement in PFS for idelalisib plus ofatumumab over ofatumumab alone (median PFS, 16.3 mo vs. 8.0 mo; HR, 0.27; $P<0.001$) [64]. Severe infections were more common in the idelalisib/ofatumumab group, which included pneumonia (13%), sepsis (6%), and *Pneumocystis jirovecii* pneumonia (5%). Out of 261 patients, 22 treatment-related deaths occurred in the idelalisib/ofatumumab group and 6 in the ofatumumab group [64].

BCL-2 INHIBITORS

After the success of ibrutinib, acquired resistance mutations were identified in the ibrutinib binding site of BTK or in phospholipase C $\gamma 2$ (PLCG2) in most patients who have progressed while on ibrutinib [65–67]. As constitutively elevated expression of BCL-2, which is a key regulator of apoptotic process, is common in CLL, agents that inhibit BCL-2 have been highlighted as a potential anti-leukemic therapy for CLL [68, 69].

Venetoclax is an orally bioavailable, highly selective inhibitor of BCL-2 that induces apoptosis *in vitro* against primary CLL cells, and inhibits tumor growth *in vivo* in xenograft models of BCL-2 overexpressed human lymphoid tumors [70]. A first-in-human phase 1 study of venetoclax in patients with relapsed or refractory CLL showed 79% of tumor responses [71]. Even though the trial included patients who were previously heavily treated and having high-risk features such as del(17p) and IGHV-UM, complete remission occurred in 20% of the patients, including 5% of MRD negativity on flow cytometry. The 15-month PFS estimated for the 400 mg dose groups was 69%. Tumor lysis syndrome developed in 3 out of 56 patients in the dose-escalation cohort, with one death. After adjustments to the dose-escalation schedule, tumor lysis syndrome did not occur in the expansion cohort of 60 patients [71].

Two multicenter, open-label, non-randomized, phase 2 trial of venetoclax for patients with relapsed or refractory CLL, who previously treated with BCR inhibitors, evaluated the efficacy and the tolerability of a single agent venetoclax [72, 73]. First, 91 patients received ibrutinib as the last BCR inhibitor [73]. Median duration of ibrutinib therapy was 20 months, and 68% of the patients had refractoriness to ibrutinib, 59 (65%) of 91 patients had an overall response. The PFS and OS at 1-year was 75% and 91%, respectively. For 57 patients assessed for MRD in peripheral blood, 24 (42%) patients were negative for MRD in peripheral blood, with 5 of 13 patients subsequently assessed for MRD in the bone marrow being negative. The most common grade 3/4 adverse events were hematologic toxicities, with neutropenia (51%), thrombocytopenia (29%), anemia (29%), and lymphopenia (15%). The most common non-hematologic toxicities were nausea (57%), diarrhea (52%), and fatigue (42%), mostly being grade 1/2. Baseline samples to assess BTK or PLCG2 mutations were obtained from 21 patients with refractory CLL to ibrutinib. BTK or PLCG2 mutations were present in 17 patients, of whom 12 patients achieved an overall response to venetoclax including one complete response with incomplete bone marrow recovery [73]. In the other trial, the last BCR inhibitor before study enrollment was idelalisib [72]. In total, 36 patients were enrolled. The estimated 12-month PFS was 79% and ORR was 67%, including 2 cases of complete response and 1 case of complete response with incomplete bone marrow recovery [72].

COMBINATION OF ANTI-CD20 ANTIBODIES WITH NOVEL SMALL MOLECULES

Given the efficacy and the tolerability of small molecules targeting BCR signaling pathways and BCL-2, adding novel small molecules to anti-CD20 antibodies, which have been thought to be indispensable in the treatment of CLL, have been widely explored.

A phase 3 trial to evaluate the efficacy of ibrutinib alone or in combination with rituximab was conducted to compare to chemoimmunotherapy, bendamustine plus rituximab (BR) for elderly patients with previously untreated CLL [74]. In total, 447 patients older than 65 years were randomly assigned to ibrutinib, ibrutinib plus rituximab (IR), and BR groups in a 1:1:1 fashion. The PFS at 2 years was 75% with BR and was higher with ibrutinib alone (87%) and with IR (88%). However, there was no significant difference between the IR group and the ibrutinib group (HR, 1.00; $P=0.49$). The rate of grade 3, 4, or 5 hematologic adverse events was higher for BR (61%) than for ibrutinib or IR (41% and 39%, respectively), whereas the rate of grades 3, 4, or 5 non-hematologic adverse events was lower for BR (63%) than for the ibrutinib containing regimens (74% with each regimen) [74].

To compare with more potent chemoimmunotherapy for young and fit patients, FCR and IR regimens were evaluated in a phase 3, randomized trial as frontline therapy for patients 70 years of age or younger [75]. In total, 354 patients were randomly assigned to the IR group and 175 to the FCR group. At a median follow-up of 33.6 months, PFS favored IR over FCR (89.4% vs. 72.9% at 3 yr; HR, 0.35; $P<0.001$). The OS also favored IR over FCR (98.8% vs. 91.5% at 3 yr; HR, 0.17; $P<0.001$). In a subgroup analysis, IGHV-UM CLL showed comparable PFS of 90.7% at 3 years for IR, whereas PFS of IGHV-M CLL was 87.7% at 3 years for IR. Although serious adverse events were similar in both groups, infectious complications of grade 3 or higher were more common for FCR than IR (20.3% vs. 10.5%, respectively; $P<0.001$).

A phase 3 trial comparing ibrutinib plus obinutuzumab with chlorambucil plus obinutuzumab (iLLUMINATE) for treatment naïve CLL patients was recently published [76]. In all, 229 patients aged 65 years or older or younger than 65 years with coexisting conditions (CIRS >6 , creatinine clearance of less than 70 mL/min, presence of del(17p) confirmed by FISH, or *TP53* mutation) were enrolled. After a median follow-up of 31.3 months, PFS was significantly higher at 30 months in the ibrutinib/obinutuzumab group (79%) than in the chlorambucil/obinutuzumab group (31%; $P<0.001$). Serious adverse event occurred in 65 (58%) of 113 patients for the ibrutinib/obinutuzumab group and 40 (35%) of 115 patients for the chlorambucil/obinutuzumab group [76].

Venetoclax has also been investigated in combination with anti-CD20 antibodies [77, 78]. Comparing venetoclax plus rituximab with BR demonstrated significant improvement

in PFS in patients with relapsed or refractory CLL in a phase 3, randomized trial [78]. After a median follow-up of 23.8 months, PFS was considerably higher in the venetoclax/rituximab group of 84.9% than in the BR group of 36.3% at 2 years (HR, 0.17; $P < 0.01$). The rate of clearance of MRD on blood was higher in the venetoclax/rituximab group than in the BR group (62.4% vs. 13.3% at 9 mo, respectively). In this trial, the threshold for MRD was defined as 1 tumor cell per 10^4 white cells using flow cytometry. The higher rate of clearance of MRD in the venetoclax/rituximab group was maintained over time [78].

Venetoclax combined with obinutuzumab for treatment naïve CLL patients with coexisting conditions (CIRS > 6 ; creatinine clearance of less than 70 mL/min) has shown favorable PFS compared with chlorambucil/obinutuzumab (88.2% vs. 64.1% at 2 yr; $P < 0.001$) in a phase 3, randomized trial

with 432 patients [77]. The trial included about 10% of CLL patients with del(17p) or *TP53* mutation. The benefit in PFS was reserved in patients with del(17p) or *TP53* mutation. Grade 3/4 neutropenia and infections occurred similarly in both groups [77].

NOVEL SMALL MOLECULES AND CLL WITH TP53 MUTATION OR DEL(17P)

TP53 mutational status is a significant prognostic factor influencing survival and treatment responses, which most of the prognostic models of CLL treat as poor prognostic marker in the era of chemoimmunotherapy [13, 35, 36, 79, 80]. The consensus guidelines of the International Workshop on CLL (iwCLL) and the European Research Initiative on

Table 2. Summary of National Comprehensive Cancer Network Clinical Practice Guidelines (accessed on 28 Nov 2019).

Category 2A if not indicated	First-line		Relapse or refractory	
	Preferred	Other	Preferred	Other
Without del(17p) or <i>TP53</i> mutation				
Fit and young (≤ 65 yr)	Ibrutinib (C1) Venetoclax+obinutuzumab	Bendamustine+anti-CD20 FCR for IGHV mutated FR HDMP+rituximab (C2B) Ibrutinib+rituximab (C2B) PCR (C3)	Acalabrutinib (C1) Ibrutinib (C1) Venetoclax+rituximab (C1) Duvelisib Idelalisib+rituximab	Alemtuzumab±rituximab BR FC+ofatumumab FCR HDMP+rituximab Idelalisib Lenalidomide±rituximab Obinutuzumab Ofatumumab PCR Venetoclax BR+ibrutinib (C2B) BR+idelalisib (C2B)
Frail or old	Ibrutinib (C1) Venetoclax+obinutuzumab	Bendamustine+anti-CD20 (not for frail) Chlorambucil+obinutuzumab HDMP+rituximab (C2B) Ibrutinib+obinutuzumab (C2B) Obinutuzumab (C2B) Chlorambucil (C3) Rituximab (C3)	Acalabrutinib (C1) Ibrutinib (C1) Venetoclax+rituximab (C1) Duvelisib Idelalisib+rituximab	Alemtuzumab±rituximab Chlorambucil+rituximab Reduced FCR HDMP+rituximab Idelalisib Lenalidomide±rituximab Obinutuzumab Ofatumumab Reduced PCR Venetoclax Dose dense rituximab (C2B) BR±ibrutinib or idelalisib (C2B for BR/BR+ibrutinib) (C3 for BR+idelalisib)
With del(17p) or <i>TP53</i> mutation	Ibrutinib Venetoclax+obinutuzumab	Alemtuzumab±rituximab HDMP+rituximab Obinutuzumab	Acalabrutinib (C1) Ibrutinib (C1) Venetoclax+rituximab (C1) Duvelisib Idelalisib+rituximab Venetoclax	Alemtuzumab±rituximab HDMP+rituximab Idelalisib Lenalidomide±rituximab ofatumumab

Abbreviations: BR, bendamustine, rituximab; C1, category 1; C2B, category 2B; C3, category 3; FC, fludarabine, cyclophosphamide; FCR, fludarabine, cyclophosphamide, rituximab; FR, fludarabine, rituximab; HDMP, high-dose methyl-prednisolone; PCR, pentostatin, cyclophosphamide, rituximab.

CLL (ERIC) recommend testing of *TP53* status or del(17p) using FISH for patients with CLL at diagnosis as well as before each line of therapy [10, 81].

In the subgroup analysis of RESONATE trial, ibrutinib has sustained survival benefits on relapse or refractory CLL with del(17p) as a single agent [53]. A prospective, single-arm, phase 2 study of ibrutinib for patients with relapsed or refractory CLL with del(17p) detected through FISH (RESONATE-17), further supported the findings from the original RESONATE trial [82]. With median follow-up of 27.6 months, ORR was reported in 120 out of 144 patients (83%). The 2-year PFS and OS were 63% and 75%, respectively. *TP53* mutational analysis were also documented with 92% of positivity out of 116 patients who had available samples [82].

From the iLLUMINATE trial, which compared ibrutinib/obinutuzumab regimen to chlorambucil/obinutuzumab in the frontline setting, about 20% of patients had *TP53* mutation of del(17p) [76]. A subgroup analysis favored ibrutinib/obinutuzumab in PFS over chlorambucil/obinutuzumab among patients with *TP53* mutation or del(17p) [HR, 0.11; 95% confidence interval (CI), 0.03–0.38] [76].

Acalabrutinib also showed promising results on CLL patients with del(17p) as 18 out of 18 patients (100%) with del(17p) showed partial response in the setting of treatment for relapse CLL in the subgroup analysis from a small, phase 1–2 trial with 61 patients enrolled [57].

The first-in-human trial of venetoclax have shown comparable anti-tumor activity with 71% of ORR in CLL with del(17p) to 80% of ORR in those without del(17p) [71]. Then, the interim analysis of a phase 2, single-arm, multi-center study of venetoclax for 107 patients with CLL with del(17p) reported a similar 79.4% ORR at a median follow-up of 12.1 months [83]. The final results with the full population of 158 patients with longer follow-up duration confirmed 77% of ORR (122 of 158 patients; 20% complete remission) and PFS at 24 months was 54%. In all, 48 (30%) patients achieved MRD below the cutoff of 10^{-4} in blood [84].

Addition of rituximab to venetoclax demonstrated benefits in PFS in the subgroup of patients with del(17p) [78]. The beneficial effect of venetoclax/rituximab was maintained with high-risk features, including del(17p). The 2-year PFS among patients with del(17p) was 81.5% in the venetoclax/rituximab group and 27.8% in the bendamustine/rituximab group. Of note, in the trial, the 2-year PFS of patients without del(17p) was 85.9% [78]. The combination of obinutuzumab with venetoclax has also shown benefits in patients with del(17p) or *TP53* mutation [77].

CONCLUSION

With advances in our understanding of the molecular pathogenesis of CLL development and progression, the landscape of CLL treatment has considerably changed (Table 2). Several targeted therapies such as anti-CD20 antibodies, BTK, PI3K δ , and BCL2 inhibitors have been developed and

shown to be improving the survival of CLL patients with tolerable safety profiles. The clinical benefit of novel agents has been maintained across subgroups with high-risk features including del(17p) or *TP53* mutations, IGHV-UM, etc. Chemoimmunotherapy is not a standard recommendation for CLL with *TP53* mutation or del(17p) in the era of novel agents. However, considering the financial toxicity of newer agents, it would be worth defining the subgroup that would benefit from chemoimmunotherapy before disregarding it.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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