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ANNUAL CLINICAL UPDATES IN HEMATOLOGICAL MALIGNANCIES

Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures

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Abstract

Disease overview: Chronic lymphocytic leukemia (CLL) is one of the most frequent types of leukemia. It typically occurs in elderly patients and has a highly variable clinical course. Leukemic transformation is initiated by specific genomic alterations that interfere with the regulation of proliferation and of apoptosis in clonal B-cells.

Diagnosis: The diagnosis is established by blood counts, blood smears, and immunophenotyping of circulating B-lymphocytes, which identify a clonal B-cell population carrying the CD5 antigen as well as typical B-cell markers.

Prognosis and staging: The clinical staging systems provide prognostic information by using the results of physical examination and blood counts. Various biological and genetic markers provide additional prognostic information. Deletions of the short arm of chromosome 17 (*del[17p]*) and/or mutations of the *TP53* gene predict resistance to chemoimmunotherapy and a shorter time to progression with most targeted therapies. The CLL international prognostic index integrates genetic, biological, and clinical variables to identify distinct risk groups of patients with CLL.

Therapy: Only patients with active or symptomatic disease or with advanced Binet or Rai stages require therapy. When treatment is indicated, several therapeutic options exist: a combination of the B-cell lymphoma 2 (BCL2) inhibitor venetoclax with obinutuzumab, monotherapy with inhibitors of Bruton tyrosine kinase (BTK) such as ibrutinib and acalabrutinib, or chemoimmunotherapy. At relapse, the initial treatment may be repeated, if the treatment-free interval exceeds 3 years. If the disease relapses earlier, therapy should be changed using an alternative regimen. Patients with a *del(17p)* or *TP53* mutation are usually resistant to chemotherapy and should, therefore, be treated with targeted agents.

Future challenges: Combinations of targeted agents are now being investigated to create efficient, potentially curative therapies of CLL with fixed duration. One of the most relevant questions currently addressed in clinical trials is the comparison of monotherapies with BTK inhibitors with fixed duration combination therapies. Moreover, the optimal sequencing of targeted therapies remains to be determined.

Alternative therapies are needed for patients with BTK and BCL2 inhibitor double-refractory disease.

1 | INTRODUCTION AND DISEASE OVERVIEW

In the most recent update of the SEER database, the age-adjusted incidence of chronic lymphocytic leukemia (CLL) was 4.9 per 100 000 inhabitants per year,[1] which makes CLL one of the most common types of leukemia. The median age at diagnosis is 70 years.[1] Only 9.1% of patients with CLL are younger than 45 years.[1] More male than female patients (1.9:1) are affected, and this gender effect seems to be stable across all ethnicities.[1]

Approximately 0.6% of men and women will be diagnosed with CLL at some point during their lifetime. By 2021, SEER estimates 21 250 new CLL cases in the United States, which represents 1.1% of all new cancer cases. In 2018, there was an estimated 195 129 people living with CLL in the United States.[1] While the incidence of CLL has been stable over the last two decades, the mortality has been declining. CLL is estimated to cause 4320 deaths in 2021, representing 0.7% of all cancer deaths. The CLL-related death rate was 1.1 per 100 000 men and women per year. The 5-year relative survival of patients with CLL was at 65.1% in 1975 and has steadily increased over the past decades; it is estimated at 87.2% in 2021.[1] Similar data regarding the epidemiology of CLL have been reported in Europe,[2] while the incidence is lower in Asian countries and ethnicities.[3,4]

CLL is characterized by the clonal proliferation and accumulation of mature, typically CD5-positive B-cells within the blood, bone marrow, lymph nodes, and spleen.[5] The capacity to generate clonal B cells seems to be acquired at the hematopoietic stem cell (HSC) stage,[6] suggesting that the primary leukemogenic event in CLL might involve multipotent, self-renewing HSCs. The process of a stepwise leukemogenic transformation is increasingly understood. CLL is often initiated by the loss or addition of large chromosomal material (e.g., deletion 13q, deletion 11q, and trisomy 12) followed later by additional mutations that render the leukemia increasingly aggressive.[7]

Approximately 80% of all patients with CLL carry at least one of four **common chromosomal alterations**: a deletion in chromosome 13q14.3 (del[13q]), del(11q), del(17p), or trisomy 12.[8] Del(13q) is the most common chromosomal alteration occurring in approx. 55% of all cases. An isolated *del(13q14)* is characterized by a benign course of the disease. The miRNAs miR-15a and 16-1 are located in the critical region of *del(13q14)*[9] and regulate the expression of proteins that can inhibit apoptosis or that are involved in cell cycle progression.[10] Deletions of the short arm of chromosome 17 (*del(17p)*) are found in 5%–8% of chemotherapy-naïve patients. These deletions almost always include band 17p13, where the prominent tumor suppressor gene *TP53* is located. Patients with CLL carrying a *del(17p)* clone show marked resistance against genotoxic chemotherapies.[11] Mutations of *TP53* are found in 4%–37% of patients with CLL, and have been associated with very poor prognosis in a number of studies.[12] Among cases with confirmed *del(17p)*, the majority shows mutations in the remaining *TP53* allele (>80%). In cases without *del(17p)*, *TP53* mutations are much rarer, but have a similarly detrimental effect on chemotherapy response and overall survival (OS).[13] Deletions of the long arm of chromosome 11 (*del(11q)*) can be found in approx. 25% of chemotherapy-naïve patients with advanced disease stages and 10% of patients with early-stage disease.[14,15] These deletions frequently encompass band *11q23* harboring the gene *ATM*, which encodes for the proximal DNA damage response kinase ATM. In addition, patients carrying a *del(11q)* clone typically show a bulky lymphadenopathy, rapid progression, and reduced OS.[16] Interestingly, some of the poor prognostic features of *del(11q)* were overcome by the use of chemoimmunotherapy.[11] Trisomy 12 is observed in 10%–20% of patients with CLL and is associated with an intermediate prognosis.[8] The genes involved in the pathogenesis of CLL carrying a trisomy 12 are largely unknown.

The use of whole-exome sequencing has allowed to characterize the **genomic landscape** of CLL. In addition to the above described chromosomal aberrations, a total number of 44 recurrently mutated genes and 11 recurrent somatic copy number variations has been identified.[7] These include the genes *NOTCH1*, *MYD88*, *TP53*, *ATM*, *SF3B1*, *FBXW7*, *POT1*, *CHD2*, *RPS15*, *IKZF3*, *ZNF292*, *ZMYM3*, *ARID1A*, and *PTPN11*.[7,15,17,18] These analyses collectively identify RNA processing and export, MYC activity, and mitogen-activated protein kinase (MAPK) signaling as central pathways involved in CLL.[7] In addition, proteins critically involved in DNA damage signaling and DNA repair are often involved.[19] Intriguingly, both *del(17p)* and *del(11q)*, as well as inactivating somatic mutations in *TP53* and *ATM* are enriched in patients with secondary resistance to DNA-damaging chemotherapy.[15,17] Mutations in an enhancer located on chromosome 9p13 may reduce the expression of the B-cell-specific transcription factor PAX5.[18]

The CLL **epigenome** has emerged as an additional disease-defining feature.[20,21] Expanding populations of CLL cells diversify by stochastic changes in DNA methylation called epimutations.[22] Multiplexed single-cell reduced representation of bisulfite sequencing of B-cells from healthy donors and patients with CLL has provided new insights into changes of DNA methylation known as epimutations.[23,24] The results suggest that the integration of genetic, epigenetic, and transcriptional information gained at a single cell level allows to chart the lineage history of individual cases of CLL and their evolution with therapy.

Survival of CLL cells depends on a **permissive microenvironment** composed of cellular components like macrophages, T cells, or stromal follicular dendritic cells[25–27] providing stimuli for activation of crucial survival and pro-proliferative signaling pathways in transformed cells. This microenvironment produces various essential proteins (chemokines, cytokines, and angiogenic factors) that interact with

leukemic cells via appropriate surface receptors or adhesion molecules to support the survival of CLL cells.[27–29] Interestingly, some of the new inhibitors also seem to exert their effects by targeting key pathways of microenvironmental cells in patients with CLL.[30–34]

As a consequence of these advances in our understanding of the pathogenesis, the management of this leukemia continues to undergo highly relevant improvements. Several new drugs have been approved during the last three decades. Chemoimmunotherapies, which combined fludarabine, cyclophosphamide with rituximab, or chlorambucil (CLB) with obinutuzumab have improved OS when used as first-line therapy for patients with CLL. More recently, specific inhibitors interrupting important pathways for CLL cell survival have been approved (ibrutinib, idelalisib, and venetoclax). These inhibitors have increasingly replaced chemoimmunotherapy in first and second-line indications. This updated review integrates the latest innovations in CLL therapy as well as diagnostic tools and provides an updated algorithm to guide the diagnostic and therapeutic decisions in daily practice.

2 | DIAGNOSIS

The latest version of iwCLL guidelines[5] gives clear recommendations on how to establish the diagnosis of CLL and is summarized here. In most cases, the diagnosis of CLL is established by blood counts, differential counts, a blood smear, and immunophenotyping. The World Health Organization classification of hematopoietic neoplasias describes CLL as leukemic, lymphocytic lymphoma, and being only distinguishable from small lymphocytic lymphoma (SLL) by its leukemic appearance.[35] CLL is always a disease of neoplastic B-cells, while the entity formerly described as T-CLL is now called T-cell prolymphocytic leukemia (T-PLL).[36]

The **diagnosis of CLL** requires the presence of ≥5000 B-lymphocytes/μL in the peripheral blood for the duration of at least 3 months. The clonality of the circulating B-lymphocytes needs to be confirmed by flow cytometry. The leukemia cells found in the blood smear are characteristically small, mature lymphocytes with a narrow border of cytoplasm, and a dense nucleus lacking discernible nucleoli and having partially aggregated chromatin. These cells may be found admixed with larger or atypical cells, cleaved cells, or prolymphocytes, which may comprise up to 55% of the blood lymphocytes.[37] Finding prolymphocytes in excess of this percentage would favor a diagnosis of PLL (B-cell PLL). Gumprecht nuclear shadows or smudge cells, found as cell debris, are other characteristic morphologic features found in CLL.

Monoclonal B-lymphocytosis (MBL)[5]: In the absence of lymphadenopathy or organomegaly (as defined by physical examination or CT scans), cytopenias, or disease-related symptoms, the presence of fewer than 5000 B-lymphocytes per μL blood is defined as "MBL."[38] The presence of a cytopenia caused by a typical marrow infiltrate defines the diagnosis of CLL regardless of the number of peripheral blood B-lymphocytes or of the lymph node involvement. MBL seems to progress to frank CLL at a rate of 1%–2% per year.[38]

The definition of SLL requires the presence of lymphadenopathy and the absence of cytopenias caused by a clonal marrow infiltrate. Moreover, the number of B-lymphocytes in the peripheral blood should not exceed 5000/μL. In SLL, the diagnosis should be confirmed by histopathological evaluation of a lymph node biopsy whenever possible.

Immunophenotyping[5]: CLL cells co-express the surface antigen CD5 together with the B-cell antigens CD19, CD20, and CD23. The levels of surface immunoglobulin, CD20, and CD79b are characteristically low compared to those found on normal B cells.[39–41] Each clone of leukemia cells is restricted to expression of either kappa or lambda immunoglobulin light chains.[39] It should be noted that the expression of CD5 can also be observed in other lymphoid malignancies, such as mantle cell lymphoma.[42] A recent, large harmonization effort has confirmed that a panel of CD19, CD5, CD20, CD23, kappa, and lambda is usually sufficient to establish the diagnosis.[43] In borderline cases, markers such as CD43, CD79b, CD81, CD200, CD10, or ROR1 may help to refine the diagnosis.[43]

3 | RISK STRATIFICATION, STAGING, AND INDICATION FOR TREATMENT

Two widely accepted clinical staging systems co-exist.[44,45] The Rai classification was later modified to reduce the number of prognostic groups from five to three.[46] Both systems describe three major prognostic groups with discrete clinical outcomes. These two staging systems are simple, inexpensive, and rely on a physical examination and standard laboratory tests. They do not require ultrasound, computed tomography, or magnetic resonance imaging.

The **Rai staging system** defines low-risk disease as patients who have lymphocytosis with leukemia cells in the blood and/or marrow (lymphoid cells >30%) (former Rai stage 0). Patients with lymphocytosis, enlarged nodes in any site, and splenomegaly and/or hepatomegaly (lymph nodes being palpable or not) are defined as having intermediate-risk disease (formerly considered Rai stage I or stage II). High-risk disease includes patients with disease-related anemia (as defined by a hemoglobin [Hb] level less than 11 g/dL) (formerly stage III) or thrombocytopenia (as defined by a platelet count of less than 100×10^9/L) (formerly stage IV).

The **Binet staging system** is based on the number of involved areas, as defined by the presence of enlarged lymph nodes of greater than 1 cm in diameter or organomegaly, and on whether there is anemia or thrombocytopenia. The areas of involvement considered are (1) head and neck, including the Waldeyer ring (this counts as one area, even if more than one group of nodes is enlarged); (2) axillae (involvement of both axillae counts as one area); (3) groins, including superficial femoral (involvement of both groins counts as one area); (4) palpable spleen; and (5) palpable liver (clinically enlarged). The Binet staging system defines stage *A as* Hb ≥10 g/dL and platelets ≥100×10^9/L and up to two of the above involved; *stage B as* Hb ≥10 g/dL and platelets ≥100×10^9/L and organomegaly greater than that defined for stage A (i.e., three or more areas of nodal or organ enlargement); and *stage C as* Hb of less than 10 g/dL and/or a platelet count of less than 100×10^9/L.

Due to recent progress in CLL therapy, the two clinical staging systems have become insufficient to distinguish prognostic subgroups.[47] In addition, a plethora of potential markers exist that provide prognostic information independent of the clinical-stage,[48] in particular, some of the above described genetic and chromosomal aberrations. To reduce the prognostic information to a few clinically relevant, essential prognostic parameters, comprehensive prognostic scores have been constructed that combine clinical, biological, and genetic information.[47,49–51] The currently most relevant prognostic score is the **CLL International Prognostic Index (CLL-IPI)**.[52] It uses a weighted grading of five independent prognostic factors: *TP53* deletion and/or mutation (collectively called *TP53* dysfunction), immunoglobulin heavy chain variable (*IGHV*) mutational status, serum β_2-microglobulin, clinical stage, and age. The CLL-IPI separates four groups with different survival at 5 years (see Table 1). The prognostic value of the CLL-IPI is currently revisited for the use of targeted agents.

Patients with a low Binet (A or B) or Rai (0-II) stage and asymptomatic disease should not be treated, because treatment of patients with early-stage disease did not result in a survival benefit so far.[53–56] Therefore, an early-intervention therapy with anti-leukemia drugs, including signaling inhibitors or BCL2 antagonists, alone or in combination with monoclonal antibodies, is currently not recommended.

A system for predicting the time to first treatment in patients with CLL with early, asymptomatic disease was recently proposed (International Prognostic Score for Early-stage CLL [IPS-E]).[57] Three covariates, unmutated IGHV gene, absolute lymphocyte count higher than 15×10^9/L, and presence of palpable lymph nodes were combined and predicted a 5-year cumulative risk for treatment start of 8.4%, 28.4%, and 61.2% for low-risk, intermediate-risk, and high-risk patients, respectively. The IPS-E will be helpful to counsel patients with early-stage CLL.

Criteria for the initiation of therapy have been proposed by the iwCLL guidelines.[5] The decision for initiating treatment depends on the presence of active/symptomatic disease. Asymptomatic patients with early-stage disease (Rai 0, Binet A), should be monitored without therapy unless they have evidence of rapid disease progression. So far, studies on early-stage disease were unable to show a benefit of early therapeutic interventions.[53–56]

TABLE 1 The different CLL International Prognostic Index (CLL-IPI) categories

CLL-IPI category	OS at 5 years	Potential clinical consequence
Low-risk	93.2%	Do not treat
Intermediate-risk	79.3%	Do not treat except if the disease is really symptomatic
High-risk	63.3%	Treatment indicated except if the disease is asymptomatic
Very high-risk	23.3%	If you need to treat, do not use chemotherapy but rather targeted agents or treatment in clinical trials

In an attempt to generate a prognostic tool for patients with CLL treated with ibrutinib, Ahn et al. identified four relevant factors[58]: TP53 aberration, prior treatment, beta-2 microglobulin ≥5 mg/L, and lactate dehydrogenase >250 U/L. These factors were used to create three prognostic subgroups with 3-year survival rates of 63%, 83%, and 93%. The model remained significant when applied to treatment-naive and relapsed/refractory cohorts individually. Richter's transformation (RT) occurred in 17% of the high-risk group, and in no patient in the low-risk group. Overall these factors may identify patients at increased risk of ibrutinib failure.

When patients progress or present with **progressive or symptomatic/active disease** treatment should be initiated. The iwCLL guidelines[5] define symptomatic or active disease by defined criteria listed in Table 2.

Hypogammaglobinemia, or monoclonal or oligoclonal paraproteinemia does not by itself constitute a basis for initiating therapy. However, it is recommended to assess the change in these protein

TABLE 2 Criteria to define symptomatic or active disease according to iwCLL guidelines[5]

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia. Cut-off levels of Hb <10 g/dL or platelet counts of <100 000/μL are generally regarded as indication for treatment. However, it should be pointed out that in some patients platelet counts of <100 000/μL may remain stable over a long-period of time; this situation does not automatically require therapeutic intervention
2. Massive (i.e., ≥6 cm below the left costal margin) or progressive or symptomatic splenomegaly
3. Massive nodes (i.e., ≥10 cm in longest diameter) or progressive or symptomatic lymphadenopathy
4. Progressive lymphocytosis with an increase of ≥50% over a 2-month period, or lymphocyte doubling time (LDT) of less than 6 months. LDT can be obtained by linear regression extrapolation of absolute lymphocyte counts (ALC) obtained at intervals of 2 weeks over an observation period of 2–3 months; patients with initial blood lymphocyte counts of <30 000/μL may require a longer observation period to determine the LDT. Factors contributing to lymphocytosis other than chronic lymphocytic leukemia (e.g., infections, steroid administration) should be excluded
5. Autoimmune complications including anemia or thrombocytopenia poorly responsive to corticosteroids
6. Symptomatic or functional extranodal involvement (e.g., skin, kidney, lung, spine)
7. Disease-related symptoms as defined by any of the following:
   (a) Unintentional weight loss ≥10% within the previous 6 months
   (b) Significant fatigue (i.e., ECOG PS 2 or worse; cannot work or unable to perform usual activities)
   (c) Fevers ≥100.5°F or 38.0°C for 2 or more weeks without evidence of infection
   (d) Night sweats for ≥1 month without evidence of infection

abnormalities, if patients are treated. In addition, patients with CLL may present with a markedly elevated leukocyte count; however, leukostasis rarely occurs in patients with CLL. Therefore, the absolute lymphocyte count should not be used as the sole indicator for treatment.

4 | RESPONSE ASSESSMENT

The iwCLL guidelines give a detailed description of the assessment of the treatment response. A detailed overview of these response criteria is beyond the scope of this manuscript. In essence, the following response categories can be separated[5]: complete remission (CR), partial remission, stable disease and progression, as well as refractory disease. In addition, the assessment of minimal residual disease (MRD) is an additional and increasingly important category of response assessment, resulting in four different response categories (Figure 1).

4.1 | Eradicating MRD

The use of sensitive multicolor flow cytometry, PCR, or next-generation sequencing can detect MRD in many patients who achieved a complete clinical response. Prospective clinical trials have provided substantial evidence that therapies that are able to eradicate MRD usually result in an improved long-term clinical outcome.[59–63] The value of MRD assessments has been compared to the evaluation of clinical response in CLL according to 554 patients treated in two randomized trials of the German CLL Study Group (CLL8 and CLL10).[59] Patients with MRD-negative CR, MRD-negative partial response (PR), MRD-positive CR, and MRD-positive PR experienced a median progression-free survival (PFS) from a landmark at the end of the treatment of 61, 54, 35, and 21 months, respectively. Interestingly, PFS did not differ significantly between MRD-negative CR and MRD-negative PR. In contrast to residual lymphadenopathy, persisting splenomegaly did not impact outcome in patients with MRD-negative PR. In a retrospective, monocentric analysis, 536 patients were analyzed who achieved at least a PR with various therapies between 1996 and 2007, and received a bone marrow MRD assessment at the end of treatment.[64] MRD negativity correlated with both PFS and OS independent of the type and line of treatment, as well as known prognostic factors, including adverse cytogenetics. The greatest impact of achieving MRD negativity was seen in patients receiving frontline treatment, with 10-year PFS of 65% versus 10% and 10-year OS of 70% versus 30% for MRD-negative versus MRD-positive patients, respectively.

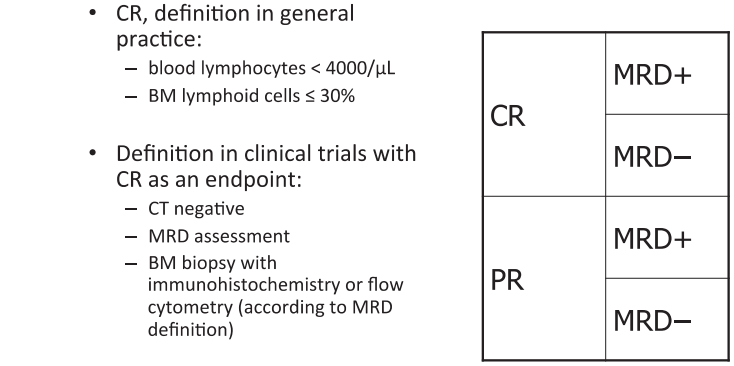

FIGURE 1 Definition of response in chronic lymphocytic leukemia, as proposed by the iwCLL.[5] Please note that the assessment of MRD is not always part of routine practice although the parameter will be increasingly used to determine response to therapy. CR, complete remission; MRD, minimal residual disease; PR, partial remission

The techniques for assessing MRD have undergone a critical evaluation and have become well standardized.[65,66] Six-color flow cytometry (MRD flow), allele-specific oligonucleotide PCR or high-throughput immunosequencing, such as by ClonoSEQ assay, are reliably sensitive down to a level of less than one CLL cell in 10 000 leukocytes.[66] Refinement and harmonization of these technologies has established that a typical flow cytometry-based assay comprises a core panel of six markers (i.e., CD19, CD20, CD5, CD43, CD79b, and CD81).[66] As such, patients will be defined as having undetectable MRD (MRD-neg) remission if they have blood or marrow with less than one CLL cell per 10 000 leukocytes. The blood generally can be used for making this assessment, as the marrow will have detectable CLL when it is also found in the peripheral blood. However, there are therapies that preferentially clear the blood but not the marrow (such as monoclonal antibodies). Therefore, it may be important to confirm that the marrow aspirate also is MRD-neg when the blood is found to be MRD-neg. Clinical trials aimed at maximizing the depth of remissions should include at least one test to assess for MRD, because the lack of leukemia persistence using these sensitive tests has a strong, positive prognostic impact. The report should be clear as to whether blood and/or marrow have been assessed and should report the proportion of MRD-neg patients on an intent-to-treat basis using the total number of patients in that treatment arm as the denominator rather than only those patients who were assessed or responded to treatment.

One approach to utilize MRD data for outcome predictions has been recently proposed with the Continuous Individualized Risk Index (CIRI).[67] The CIRI is able to predict PFS and OS based on baseline CLL-IPI and choice of therapy, but also longitudinal knowledge like interim MRD or final MRD status, which allows to refine the prediction using the MRD response. The algorithm was recently tested and validated also for a fixed-duration therapy with venetoclax and obinutuzumab.[68]

Collectively, there is overwhelming evidence to suggest that MRD quantification allows for improved PFS prediction in both patients who achieve a PR and CR, supporting its application in all responders. Although evaluation of MRD is still not generally recommended for routine clinical practice,[5] I anticipate that MRD assessment will be the key variable with regard to the decision to halt therapies with novel inhibitors.[69] In my own practice, I use MRD levels at increased frequency for the following treatment decisions:

(a) Should I continue therapies in a high-risk patient? or (b) Should I stop therapy with targeted inhibitors?

5 | TREATMENT OF CLL

5.1 | Active agents in CLL and their use as monotherapy

5.1.1 | Cytostatic agents

Monotherapy with **alkylating agents** has served as initial, front-line therapy for CLL, and CLB was the therapeutic "gold standard" for several decades.[56] The advantages of CLB are its low toxicity, low cost, and convenience as an oral drug; the major disadvantages are its low to nonexistent CR rate and some side effects that occur after extended use (prolonged cytopenia, myelodysplasia, and secondary acute leukemia). Today, CLB monotherapy may be used as an inexpensive option to achieve palliation in elderly or unfit patients with an inexpensive cytostatic drug.

Three **purine analogs** are used in CLL: fludarabine, pentostatin, and cladribine (2-CdA). Fludarabine remains by far the best-studied compound of the three in CLL. Fludarabine monotherapy produces superior overall response rates (ORRs) compared with other treatment regimens containing alkylating agents or corticosteroids.[70–72] Fludarabine induced more remissions and more CRs (7%–40%) than other conventional chemotherapies, like CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), CAP (cyclophosphamide, doxorubicin, prednisone), or CLB, but did not improve OS when used as single agent.[72–75] Similarly, cladribine monotherapy was shown to produce a higher CR rate than CLB plus prednisone (47% vs. 12%) without resulting in a longer survival.[76]

Bendamustine was first described in 1963 by Ozegowski and Krebs.[77] It was used in East Germany for treating a variety of cancers and became available in West Germany after 1990. Later, bendamustine was compared to CLB in a randomized trial and produced improved responses but greater toxicity and no survival benefit.[78] The OR and median PFS rates were 67% and 22 months, respectively, for bendamustine versus 30% and 8 months for CLB (both $p < .0001$). Another trial compared bendamustine to fludarabine in 96 patients with relapsed CLL requiring treatment after one previous systemic regimen.[79] ORRs were 76% on bendamustine and 62% on fludarabine, with clinical complete response rates of 27% and 9%, respectively. Median PFS was 20.1 and 14.8 months, median OS was 43.8 and 41.0 months. Collectively, these results showed that bendamustine is a potent single agent for the treatment of CLL.

5.1.2 | Monoclonal antibodies

Anti-CD20 antibodies

CD20 is an activated, glycosylated phosphoprotein expressed on the surface of mature B-cells. The protein has no known natural ligand[80] and its function is not yet discovered. It is suspected to act as a calcium channel in the cell membrane. As CD20 is expressed on most B-cell malignancies, the introduction of the anti-CD20 antibody rituximab in 1998 improved the treatment of most CD20-positive non-Hodgkin lymphomas, including CLL.[81] Some newer CD20-antibodies challenge rituximab.[82–84]

Rituximab: In CLL, rituximab is less active as a single agent than in follicular lymphoma, unless very high doses are used.[85,86] In contrast, combinations of rituximab with chemotherapy have proven to be very efficacious therapies for CLL (see below).

Ofatumumab is a fully-humanized antibody targeting a unique epitope on the CD20 molecule expressed on human B-cells, resulting in increased binding affinity to CD20, prolonged dissociation rate, and increased cell kill due to greater complement-dependent cytotoxicity (CDC) activity and similar antibody-dependent cellular cytotoxicity (ADCC) activity compared to Rituximab, especially in cells expressing low levels of CD20.[87] In a study on 201 patients that were either fludarabine- and alemtuzumab-refractory (FA-refractory) or only fludarabine-refractory and suffered from bulky disease (>5 cm), ofatumumab yielded an ORR of 51% in the FA-refractory group and of 44% in the bulky disease group.[88] On 28 February 2019, the European Commission withdrew the marketing authorization for ofatumumab in Europe at the request of the marketing authorization holder, Novartis Europharm Limited. The drug is now approved for a different indication, multiple sclerosis.

Obinutuzumab (GA101): The humanized and glycoengineered monoclonal antibody obinutuzumab showed impressive results in vitro with higher rates of apoptosis in B-cells in comparison to rituximab.[89] The humanization of the parental B-Ly1 mouse antibody and subsequent glycoengineering lead to higher affinity binding to a CD20 type II epitope, increased ADCC, low CDC activity, and increased direct cell death induction.[90] The GAUGUIN trial a phase 1/2 trial testing obinutuzumab monotherapy in patients with relapsed/refractory patients with CLL showed that obinutuzumab was an active drug in CLL.[91] ORR was 62% (phase 1) and 30% (phase 2), respectively. Phase 2 median PFS was 10.7 months.

Other monoclonal antibodies

Alemtuzumab is a recombinant, fully humanized, monoclonal antibody against the CD52 antigen. Monotherapy with alemtuzumab has produced response rates of 33%–53%, with a median duration of response ranging from 8.7 to 15.4 months, in patients with advanced CLL previously treated with alkylating agents who had failed or relapsed after second-line fludarabine therapy.[92–94] Alemtuzumab has also proven effective in patients with high-risk genetic markers.[95,96] Therefore, alemtuzumab is a reasonable therapeutic option for patients with poor prognostic features. In a prospective randomized study alemtuzumab was tested against CLB.[97] Alemtuzumab led to a greater OR and CR ($p < .0001$), superior PFS with a 42% reduction in risk of progression or death ($p < .0001$) and significantly longer median time to progression (TTP) ($p = .0001$). Therefore, the drug was approved for CLL front-line therapy. A strategic decision of Sanofi led to the withdrawal of the license of alemtuzumab for CLL in 2012. The

drug continues to be available through international compassionate use programs and is now approved for the treatment of multiple sclerosis under a new tradename. However, following the arrival of new oral agents, alemtuzumab became less relevant in CLL therapy.

5.1.3 | Agents targeting the signaling in CLL cells and in their microenvironment

B-cell receptor (BCR) signaling seems to play an important role in the survival of CLL cells.[98,99] Different aspects of the BCR have been recognized as a prognostic marker in CLL, such as IGHV mutational status or stereotypy. Continuous or repetitive BCR signaling supports CLL cell survival (reviewed by Stevenson et al.[99]). This might explain why inhibition of BCR signaling is a new and potent strategy to treat CLL.[100] The BCR signaling in CLL cells is supported by different tyrosine kinases, such as Bruton tyrosine kinase (BTK), spleen tyrosine kinase (Syk), ZAP70, Src family kinases (in particular Lyn) as well as phosphatidylinositol 3-kinase (PI3K).[100] Targeting of these B cell receptor-associated kinases (BAKs), in particular of BTK or PI3K delta, by specific inhibitors, has revolutionized the therapy of B lymphoid malignancies. In addition, results obtained by targeted deletion of BAKs such as Lyn and Btk in murine CLL models suggest that BAKs may also shape the dialogue between malignant B cells and the tumor microenvironment (TME).[30] Since BAKs are expressed in multiple cell types, BAK inhibitors may disrupt the lymphoma supportive microenvironment.[101] This concept provides a mechanistic understanding of the typical clinical response to BAK inhibitor treatment, which is characterized by a long-lasting increase of peripheral blood lymphoid cells, due to a redistribution from lymphoid homing compartments.

PI3K inhibitors

Idelalisib. Class I PI3Ks regulate cellular functions relevant to oncogenesis.[102] Expression of the PI3K p110 δ isoform (PI3K-δ) is restricted to cells of hematopoietic origin where it plays a key role in B cell proliferation and survival. In CLL, the PI3K pathway is constitutively activated and dependent on PI3K δ.[103] Idelalisib is an oral PI3Kδ-isoform-selective inhibitor, which promotes apoptosis in primary CLL cells in a time- and dose-dependent manner without inducing apoptosis in normal T cells or natural killer cells and without diminishing ADCC. Idelalisib inhibits CLL cell chemotaxis toward CXCL12 and CXCL13 and migration beneath stromal cells (pseudoemperipolesis). Idelalisib also down-regulates secretion of chemokines in stromal cocultures and after BCR triggering.[103] Idelalisib reduces survival signals derived from the BCR or from nurse-like cells, and inhibits BCR- and chemokine-receptor-induced AKT and MAP kinase (ERK) activation.[103]

In a phase 1 trial, idelalisib was evaluated in 54 patients with relapsed/refractory CLL with adverse characteristics, including bulky lymphadenopathy (80%), extensive prior therapy (median 5 [range 2–14] prior regimens), treatment-refractory disease (70%), unmutated IGHV (91%), and del(17p) and/or TP53 mutations (24%).[104] Patients were treated at six dose levels of oral idelalisib (range 50–350 mg once or twice daily) and remained on continuous therapy while deriving clinical benefit. The most commonly observed grade ≥3 adverse events (AEs) were pneumonia (20%), neutropenic fever (11%), and diarrhea (6%). Idelalisib treatment resulted in nodal responses in 81% of patients. The ORR was 72% and the median PFS 15.8 months.

Duvelisib. Duvelisib is an orally available inhibitor of both the delta and gamma isoform of PI3K. A phase 1 trial included 55 relapsed/refractory patients with CLL, 56% showed an ORR.[105] In the phase 3 trial, patients with relapsed/refractory CLL were randomized to receive duvelisib 25 mg twice daily or ofatumumab. Median PFS was 13 months with duvelisib compared to 10 months with ofatumumab.[106] The most frequent toxicities with duvelisib include hematologic toxicities, but also elevated transaminases and diarrhea in almost a quarter of patients, as well as pneumocystis jirovecii and cytomegalovirus infections. This suggests that the toxicity profile is similar to idelalisib. Duvelisib is approved for the treatment of CLL after at least two prior lines of therapy.

Umbralisib. Umbralisib is a dual inhibitor of PI3Kdelta and CK1ε that has shown good efficacy in relapsed/refractory CLL with 62% ORR in an early phase 1 study in combination with the CD20 antibody ublituximab.[107] Subsequent phase 2 studies confirmed its activity in CLL[108] and showed a low rate of transaminitis (2%–3%) or diarrhea (3%–10%). Otherwise, the overall toxicity profile was similar to idelalisib or duvelisib. Recently, the UNITY study explored umbralisib in combination with ublituximab (U2 regimen) in treatment-naïve as well as relapsed/refractory CLL and reported a median PFS of 32 versus 18 months in treatment-naïve CLL after a median follow-up of 36 months.[109] Transaminitis, diarrhea, and pneumonitis occurred more frequently with umbralisib than with CLB (8% vs. 2%, 12% vs. 3%, and 3% vs. 0%). In light of the limited efficacy in the frontline setting and the toxicity profile, the U2 regimen, which is not yet approved for CLL, should be considered for later lines of therapy, similar to other PI3K inhibitors.

BTK inhibitors

BTK leads to downstream activation of B-cell survival pathways such as NF-κB and MAP kinases via Src family kinases.[110] These pathways play a relevant role in the signal transduction of the BCR. Inhibitors of BTK have become a new class of very active therapeutic agents in B-cell malignancies.[111]

Ibrutinib. Ibrutinib is an orally active, small molecule BTK inhibitor that induces apoptosis in B-cell lymphomas and CLL-cells.[110] In one of the first trials, 56 patients with relapsed or refractory B-cell lymphoma and CLL received escalating oral doses of ibrutinib, at two schedules: one, 28 days on, 7 days off; and two, once-daily continuous dosing. The ORR in 50 evaluable patients was 60%, including 16% CR. Median PFS in all patients was 13.6 months.[112] The most relevant treatment-related side effects were viral infections.

Thereafter, ibrutinib was investigated in 85 patients with relapsed or refractory CLL or SLL, the majority of whom with high-risk

disease.[113] Fifty-one patients received a daily dose of 420 mg ibrutinib, and 34 patients, a dose of 840 mg. In this early study, the reported side effects were predominantly grade 1 or 2 and included transient diarrhea, fatigue, and upper respiratory tract infection. Interestingly, in this study, there was no recognition of cardio-vascular events or bleeding episodes, potentially due to the unexpected nature of these events that remained, therefore, unrecognized. The ORR was the same in both dose groups, 420 and 840 mg (71%). An additional 20% and 15% of patients in the respective groups had a PR with lymphocytosis. An important finding was that the response seemed independent of clinical and genomic risk factors, including advanced-stage disease, the number of previous therapies, and the presence of a *del (17p)*. At 26 months, the estimated PFS rate was 75% and the rate of OS was 83%. These results were encouraging in that ibrutinib yielded durable remissions in CLL/SLL patients with relapsed, refractory or high-risk disease.

Ahn et al. reported a long term observation of 34 patients who had CLL with *TP53* alterations and were treated with ibrutinib as first-line therapy in the context of a phase 2 trial.[114] At 6 years of treatment, the estimated percentage of patients with PFS and OS was 61% and 79%, respectively. Of the 12 patients who had disease progression while receiving ibrutinib, 4 had histologic transformation and 8 had progressive CLL. The data indicate that BTK inhibitor monotherapy holds the potential to control high-risk, *TP53* aberrant CLL over extended periods of time in some patients. It should be noted, however, that genetic *TP53* aberrations remain an unfavorable prognostic factor in the context of continuous BTK inhibitor monotherapy when compared to other factors.[58,115]

Ibrutinib was compared to ofatumumab in phase 3 study.[116] Three hundred and ninety-one patients with relapsed or refractory CLL or SLL were included. At a median follow-up of 9.4 months, ibrutinib significantly improved PFS; the median duration was not reached in the ibrutinib group (with a rate of PFS of 88% at 6 months), as compared with a median of 8.1 months in the ofatumumab group ($p < .001$). Ibrutinib also significantly improved OS ($p = .005$). At 12 months, the OS rate was 90% in the ibrutinib group and 81% in the ofatumumab group.

As with any targeted treatment of cancer, this initial success was followed by the occurrence of resistance to ibrutinib, the mechanisms of which are now partially understood. Whole-exome sequencing studies in six patients with acquired resistance to ibrutinib therapy identified a cysteine-to-serine mutation in BTK at the binding site of ibrutinib in five patients and three distinct mutations in PLCgamma2 in two patients.[117] The C481S mutation of BTK results in a protein that is only reversibly inhibited by ibrutinib. The R665W and L845F mutations in PLCgamma2 are both potentially gain-of-function mutations leading to autonomous activity of the BCR stimulated pathways.

A large single-center analysis on 308 ibrutinib-treated patients determined the features associated with discontinuation of ibrutinib therapy and subsequent outcomes.[118] For patients who discontinued therapy because of disease progression, targeted deep sequencing was performed in samples at baseline and time of relapse. At a median follow-up of 20 months, 232 patients remained on therapy, 31 had discontinued because of disease progression, and 45 had discontinued for other reasons. Disease progression includes RT or progressive CLL. RT appeared to occur early and CLL progressions later (cumulative incidence at 12 months, 4.5% and 0.3%, respectively. Median survival following RT was 3.5 months only and 17.6 months following CLL progression. Sequencing on peripheral blood from eight patients with RT revealed two with mutations in BTK, and a lymph node sample showed no mutations in BTK or PLCgamma2. Deep sequencing on 11 patients with CLL progression revealed BTK or PLCgamma2 mutations in all. These mutations were not identified before treatment in any patient.

A later analysis of the same institution with a median follow-up time of 3.4 years showed a cumulative incidence of progression at 4 years of 19%.[119] Baseline karyotypic complexity, presence of del (17)(p13.1), and age less than 65 years were risk factors for progression. Among patients who experienced relapse, acquired mutations of BTK or PLCgamma2 were found in 85%. These mutations were detected at an estimated median of 9.3 months before relapse. Of a group of 112 patients examined prospectively, 8 patients have experienced relapse, and all of these patients had acquired resistance mutations before relapse. A resistance mutation was detected in an additional eight patients who did not meet criteria for clinical relapse. Together, these findings underscore the importance of the BCR pathway in the mechanism of action of ibrutinib in CLL. Moreover, these mutations may be detected prior to clinical relapse and serve as an anchor point for additional, targeted interventions.

The long-term follow-up of patients treated with ibrutinib in multiple clinical trials and registries revealed a distinct toxicity pattern of ibrutinib. This particularly relates to off-target effects and multi-kinase inhibition that lead to an increased risk of cardiac arrhythmia, in particular, atrial fibrillation (AF), cardiac failure, bleeding, and hypertension.[120–122] The occurrence of AF, which occurs typically in elderly patients with CLL regularly necessitates therapeutic anticoagulation, which potentially increases the risk of bleeding events. This cardiac toxicity does not seem to be associated with AF-associated thromboembolism or acute myocardial infarction.[123]

Acalabrutinib. Acalabrutinib (ACP-196) is a more selective, irreversible BTK inhibitor when compared to ibrutinib. It was designed to improve the safety and efficacy of BTK inhibitors. In a phase 1–2 study, 61 patients with relapsed CLL were treated with acalabrutinib at 100–400 mg once daily in the dose-escalation (phase 1) portion of the study and 100 mg twice daily in the expansion (phase 2) portion.[124] No dose-limiting toxic effects occurred during the dose-escalation portion of the study. An updated and expanded analysis of the study confirmed the efficacy, durability of response, and safety profile of acalabrutinib.[125] Overall, 134 patients with relapsed/refractory CLL or SLL received acalabrutinib 100 mg twice daily for a median of 41 months. Most AEs were mild or moderate, and were most commonly diarrhea (52%) and headache (51%). Grade ≥3 AEs (occurring in ≥5% of patients) were neutropenia (14%), pneumonia (11%), hypertension (7%), anemia (7%), and diarrhea (5%). AF and major bleeding AEs (all grades) occurred in 7% and 5% of patients,

respectively. The ORR was 94%; responses were similar regardless of genomic features (presence of del(11q), del(17p), complex karyotype, or IGHV mutation status). The estimated 45-month PFS was 62%. BTK mutations were detected in six of nine patients (67%) at relapse.

Acalabrutinib was also studied in 99 patients with treatment-naive CLL[126] with doses of 200 mg once daily, or 100 mg twice daily until progression or intolerance. Fifty-seven (62%) had unmutated IGHV genes, and 12 (18%) TP53 aberrations. After a median follow-up of 53 months, 85 patients remained on treatment; 14 discontinued treatment, mostly because of AEs ($n = 6$) or disease progression ($n = 3$). The ORR was 97% (7% complete responses), with similar outcomes among all prognostic subgroups. Because of improved trough BTK occupancy with twice-daily dosing, all patients were transitioned to 100 mg twice daily. Serious AEs were reported in 38 patients (38%). AEs required discontinuation in six patients (6%) because of second primary cancers ($n = 4$) and infection ($n = 2$). Grade ≥3 events of special interest included infection (15%), hypertension (11%), bleeding events (3%), and AF (2%).

Phase 2 studies of acalabrutinib were also conducted in patients with relapsed/refractory CLL who were ibrutinib-intolerant and had continued disease activity. In one study,[127] intolerance was defined as having discontinued ibrutinib due to persistent grade 3/4 AEs or persistent/recurrent grade 2 AEs despite dose modification/interruption. Treatment consisted of oral acalabrutinib 100 mg twice daily until disease progression or intolerance. Sixty patients were treated. The ORR to acalabrutinib was 73% and three patients (5%) achieved CR. At median follow-up of 35 months, the median progression-free and OS were not reached; 24-month estimates were 72% and 81%, respectively. The most frequent AEs with acalabrutinib were diarrhea (53%), headache (42%), contusion (40%), dizziness (33%), upper respiratory tract infection (33%), and cough (30%). Most common reasons for acalabrutinib discontinuation were progressive disease (23%) and AEs (17%).

In another report, acalabrutinib (100 mg twice daily or 200 mg once daily) was tested in 33 patients with ibrutinib intolerance as determined by the investigators.[127] Patients had been treated with ibrutinib for a median of 11.6 months. The median time from ibrutinib discontinuation to acalabrutinib start was 47 days. After a median of 19.0 months, 23 patients remained on acalabrutinib, and 10 had discontinued (progressive disease, $n = 4$; AEs, $n = 3$). During acalabrutinib treatment, the most frequent AEs included diarrhea (58%), headache (39%), and cough (33%). Grade 3/4 AEs occurred in 58%, most commonly neutropenia (12%) and thrombocytopenia (9%). Of 61 ibrutinib-related AEs associated with intolerance, 72% did not recur and 13% recurred at a lower grade with acalabrutinib. The ORR was 76%, including 1 complete, 19 PRs, and 5 PRs with lymphocytosis. Among 25 responders, median duration of response and PFS was not reached; 1-year PFS was 83.4% (95% confidence interval, 64.5%–92.7%). A similar pattern was observed in a separate phase 2 study, which confirmed good tolerance and high response to acalabrutinib after ibrutinib intolerance.[128] Together, these studies indicate that acalabrutinib may be beneficial for patients who are ibrutinib intolerant.

Following these encouraging results, acalabrutinib was then investigated in a randomized, open-label, phase 3 study in relapsed/refractory CLL comparing acalabrutinib monotherapy to idelalisib plus rituximab [I-R] or bendamustine plus rituximab (BR), depending on the choice of investigator.[129] Three hundred and ten patients with a median of two prior therapies received acalabrutinib monotherapy ($n = 155$) versus investigator's choice ($n = 155$; I-R, $n = 119$; BR, $n = 36$). After a median follow-up of 16.1 months, median PFS was significantly longer with acalabrutinib monotherapy (PFS not reached) compared with investigator's choice (16.5 months). Serious AEs occurred in 29% of patients treated with acalabrutinib monotherapy, 56% with I-R, and 26% with BR. Deaths occurred in 10% ($n = 15$ of 154), 11% ($n = 13$ of 118), and 14% ($n = 5$ of 35) of patients receiving acalabrutinib monotherapy, I-R, and BR, respectively. As these results were quite promising, acalabrutinib monotherapy was established and approved for the treatment of relapsed/refractory CLL.

In order to establish whether the higher specificity of acalabrutinib leads to clinically meaningful reductions of ibrutinib-related toxicity, a phase 3 ELEVATE-RR study was conducted in patients with high-risk CLL (defined as presence of del(17p) and/or del(11q)) and at least one prior line of therapy. Patients were randomized to receive either ibrutinib (420 mg/day) or acalabrutinib (100 mg twice daily) until nontolerance or disease progression. The primary study hypothesis was noninferiority regarding PFS of acalabrutinib compared to ibrutinib. Key secondary endpoints included cardiovascular toxicity, including AF. The study met its primary endpoint with a median PFS of 38.4 months in both arms, respectively.[130] AF/flutter of any grade occurred in 16% of patients in the ibrutinib arm and 9% in the acalabrutinib arm ($p = .02$). While bleeding remained a frequent AE with acalabrutinib, its relative frequency was significantly lower than with ibrutinib (38% versus 51%), whereas hypertension was a less frequent event with acalabrutinib (9% versus 23%). Hence, acalabrutinib seems to show a favorable toxicity profile compared with ibrutinib.

Pirtobrutinib. Pirtobrutinib (LOXO-305) is a highly selective, but reversible (non-covalent) BTK inhibitor, which also has activity in patients with C481S mutation of BTK. In a recent phase 1/2 trial, 323 patients with previously treated B-cell malignancies were treated with pirtobrutinib across seven dose levels (25, 50, 100, 150, 200, 250, and 300 mg once per day).[131] No dose-limiting toxicities were reported, and the maximum tolerated dose was not reached. The study continued with a recommended phase 2 dose of 200 mg/day. AEs occurring in at least 10% of 323 patients were fatigue (65 [20%]), diarrhea (55 [17%]), and contusion (42 [13%]). The most common AE of grade 3 or higher was neutropenia (32 [10%]). Of particular importance, the study did not report any grade 3 AF or flutter. A grade 3 hemorrhage was observed in one patient in the setting of mechanical trauma. Only five (1%) patients discontinued treatment due to a treatment-related AE. In 121 efficacy evaluable CLL or SLL patients who had received a covalent BTK inhibitor prior to the study, the ORR with pirtobrutinib was 62%. The ORR was similar in patients with CLL with previous covalent BTK inhibitor resistance (53 [67%] of

79), covalent BTK inhibitor intolerance (22 [52%] of 42), BTK C481-mutant (17 [71%] of 24), and BTK wild-type (43 [66%] of 65) disease. The results indicate that reversible BTK inhibitors such as pirtobrutinib might address a growing unmet need for patients with intolerance of or resistance to conventional BTK inhibitors.

Zanubrutinib. Like acalabrutinib, zanubrutinib is a second-generation, covalent BTK inhibitor with higher specificity and less off-target inhibition than ibrutinib. It was initially tested in a phase 1 study of various B cell malignancies.[132] Additional data were gained from a phase 2 trial using zanubrutinib 160 mg twice daily in 91 Chinese patients with relapsed CLL.[133] The study reported an ORR of 82%–86% in patients with low- and high-risk CLL. While bleeding-associated AEs, including petechiae or contusions, were quite common (35%), AF was not observed. To perform a head-to-head comparison between ibrutinib and zanubrutinib, the ALPINE phase 3 study included patients with relapsed/refractory CLL, who were treated with zanubrutinib or ibrutinib until nontolerance or disease progression.[134] The primary hypothesis was superiority of zanubrutinib compared to ibrutinib in terms of ORR, excluding those patients with PR with lymphocytosis. After a median follow-up of 15 months, a statistically significantly higher, modified ORR of 78% versus 63% was observed. A pre-planned subgroup analysis also indicated a longer PFS with zanubrutinib, although the follow-up was short and the analysis excluded approximately 200 patients. AF occurred less frequently with zanubrutinib than with ibrutinib (3% vs. 10%), while the rates of bleeding or hypertension were not different (2%–9% vs. 3.9%; 17% vs. 11%). Overall, the studies so far demonstrate a high activity of zanubrutinib and indicate a lower rate of AF than with ibrutinib.

Phase 3 trials comparing BTK inhibitor monotherapy with combination therapies

Ibrutinib plus anti-CD20 antibodies. Several phase 3 studies have compared targeted agents (alone or in combination) to conventional chemoimmunotherapy (Table 3). The RESONATE-2 trial established ibrutinib monotherapy as a first-line option in patients with CLL by demonstrating a significant improvement in survival.[135] The results were impressive, especially for patients with CLL with high-risk genetics. However, the control arm (CLB monotherapy) was no longer considered appropriately potent. Therefore, newer trials compared ibrutinib to more potent therapies.

The intergroup trial E1912 of the Eastern Cooperative Oncology Group (ECOG) and the American College of Radiology Imaging Network (ACRIN) compared an indefinite ibrutinib-based treatment with fludarabine, cyclophosphamide, and rituximab (FCR).[136] Five hundred and twenty-nine patients with previously untreated CLL of 70 years or younger were randomly assigned (2:1 ratio) to receive either ibrutinib and rituximab for six cycles (after a single cycle of ibrutinib alone), followed by ibrutinib until disease progression, or six cycles of FCR chemoimmunotherapy. Results of PFS favored ibrutinib-rituximab over chemoimmunotherapy (89.4% vs. 72.9% at 3 years), as well as the analysis of OS (98.8% vs. 91.5% at 3 years). In patients without IGHV mutation, ibrutinib-rituximab resulted in better PFS than chemoimmunotherapy (90.7% vs. 62.5% at 3 years). The 3-year PFS among patients with IGHV mutation was similar in both groups. The incidence of AEs of grade 3 or higher was similar in both groups. Infectious complications of grade 3 or higher were less common with ibrutinib-rituximab than with chemoimmunotherapy (10.5% vs. 20.3%).

This important trial has led to a reassessment of the first-line treatment recommendations for young, fit patients with CLL, because PFS and OS were improved by ibrutinib in most subgroups except in IGHV mutated patients. However, the relatively short follow-up time and the very small number of events still justify some caution. Moreover, a surprisingly high number of early deaths from CLL was observed in the FCR arm, raising the question of appropriate second-line therapy. Longer follow-up is needed to consolidate the recommendation of the first-line use of ibrutinib in fit, young patients with CLL.

Woyach et al. compared ibrutinib alone or in combination with rituximab to a first-line therapy with bendamustine and rituximab (BR) for patients with CLL ≥ 65 years of age.[115] The study showed a superior PFS for ibrutinib and IR compared to BR. The addition of rituximab to ibrutinib did not result in prolonged PFS. There was no significant PFS advantage observed in patients with mutated IGHV. No OS benefit was seen for any of the arms.

The Illuminate study tested CLB-obinutuzumab against a combination of ibrutinib and obinutuzumab in elderly and comorbid patients.[137] This combination had shown promising results with MRD-negative responses in a phase 2 trial.[138] The Illuminate study produced a significant PFS benefit for the combination of ibrutinib and obinutuzumab versus CLB-obinutuzumab. As the study did not contain an ibrutinib monotherapy arm, the benefit of adding obinutuzumab to ibrutinib remains unclear.

Acalabrutinib plus Obinutuzumab. A recent analysis and phase 1b/2 study generated some rationale of combining acalabrutinib and obinutuzumab[139] in 19 treatment-naive and 26 relapsed/refractory patients with CLL, who were treated with acalabrutinib (100 mg twice daily) until progression and obinutuzumab (cycle 1: 100 mg day 1, 900 mg day 2, 1000 mg days 8 and 15; cycles 2–6: 1000 mg day 1). Grade 3/4 AEs occurred in 71% of patients. ORRs were 95% (treatment-naive) and 92% (relapsed/refractory). Thirty-two percent of treatment-naive and 8% of relapsed/refractory patients achieved CR. At 36 months, 94% (treatment-naive) and 88% (relapsed/refractory) were progression-free. These results support the evaluation of this combination in larger comparative studies in CLL.

The ELEVATE-TN trial compared acalabrutinib (100 mg twice daily) with or without obinutuzumab against CLB with obinutuzumab in 535 patients with treatment-naive CLL (aged 65 years or older, or younger than 65 years with creatinine clearance of 30–69 mL/min or CIRS >6).[140] Importantly, patients with significant cardiovascular disease were excluded, and concomitant treatment with warfarin or equivalent vitamin K antagonists was prohibited. Treatments were administered in 28-day cycles. To reduce infusion-related reactions, acalabrutinib was administered for one cycle before obinutuzumab

TABLE 3 Randomized studies using targeted agents ibrutinib, idelalisib, or venetoclax, alone or in combination, as *first or second-line* therapy for chronic lymphocytic leukemia (CLL)

Treatment	*N*	Age[a]	ORR	CR %	PR %	uMRD, %	PFS[b]	2 years-PFS	2 years-OS	References
Randomized studies in first-line treatment										
Ibrutinib	136	73	86%	4%	82	NA	NR	89%	98%	Burger et al.[135]
Chlorambucil (CLB)	133	72	35%	2%	22	NA	18.9	34%	85%	
Ibrutinib + rituximab	354	58	NA	NA	NA	NA	NA	3 years: 89%	NA	Shanafelt et al.[136]
FCR	175	57	NA	NA	NA	NA	NA	3 years: 73%	NA	
Ibrutinib	182	71	93%	7%	NA	1%	NR	87%	90%	Woyach et al.[115]
Ibrutinib + rituximab	182	71	94%	12%	NA	4%	NR	88%	94%	
BR	183	70	81%	26%	NA	8%	41.0	74%	95%	
Ibrutinib + obinutuzumab	113	70	88%	19%	69%	35%	NR	30 months: 79%	30 months-OS: 86%	Moreno et al.[137]
CLB + obinutuzumab	116	72	73%	8%	66%	25%	19.0	30 months: 31%	30 months-OS: 85%	
Venetoclax + obinutuzumab	216	72	85%	50%	35%	76%	NR	88%	92%	Fischer et al.[220]
CLB + obinutuzumab	216	71	71%	23%	48%	35%	NR	64%	93%	
Randomized studies in treatment of relapsed/refractory CLL										
BR + ibrutinib	289	64	83%	10%	72%	26%	NR	18 months: 79%	3 years-OS: 82%	Chanan-Khan et al.[211,247]
BR	289	63	68%	3%	65%	6%	13.3	18 months: 24%	3 years-OS: 73%	
Venetoclax + rituximab	194	65	92%	8%	84%	62%	NR	85%	92%	Seymour et al.[216]
BR	195	65	72%	4%	69%	13%	17.0	63%	87%	
Idelalisib + rituximab	110	71	81%	0	81%	NA	NR	6 months: 93%	1 year-OS: 92%	Furman et al.[202]
Rituximab	110	71	13%	0	13%	NA	5.5	6 months: 46%	1 year-OS: 80%	
BR + idelalisib	207	62	70%	1%	69%	NA	20.8	NA	NA	Zelenetz et al.[248]
BR	209	64	45%	0	44%	NA	11.1	NA	NA	

Note: Only fully published studies are listed.
Abbreviations: BR, bendamustine, rituximab; CR %, complete response rate; FCR, fludarabine, cyclophosphamide, rituximab; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PR % partial response rate; uMRD %, rate of patients with undetectable MRD ($<10^{-4}$) in PB.
[a]Median, years.
[b]Median, months.

administration. At median follow-up of 28.3 months, median PFS was longer with acalabrutinib-obinutuzumab and acalabrutinib monotherapy compared with obinutuzumab-CLB (median not reached with acalabrutinib and obinutuzumab vs. 22.6 months with obinutuzumab; and not reached with acalabrutinib monotherapy vs. 22.6 months with obinutuzumab). Estimated PFS at 24 months was 93% with acalabrutinib-obinutuzumab, 87% with acalabrutinib monotherapy, and 47% with obinutuzumab-CLB. The most common grade 3 or higher AE across groups was neutropenia (30% in the acalabrutinib-obinutuzumab group, 9% in the acalabrutinib group, and 41% in the obinutuzumab-CLB group). Infusion reactions were less frequent with acalabrutinib-obinutuzumab (13%) than obinutuzumab-CLB (40%). These results led to the recent approval of acalabrutinib (alone or in combination with obinutuzumab) as first-line treatment of symptomatic CLL.

5.1.4 | Lenalidomide

Lenalidomide is a thalidomide analog with therapeutic activity in myelodysplastic syndrome and multiple myeloma. It showed encouraging results in the treatment of high-risk patients with CLL, including carriers of a *del(17p)*.[141] In 58% of patients with CLL lenalidomide causes a so-called tumor flare reaction, which leads to a sensation of heat and burning in the lymph nodes.[142,143] This phenomenon is much less frequently observed in other neoplasias. In CLL, the ORR of

lenalidomide monotherapy varied between 32% and 54%.[143,144] The long-term outcomes of 60 patients with CLL treated with lenalidomide were reported as a single-center experience.[145] At a median follow-up of 4 years, time-to-treatment failure was reached, with an OS of 82%. Thirty-five (58%) patients had a response lasting >36 months (long-term responders [LTRs]). Best LTR responses consisted of 25 (71%) CR and 10 (29%) PR. In addition to clinical responses, an increase in IgA, IgG, and IgM levels of >50% from baseline was reported in 61%, 45%, and 42% of LTRs. Normalization in the percentage of CD4+ and CD8+ cells and T-cell numbers was observed in 48%, 71%, and 99% of LTRs. Compared with other patients in the study, LTRs had lower baseline plasma levels of beta$_2$-microglobulin, were more likely to have trisomy 12, and were less likely to have a deletion 17p.

A promising approach seemed the use of lenalidomide as maintenance therapy in high-risk CLL. In one trial, patients with CLL with at least a PR after chemoimmunotherapy were eligible, if they had presence of MRD (at intermediate or high levels combined with an unmutated IGHV gene status or TP53 alterations).[146] While this approach was able to prolong the PFS substantially, it carried the risk of transformation to acute lymphoblastic leukemia.[147] Similar observations were made in a phase 3 study of lenalidomide versus placebo maintenance following second-line therapy, in which no OS was observed.[148] Lenalidomide is, therefore, not recommended as a maintenance therapy for CLL.

5.1.5 | BCL-2 inhibitors

Proteins in the B-cell lymphoma 2 (Bcl-2) family are key regulators of the apoptotic process.[149] The Bcl-2 family comprises proapoptotic and prosurvival proteins. Shifting the balance toward the latter is an established mechanism whereby cancer cells evade apoptosis. Bcl-2, the founding member of this protein family, is encoded by the BCL2 gene initially described in follicular lymphoma as a protein in translocations involving chromosomes 14 and 18.[150]

Venetoclax

Venetoclax is a BH3-mimetic drug designed to block the function of the Bcl-2 protein.[151] Venetoclax inhibits the growth of BCL-2 dependent tumors in vivo but spares human platelets. A single oral dose of venetoclax in three patients with refractory CLL resulted in tumor lysis within 24 h.[151] Therefore, a dose escalation scheme was installed to prevent these incidents,[152] with a weekly dose ramp-up schedule (20, 50, 100, 200, and 400 mg) over 4–5 weeks. Thereafter, patients should take daily 400 mg continuously dosing until disease progression or side effects occur.[153] In a pivotal phase 1/2 trial, 56 patients received venetoclax in one of eight dose groups that ranged from 150 to 1200 mg per day.[154] In an expansion cohort, 60 additional patients were treated with venetoclax using a weekly stepwise ramp-up in doses as high as 400 mg per day. The majority of the patients had received multiple previous treatments, and 89% had poor prognostic clinical or genetic features. Venetoclax was active at all dose levels. Clinical tumor lysis syndrome occurred in 3 of 56 patients in the dose-escalation cohort, with one death. After adjustments to the dose-escalation schedule, clinical tumor lysis syndrome did not occur in any of the 60 patients in the expansion cohort. Other toxic effects included mild diarrhea (in 52% of the patients), upper respiratory tract infection (in 48%), nausea (in 47%), and grade 3 or 4 neutropenia (in 41%). A maximum tolerated dose was not identified. Among the 116 patients who received venetoclax, 92 (79%) had a response. Response rates ranged from 71% to 79% among patients in subgroups with an adverse prognosis, including those with resistance to fludarabine, or del(17p) or unmutated IGHV. CR occurred in 20%, including 5% MRD negative remissions. The 15-month PFS estimate for the 400-mg dose groups was 69%.

Another trial was conducted in 107 patients with CLL with relapsed or refractory del(17p) CLL.[153] At a median follow-up of 12.1 months, an OR by independent review was achieved in 85 patients (79.4%). The most common grade 3–4 AEs were neutropenia (40%), infection (20%), anemia (18%), and thrombocytopenia (15%). Serious AEs occurred in 55% of patients, with the most common (≥5% of patients) being pyrexia and autoimmune hemolytic anemia (7% each), pneumonia (6%), and febrile neutropenia (5%). Eleven patients died in the study within 30 days of the last dose of venetoclax; seven due to disease progression and four from an AE (none assessed as treatment related). Taken together the results of the two trials show that venetoclax monotherapy is active and well tolerated in patients with relapsed or refractory del(17p) CLL, providing a new therapeutic option for this very poor prognosis population.

5.1.6 | Pembrolizumab and checkpoint inhibition

Preclinical evidence suggested that the programmed death 1 (PD-1) pathway is critical for inhibiting the immune surveillance of CLL. Therefore, a phase 2 trial was performed with pembrolizumab, a humanized PD-1-blocking antibody, at a dose of 200 mg every 3 weeks in relapsed and transformed CLL.[155] Twenty-five patients (16 relapsed CLL and 9 RTs) were enrolled, 60% received prior ibrutinib. Objective responses were observed in 4 out of 9 RT patients (44%) and in 0 out of 16 patients with CLL (0%). Treatment-related grade 3 or above AEs were reported in 15 (60%) patients and were manageable. Analyses of pretreatment tumor specimens from available patients revealed increased expression of PD-L1 and a trend of increased expression in PD-1 in the TME in patients who had confirmed responses. The results of this study suggest a benefit of PD-1 blockade in patients with CLL with RT. As the efficacy of monotherapy does not appear sufficiently durable,[156] several studies are exploring combinations of checkpoint inhibitors with kinase inhibitors for RT therapy, such as ibrutinib (NCT04781855) or zanubrutinib (NCT04271956).

5.1.7 | CART cells

An initial report using a lentiviral vector expressing a chimeric antigen receptor (CAR) with specificity for the B-cell antigen CD19, coupled with CD137 (a costimulatory receptor in T cells [4-1BB]) and CD3-zeta (a signal-transduction component of the T-cell antigen

receptor) signaling domains showed a very impressive efficacy.[157] A low dose (approximately 1.5×10^5 cells per kilogram of body weight) of autologous CAR-modified T cells reinfused into a patient with refractory CLL expanded to a level that was more than 1000 times as high as the initial engraftment level in vivo, with delayed development of a tumor lysis syndrome and subsequent CR.

An anti-CD19 CAR-T cell therapy was applied to 24 patients with CLL who had previously received ibrutinib.[158] Patients received lymphodepleting chemotherapy and anti-CD19 CAR-T cells at one of three dose levels (2×10^5, 2×10^6, or 2×10^7 CAR-T cells/kg). Four weeks after CAR-T cell infusion, the ORR was 71% (17 of 24). In 19 of these patients who were restaged, the ORR 4 weeks after infusion was 74% (CR, 4/19, 21%; PR, 10/19, 53%), and 15/17 patients (88%) with marrow disease before CAR-T cells had no disease by flow cytometry after CAR-T cells, and seven (58%) had no malignant IGH sequences detected in the bone marrow. Absence of the malignant IGH clone in marrow of patients with CLL who responded by international workshop on CLL (iwCLL) criteria was associated with 100% PFS and OS (median 6.6 months follow-up).

More recently, a longer follow-up of anti-CD19 chimeric antigen receptor T (CART) cell therapy was reported in patients with relapsed or refractory CLL.[159] Between 2013 and 2016, 42 patients with relapsed or refractory CLL were enrolled in this study and 38 were infused with anti-CD19 CART cells (CART-19). Of these, 28 patients were initially randomly assigned to receive a low (5×10^7) or high (5×10^8) dose of CART-19. Twenty-four patients were evaluable for response assessment. After an interim analysis, 10 additional patients received the selected, high dose and of these, 8 were evaluable for response. Patients were followed for a median of 31.5 months. At 4 weeks, the complete and ORs for the 32 evaluable patients were 28% and 44%, respectively. The median OS for all patients was 64 months; there was no statistically significant difference between low- and high-dose groups ($p = .84$). Regardless of dose, prolonged survival was observed in patients who achieved a CR versus those who did not ($p = .035$), with median OS not reached in patients with CR versus 64 months in those without CR. The median PFS was 40.2 months in patients with CR and 1 month in those without a CR. Toxicity was comparable in both dose groups. The results illustrate that attainment of a CR after CART-19 infusion is associated with longer OS and PFS in patients with relapsed CLL. Multiple other CART constructs are under development for treatment of CLL. Recently, the TRANSCEND-CLL 004 study reported its first readout of patients with relapsed/refractory CLL, who were either treated with liso-cel as a monotherapy given at equal doses of CD8+ and CD4+ CART cells (23 patients) or liso-cel in combination with ibrutinib with the aim of improving engraftment by the BTK inhibitors (19 patients). An ORR >90% was reported in both cohorts and uMRD was observed in >70% of evaluable patients.[160,161]

Overall, these observations highlight the potential of CD19 CAR-T cells in CLL, but more substantial clinical studies need to be performed before recommending this modality on a broader basis or outside of clinical trials for relapsed or refractory patients with CLL.

As a principle, some of the most relevant advances in CLL treatment have been achieved by the combined use of different treatment modalities. The subsequent sections will summarize the most relevant results obtained with different drug combinations in CLL.

5.2 | Combination therapies

One of the key principles of designing more efficient treatments of CLL has been the use of drug combinations with synergistic or at least additive efficacy but nonoverlapping toxicity. This principle has recently been expanded to the use of targeted agents that usually do not have identical toxicity profiles and hold the promise of a long-term control of CLL following a short, fixed-duration treatment with the most potent inhibitors.[69,162]

5.2.1 | Chemotherapy combinations

Since purine analogs and alkylating agents have different mechanisms of action and partially nonoverlapping toxicity profiles, it seemed logical to combine the two modalities for achieving synergistic effects. Preclinical studies demonstrated that exposure of CLL cells to fludarabine and cyclophosphamide resulted in synergistic cytotoxicity.[163] Fludarabine has been evaluated in a variety of combination regimens. The combination of fludarabine with another purine analog, cytarabine, appeared to be less effective than fludarabine alone, while the combination of fludarabine with CLB or prednisone increased the hematological toxicity but not the response rate.[72,164] The most thoroughly studied combination chemotherapy for CLL is **fludarabine plus cyclophosphamide (FC)**, which generated very promising results in phase 2 trials.[164,165] A Phase 2 study of cladribine in combination with cyclophosphamide also demonstrated activity in advanced CLL, but the results seemed inferior to FC.[166]

Later, three randomized trials showed that FC combination chemotherapy improves the CR and ORR and PFS as compared to fludarabine monotherapy.[167–169] The rate of severe infections was not significantly increased by the FC combination despite a higher frequency of neutropenias. A re-analysis of the CLL4 trial of the GCLLSG suggested that the first-line treatment of patients with CLL with FC combination may improve the OS of the nonhigh risk patients with CLL (all patients *not* exhibiting a *del(17p)* or *TP53* mutation).

A Polish study group compared 2-CdA alone to 2-CdA combined with cyclophosphamide (CC) or to cyclophosphamide and mitoxantrone (CMC) in 479 cases with untreated progressive CLL.[170] Surprisingly, the CC or CMC combination therapies did not produce any benefit in terms of PFS or response rates when compared to 2-CdA alone.

5.2.2 | Chemoimmunotherapy using rituximab

Since preclinical studies showed evidence for a synergy between rituximab and fludarabine,[171] rituximab combinations with fludarabine were investigated in phase 2 trials. A GCLLSG trial on 31 previously

treated or untreated patients with CLL showed 27 (87%) responses and 10 (32%) CR.[172] The CALGB 9712 protocol combined rituximab with fludarabine in either a sequential or concurrent regimen in a randomized study. Patients ($n = 104$) with previously untreated CLL received six cycles of fludarabine, with or without rituximab, followed by four once-weekly doses of rituximab.[173] Overall and complete response rates were higher in the concurrent group (90% and 47% vs. 77% and 28%). In a retrospective analysis, all patients of the CALGB 9712 protocol treated with fludarabine and rituximab were compared with 178 patients from the previous CALGB 9011 trial, who received only fludarabine.[174] The patients receiving fludarabine and rituximab had a better PFS and OS than patients receiving fludarabine alone. Two-year PFS probabilities were 67% versus 45% and 2-year OS probabilities were 93% versus 81%. Similarly, in a Phase 2 trial conducted at the MD Anderson Cancer Center on 300 patients with previously untreated CLL, **rituximab combined with fludarabine and cyclophosphamide (FCR)** achieved an ORR of 95%, with CR in 72%, nPR in 10%, PR due to cytopenia in 7%, and PR due to residual disease in 6%.[175] Six-year overall and failure-free survival was 77% and 51%, respectively. Median TTP was 80 months.

These results led the GCLLSG to conduct a randomized trial, the CLL8 protocol.[11] Eight hundred and seventeen patients (median age 61 years) with good physical fitness were randomly assigned to receive six courses of FC ($n = 409$) or FC plus rituximab (FCR) ($n = 408$). Sixty-four percent were at Binet stage B, 32% Binet C, and 5% Binet A. FCR induced a higher ORR than FC (92.8 versus 85.4%) and more CR (44.5 versus 22.9) ($p < .001$). PFS at 2 years was 76.6% in the FCR arm and 62.3% in the FC arm ($p < .01$). FCR treatment was more frequently associated with CTC grade 3 and 4 neutropenia (FCR 34%; FC 21%), while other side effects were not increased. Treatment-related mortality occurred in 2.0% in the FCR and 1.5% in the FC arm. A systematic analysis of prognostic factors, including molecular cytogenetics showed that the positive effect of FCR applied for most prognostic subgroups. However, FCR did not improve the survival of patients with a *del(17p)*. Similar results were obtained in a trial comparing FCR to FC in second-line treatment of CLL.[176] Two hundred and seventy-two patients were treated with FC and 274 with FCR. ORRs were 58% and 70% for FC and FCR, respectively, with 13% and 24.3% CR. TTF was 20.6 versus 30.6 months.

In recent up dates of the CLL8 trial and the MD Anderson patient cohort treated with FCR, a very good outcome was demonstrated for specific subgroups of patients, in particular, those with a mutated IGVH, del(13q), trisomy 12 or del(11q), or those patients achieving an MRD negative remission.[177,178] These patients seemed to achieve very durable remissions and a very good OS rate following FCR treatment. In the MD Anderson trial, a plateau was seen on the PFS curve in patients with IGHV-M, with no relapses beyond 10.4 years in 42 patients.[177]

A dose-modified FCR-Lite regimen was designed to decrease the toxicity of the FCR regimen.[179] This regimen reduced the dose of the two cytostatic agents, (fludarabine to 20 mg/m^2 and CC to 150 mg/m^2 days 2–4 during cycle 1 and days 1–3 in cycle 2–5) and increased the dose of rituximab (day 1 of cycle 1 at a dose of 375 mg/m^2; cycles 2–5 on day 1 at 500 mg/m^2 preceding chemotherapy and on day 14 of each cycle). Maintenance rituximab at 500 mg/m^2 was given every 3 months until progression. The CR rate was 77% for 50 previously untreated patients with CLL with an ORR of 100%. At a median follow-up of 2.4 years all complete responders remain in CR except for one patient who died of a myocardial infarction while still in remission. Five patients with PRs died within 2 years of completing FCR-Lite. Grade 3/4 neutropenia was documented in only 13% of cycles, which is lower than observed with the usual FCR regimen.

More recently, it has become popular to combine **bendamustine with rituximab (BR)**. The BR protocol was initially tested in 81 patients with relapsed CLL.[180] Patients received 70 mg/m^2 of bendamustine on days 1 and 2 and 375 mg/m^2 of rituximab on day 1 of the first cycle and 500 mg/m^2 on day 1 of subsequent cycles administered every 28 days for up to 6 cycles. On the basis of intent-to-treat analysis, the ORR was 59.0%. Complete response, PR, and nodular PR were achieved in 9.0%, 47.4%, and 2.6% of patients, respectively. ORR was 45.5% in fludarabine-refractory patients and 60.5% in fludarabine-sensitive patients. Among genetic subgroups, 92.3% of patients with *del(11q)*, 100% with trisomy 12, 7.1% with *del(17p)*, and 58.7% with unmutated IGHV status responded to treatment. After a median follow-up time of 24 months, the median event-free survival was 14.7 months. Severe infections occurred in 12.8% of patients. Grade 3 or 4 neutropenia, thrombocytopenia, and anemia were documented in 23.1%, 28.2%, and 16.6% of patients, respectively.

The BR regimen was also investigated as first-line therapy in 117 patients with CLL.[181] Bendamustine was administered at a dose of 90 mg/m^2 on day 1 and 2 combined with 375 mg/m^2 rituximab on day 0 of the first course and 500 mg/m^2 on day 1 during subsequent courses for up to six courses. In all, 117 patients, age 34–78 years, 46.2% of patients at Binet stage C, and 25.6% of patients age 70 years or older received BR chemoimmunotherapy for first-line treatment of CLL. ORR was 88.0% with a complete response rate of 23.1% and a PR rate of 64.9%. Ninety percent of patients with *del(11q)*, 94.7% with trisomy 12, 37.5% with *del(17p)*, and 89.4% with unmutated IGHV status responded to treatment. After a median observation time of 27.0 months, median event-free survival was 33.9 months, and 90.5% of patients were alive. Grade 3 or 4 severe infections occurred in 7.7% of patients. Grade 3 or 4 AEs for neutropenia, thrombocytopenia, and anemia were documented in 19.7%, 22.2%, and 19.7% of patients, respectively.

Using this information, the CLL10 study of the GCLLSG was designed to compare BR to FCR, each given for six cycles, as frontline therapy for fit patients with CLL without *del(17p)*.[182] Five hundred and sixty-one patients were included in the intention-to-treat population, 282 patients in the FCR group and 279 in the BR group. After a median observation time of 37.1 months, median PFS was 41.7 months with BR and 55.2 months with FCR, showing that BR was inferior to FCR. The number of patients achieving an MRD negative response was also higher for FCR than for BR. On the other hand, severe neutropenia and infections were more frequently observed with FCR (235 [84%] of 279 vs. 164 [59%] of 278, and 109 [39%] vs. 69 [25%], respectively) during the study. The increased frequency

of infectious complications with FCR was more pronounced in patients >65 years. In conclusion, the CLL10 study shows that FCR remains the standard therapy in very fit patients with CLL, because it yields higher CR rates, more MRD negativity and longer PFS when compared to BR. However, elderly fit patients with CLL might benefit from BR as alternative regimen.

Alemtuzumab and mitoxantrone have been added to FCR to further improve the efficacy of this regimen.[183,184] Since both regimen yielded limited improvements of therapeutic efficacy but a relevant increase of toxicity, their use is not justified outside of clinical trials. Similarly, attempts replace fludarabine in the FCR regimen by pentostatin (PCR) failed to show statistically significant improvements in response or infection rates.[185] Several other combinations have been investigated, like cladribine with rituximab, methylprednisolone plus rituximab followed by alemtuzumab, or rituximab plus alemtuzumab. Their detailed description is beyond the scope of this paper, since none of them has proven to result in higher efficacy compared to FCR.

5.2.3 | Chemoimmunotherapy using obinutuzumab

The CLL11 protocol of the GCLLSG investigated chemoimmunotherapies with anti-CD20 antibodies combined with a milder chemotherapeutic component, CLB, in previously untreated patients with CLL with comorbidities.[186] The rationale of this study was based on encouraging results of phase 2 trials using **CLB in combination with rituximab** (R-CLB)[187,188] and on the run-in phase of the CLL11 trial, where patients with CLL with increased comorbidity were treated with a **combination of CLB and obinutuzumab**.[189] In the CLL11 trial, 781 patients with previously untreated CLL and a score higher than 6 on the Cumulative Illness Rating Scale (CIRS) (range, 0–56, with higher scores indicating worse health status) or an estimated creatinine clearance of 30–69 mL per minute were assigned to receive CLB, obinutuzumab plus CLB, or rituximab plus CLB. The patients had a median age of 73 years, creatinine clearance of 62 mL/min, and a CIRS score of 8 at baseline. Treatment with obinutuzumab–CLB or R-CLB, as compared with CLB monotherapy, significantly increased response rates and prolonged PFS (median PFS, 26.7 months with obinutuzumab–CLB vs. 11.1 months with CLB alone; 16.3 months with R–CLB; $p < .001$). Treatment with obinutuzumab–CLB, as compared with CLB alone, prolonged OS ($p = .002$). Treatment with obinutuzumab–CLB, as compared with R-CLB, resulted in prolongation of PFS and higher rates of complete response (20.7% vs. 7.0%) and molecular response. Importantly, the final analysis of the CLL11 study revealed a significant OS advantage of obinutuzumab compared to rituximab.[190] Infusion-related reactions and neutropenia were more common with obinutuzumab–CLB than with R–CLB, but the risk of infection was not increased. Taken together, these results show that combining an anti-CD20 antibody with chemotherapy improved outcomes in patients with CLL and coexisting conditions. Moreover, in this patient population, obinutuzumab was superior to rituximab when combined with CLB.

5.2.4 | Chemoimmunotherapy using ofatumumab

Given the superior preclinical activity of Ofatumumab (O) compared to rituximab, it was assumed that the addition of this antibody to CLB would provide superior clinical outcomes in CLL. A randomized, open-label, phase 3 trial was conducted in 447 treatment-naïve patients with CLL (median age 69 years; range 35–92) who had active disease needing treatment, but in whom fludarabine-based treatment was not possible.[191] Median PFS was 22.4 months in the group assigned to CLB-O arm, compared with 13.1 months in the CLB group ($p < .0001$). Grade 3 or greater AEs were more common in the CLB-O group (109 [50%] patients; vs. 98 [43%] given CLB alone), with neutropenia being the most common event (56 [26%] vs. 32 [14%]). Grade 3 or greater infections had similar frequency in both groups. The results show that the addition of ofatumumab to CLB induces a relevant extension of the PFS in elderly patients with CLL.

5.2.5 | Chemoimmunotherapy using alemtuzumab

The synergistic activity of **fludarabine and alemtuzumab** was initially suggested by the induction of responses, including one CR, in 5 of 6 patients who were refractory to each agent alone,[192] and a subsequent phase 2 trial showed encouraging efficacy and safety.[193] The combination of alemtuzumab with rituximab has also been studied in patients with lymphoid malignancies, including those with refractory/relapsed CLL, producing an ORR of 52% (8% CR; 4% nodular PR, nPR; 40% PR).[194]

Two phase 3 trials tested alemtuzumab in combination with FC (FCA) or fludarabine (FA). FCA showed a much higher treatment-related mortality than FCR in first-line therapy and should not be given outside of clinical trials.[195] A second randomized trial compared FA to fludarabine monotherapy in previously treated patients with relapsed or refractory CLL.[196] In this trial, alemtuzumab was given intravenously (i.v.). FA ($n = 168$) resulted in better PFS than fludarabine monotherapy ($n = 167$; median 23.7 months vs. 16.5 months; $p = .0003$) and OS (median not reached vs. 52.9 months; $p = .021$) compared with fludarabine alone. Despite these interesting results, the use of this FA regimen in relapsed CLL has been widely replaced by the novel kinase or Bcl2 inhibitors.

5.2.6 | Combinations using lenalidomide

The combination of **lenalidomide and rituximab** seems to increase the response rate without increasing the toxicity, even in patients with *del (17p)* and/or unmutated IGHV-status. In a phase 2 trial, 59 patients with relapsed or refractory CLL received a combination of lenalidomide and rituximab.[197] In this trial, oral daily therapy with 10 mg lenalidomide was started on day 9 of cycle one. Rituximab was administered at 28-day intervals for up to 12 cycles; lenalidomide could continue indefinitely if patients benefited clinically. The ORR was 66%, including 12% complete responses and 12% nodular

PR. Time to treatment failure was 17.4 months. The most frequent grade 3 or 4 toxicity was neutropenia (73% of patients). Fourteen patients (24%) experienced a grade 3–4 infection or febrile episode. In essence, this combination is a helpful alternative for patients with refractory CLL and warrants further investigation.

The combination of lenalidomide, rituximab, and fludarabine in previously untreated patients with CLL yielded very relevant side effects.[198] The initially high toxicity rate observed with this regimen was potentially explained by a simultaneous start of all three drugs. Flinn et al.[199] tested a similar treatment regimen consisting of fludarabine, rituximab, and lenalidomide; 3 out of 4 patients who received all three drugs on day 1 experienced severe side effects. After amending the protocol, starting with lenalidomide on day 8 of the first cycle, the regimen was better tolerated. This observation was later confirmed.[200] Finally, the GCLLSG has investigated the combination of bendamustine, rituximab, and lenalidomide (BRL) in 17 relapsed or refractory (R/R) and 5 previously untreated (FL) patients with CLL.[201] The response rate was 47.1% in R/R and 60% in FL patients. Grade 3/4 hematological toxicity was observed in 71.4%, and severe infections in 47.6% of patients. Due to this high toxicity and the disappointingly low response rate of BRL, the trial was closed prematurely.[201]

5.2.7 | Combinations using idelalisib

The PI3K delta inhibitor, idelalisib, was investigated in a multicenter, randomized, double blind, placebo-controlled, phase 3 study, in combination with rituximab versus rituximab plus placebo.[202] The trial included 220 patients with decreased renal function, previous therapy-induced myelosuppression, or major coexisting illnesses to receive rituximab and either idelalisib (at a dose of 150 mg) or placebo twice daily. Patients receiving idelalisib versus those receiving placebo had improved rates of OR (81% vs. 13%; $p < .001$) and OS at 12 months (92% vs. 80%; $p = .02$). These results led to the approval of idelalisib and rituximab for patients with relapsed CLL who are unfit for receiving chemotherapy.

The long-term efficacy and safety of this treatment were reported in 110 patients who received at least one dose of the drug in the primary study, 75 of whom enrolled in the extension study with idelalisib monotherapy.[203] The idelalisib/rituximab-to-idelalisib group had a median PFS of 20.3 months. The ORR was 85.5% (94 of 110 patients; $n = 1$ complete response). The median OS was 40.6 and 34.6 months for patients randomly assigned to the idelalisib/rituximab and placebo/rituximab groups, respectively. Prolonged exposure to idelalisib increased the incidence of all-grade, grade 2, and grade 3 or greater diarrhea (46.4%, 17.3%, and 16.4%, respectively), all-grade and grade 3 or greater colitis (10.9% and 8.2%, respectively), and all-grade and grade 3 or greater pneumonitis (10.0% and 6.4%, respectively) but did not increase the incidence of elevated hepatic aminotransferases.

Idelalisib was tested also in combination with ofatumumab in 261 patients (median age 68 years) with 3 median previous therapies.[204] Median PFS was 16.3 months in the Idela-O group and 8.0 months in the ofatumumab group. The most frequent grade 3 or worse AEs in the Idela-O group were neutropenia (59 [34%] patients vs. 14 [16%] in the ofatumumab group), diarrhea (34 [20%] vs. one [1%]), and pneumonia (25 [14%] vs. seven [8%]). Serious infections were generally more common in the Idela-O group and included pneumonia (in 13% patients, sepsis in 6% and Pneumocystis jirovecii pneumonia in 5%).

These and additional data led to a warning of the FDA regarding the following toxicities, for which patients need to be monitored during under idelalisib therapy[205]: (1) Fatal and/or serious hepatotoxicity (in 16%–18% of idelalisib-treated patients). (2) Fatal and/or serious and severe diarrhea or colitis (14%–20%). (3) Fatal and/or serious pneumonitis (4%). (4) Fatal and/or serious infections (21%–48%). (5) Fatal and serious intestinal perforation. It should be noted that patients should be monitored in particular for opportunistic infections (CMV, Pneumocystis jirovecii). This safety profile has led to a somewhat reduced use of idelalisib in CLL, although the drug has some very useful features, in particular, in controlling high risk disease.[206]

5.2.8 | Combinations using ibrutinib

Despite preclinical findings suggesting that ibrutinib might antagonize the antibody-dependent cell killing by rituximab,[207,208] the combination of **ibrutinib with rituximab** was tested in patients with high-risk CLL.[209] Treatment consisted of 28-day cycles of once-daily ibrutinib 420 mg together with rituximab (375 mg/m^2, i.v., every week during cycle 1, then once per cycle until cycle 6), followed by continuous daily single-agent ibrutinib 420 mg until disease progression or until toxicities or complications precluded further treatment. Forty patients with CLL with high-risk disease features were enrolled, 20 of whom had del(17p) or TP53 mutations (16 previously treated, four untreated), 13 had relapsed CLL with del(11q), and 7 a PFS less than 36 months after first-line chemoimmunotherapy. Toxicity was mainly mild to moderate in severity (grade 1–2). Diarrhea occurred in 10 (25%) patients (grade 1 in nine patients and grade 2 in one), bleeding events in 14 (33%) patients (8 grade 1 and 5 grade 2), nausea or vomiting in 15 patients (38%) (10 grade 1 and 5 grade 2), and fatigue in 7 (18%) patients (4 grade 1 and 3 grade 2). Five patients (13%) had grade 3 infections (two lung infections, one upper respiratory tract infection, one sepsis, and one mucositis), and no grade 4 or 5 infections occurred. One patient had grade 4 neutropenia. A long-term follow-up (median 47 months) of this trial was recently reported.[210] At this time, the median duration on treatment was 41 months. ORR was 95%, and 9 patients (23%) were reported to show a CR. Twenty-one patients discontinued treatment, 10 due to disease progression, 9 for other causes, and 2 due to stem cell transplantation; the remaining 19 patients continue on ibrutinib. Median PFS for all patients was 45 months compared to 32 months in the subgroup of patients with del(17p) ($n = 21$, $p = .02$). Fourteen patients (35%) died, five from progressive disease, five from infections, and four from other causes. Median OS has not been reached. Taken together, the IR combination therapy leads to durable remissions in high-risk CLL.

The HELIOS trial was a phase 3 study conducted with 578 patients with active, relapsed or refractory CLL/SLL to receive six courses of BR combined with either ibrutinib (420 mg daily orally) or placebo given until disease progression or unacceptable toxicity.[211] At a median follow-up of 17 months, PFS was significantly improved in the ibrutinib group compared with the placebo group (not reached vs. 13.3 months; $p < .0001$). IRC-assessed PFS at 18 months was 79% in the ibrutinib group and 24% in the placebo group. The most frequent all-grade AEs were neutropenia and nausea. A total of 222 (77%) of 287 patients in the ibrutinib group and 212 (74%) of 287 patients in the placebo group reported grade 3–4 events; the most common grade 3–4 AEs in both groups were neutropenia and thrombocytopenia. A safety profile similar to that previously reported with ibrutinib and BR individually was noted. The results show that the addition of ibrutinib to BR results improves outcome with no new safety signals identified from the combination.

Another trial evaluated the combination of **ibrutinib with ofatumumab** in three different administration sequences.[212] Patients with CLL/SLL, PLL, or RT who failed at least two prior therapies were enrolled. Patients received ibrutinib 420 mg daily and 12 doses of ofatumumab 300/2000 mg in 3 schedules: ibrutinib lead-in (group 1; $n = 27$), concurrent start (group 2; $n = 20$), or ofatumumab lead-in (group 3; $n = 24$). Seventy-one patients were included, most with del (17p) (44%) or del(11q) (31%). The most common AEs (any grade) were diarrhea (70%), infusion-related reaction (45%), and peripheral sensory neuropathy (44%). ORRs in CLL/SLL patients ($n = 66$) were 100%, 79%, and 71% in groups 1, 2, and 3, respectively. Estimated 12-month PFS for all patients was 89%, 85%, and 75%, respectively. The results show a good tolerability and clinical activity of this combination, with durable responses.

5.2.9 | Combinations using venetoclax or other Bcl2-antagonists

In a first attempt to introduce Bcl2-antagonists into CLL therapies, oblimersen was tested in combination with fludarabine and CC in 241 patients with CLL.[213,214] This combination achieved deep responses (CR/nPR) of 17% compared to 7% in the chemotherapy-only group ($p = .025$). The most interesting result of this study was that the OS and the PFS were improved in patients that achieved at least a PR. This study already heralded the potential of combination therapies using Bcl-2 targeted agents.

A combination of **venetoclax and rituximab** was investigated in 49 patients with CLL with relapsed or refractory CLL or SLL and achieved encouraging results.[215] Overall, 42 (86%) of 49 patients achieved a response, including a complete response in 25 (51%) of 49 patients. Two-year estimates for PFS and ongoing response were 82% and 89%, respectively. Negative marrow MRD was achieved in 20 (80%) of 25 complete responders and 28 (57%) of 49 patients overall.

In the phase 3 Murano trial, 389 patients received venetoclax for up to 2 years (from day 1 of cycle 1) plus rituximab for the first 6 months (VR group) or BR for 6 months (BR).[216] At the 5-year follow-up, median PFS was 53.6 months in the VR arm and 17 months in the BR arm, with a significant 5-year-OS advantage for VR (82.1% vs. 62.2%).[217] The benefit was maintained across all clinical and biologic subgroups, including patients with del(17p). The rate of grade 3 or 4 neutropenia was higher in the VR group than in the BR group, but the rates of grade 3 or 4 febrile neutropenia and infections or infestations were lower with venetoclax than with bendamustine. These results established venetoclax plus rituximab as a new second-line treatment in CLL.

Venetoclax and obinutuzumab were initially evaluated in 12 patients with previously untreated CLL and coexisting medical conditions as part of a run-in phase of the CLL14 phase 3 protocol and showed very encouraging results,[218] in particular, an ORR of 100% and no detectable ($<10^{-4}$) MRD in peripheral blood in 11 or 12 patients. The full data of the CLL14 protocol, a phase 3 comparing a fixed-duration treatment with venetoclax and obinutuzumab in patients with previously untreated CLL and coexisting conditions and first follow-up analyses were recently published.[219,220] Patients with a score of greater than 6 on the CIRS or a calculated creatinine clearance of less than 70 mL per minute were randomly assigned to receive venetoclax-obinutuzumab or CLB-obinutuzumab. In total, 432 patients (median age, 72 years; median CIRS, 8; median creatinine clearance, 66.4 mL per minute) underwent randomization, with 216 assigned to each group. After a median follow-up of 52.4 months, the 4-year-PFS rate was significantly higher in the venetoclax-obinutuzumab arm than in the CLB-obinutuzumab arm (74% vs. 35%). Median PFS was not reached with venetoclax-obinutuzumab and was 36 months in the CLB-obinutuzumab arm. This benefit was also observed in patients with TP53 deletion, mutation, or both and in patients with unmutated immunoglobulin heavy-chain genes. Grade 3 or 4 neutropenia occurred in 52.8% of patients in the venetoclax-obinutuzumab group and in 48.1% of patients in the CLB-obinutuzumab group, and grade 3 or 4 infections occurred in 17.5% and 15.0%, respectively. One of the most important results of this trial was the very high rate of MRD negative remissions at 76% (peripheral blood) achieved in the venetoclax-obinutuzumab group.[220]

Two phase 2 trials testing combinations of **venetoclax plus ibrutinib** were published recently, partially with preliminary data. The CLARITY trial combined ibrutinib with venetoclax to eradicate detectable CLL in patients with relapsed or refractory CLL.[221] The primary end point was eradication of MRD after 12 months of combined therapy. In 53 patients after 12 months of ibrutinib plus venetoclax, MRD negativity was achieved in the blood of 28 (53%) and the marrow of 19 (36%). Forty-seven patients (89%) responded, and 27 (51%) achieved a CR. After a median follow-up of 21.1 months, one patient progressed, and all patients were alive. A single case of biochemical tumor lysis syndrome was observed. Other adverse effects were mild or manageable and most commonly were neutropenia or GI events.

Another phase 2 study of combined ibrutinib and venetoclax in a total of 80 previously untreated high-risk and older patients with CLL.[222] All patients had at least one of the following features: chromosome 17p deletion, mutated *TP53*, chromosome 11q deletion,

unmutated *IGHV*, or an age of 65 years or older. Patients received ibrutinib monotherapy (420 mg once daily) for 3 cycles, followed by the addition of venetoclax (weekly dose escalation to 400 mg once daily). Combined therapy was administered for 24 cycles. Response assessments were performed according to iwCLL 2008 criteria. MRD assessment was performed by multicolor flow cytometry in the bone marrow (sensitivity 10e-4). The median age was 65 years (range, 26–83). A total of 30% of the patients were 70 years of age or older. Overall, 92% of the patients had unmutated *IGHV*, *TP53* aberration, or del(11q). After 12 cycles of combined treatment, 88% of the patients had CR or CR with incomplete count recovery, and 61% had achieved MRD negativity. Three patients had laboratory evidence of tumor lysis syndrome. Both studies highlight the potential of combining venetoclax with ibrutinib.

The first data of a randomized trial comparing ibrutinib and venetoclax to CLB and obinutuzumab in previously untreated CLL/SLL was presented recently.[223] The study enrolled patients aged ≥65 years or 18–64 years with CIRS score >6 or creatinine clearance <70 mL/min. One hundred and six patients received 3 cycles of ibrutinib 420 mg/day, followed by 12 cycles ibrutinib plus venetoclax with a venetoclax ramp-up to 400 mg/day and 105 patients received 6 cycles of standard dose CLB plus obinutuzumab. Median age was 71.0 years (34.1% ≥75 years). With a median follow-up of 27.7 months, PFS for ibrutinib and venetoclax was superior to CLB and obinutuzumab. Median PFS was not reached for ibrutinib and venetoclax and 21.0 months for CLB and obinutuzumab. PFS improvement with ibrutinib and venetoclax versus CLB and obinutuzumab was consistent across predefined subgroups. The rate of uMRD was significantly higher for ibrutinib and venetoclax versus CLB and obinutuzumab in the bone marrow (51.9% vs. 17.1%) and peripheral blood (54.7% vs. 39.0%). The CR rate was significantly higher for ibrutinib and venetoclax versus CLB and obinutuzumab (38.7% vs. 11.4%). Most common grade ≥3 treatment-emergent AEs were neutropenia (34.9%), diarrhea (10.4%), and hypertension (7.5%) for ibrutinib and venetoclax, and neutropenia (49.5%), thrombocytopenia (20.0%), and pneumonia and tumor lysis syndrome (5.7% each) for CLB and obinutuzumab. Grade 5 AEs occurred in 7 pts on ibrutinib and venetoclax and 2 pts on CLB and obinutuzumab. At time of analysis, OS was immature, with 11 deaths in the ibrutinib and venetoclax arm and 12 in the CLB and obinutuzumab arm (HR 1.048). The current status of the data showing a relevant mortality and a lack of survival differences in the ibrutinib and venetoclax arm does not allow to firmly recommend ibrutinib and venetoclax as a standard first-line therapy for unfit CLL/SLL patients.

6 | SELECTING THE RIGHT TREATMENT: HOW TO TREAT CLL?

6.1 | Parameters to be considered

Given the impressive number of choices, the selection of the optimal treatment of a given CLL, a patient has become a task that requires experience, a good clinical judgment, and an appropriate use of diagnostic tools.

In addition to leukemia-related parameters, the newer agents may induce a number of specific effects. Therefore, the pre-existing comorbidities (e.g., cardiomyopathies, arrhythmia, renal failure), the comedication (e.g., CYP inhibitors, anticoagulants), and also the individual preference (time-limited vs. indefinite treatment), and finally even economic considerations need to be discussed with the patient when it comes to the decision of treatment initiation.

Despite its efficacy and widespread use, indefinite ibrutinib monotherapy of patients with CLL comes with some essential drawbacks: an increased financial burden, relatively high rates of cardiac arrhythmias as well as resistance mutations and rapid relapses after discontinuation of the drug in some cases.[224–226] Therefore it appears highly important to create fixed-duration combination therapies with venetoclax, ibrutinib, and/or obinutuzumab that aim to achieve MRD-negative, durable responses while being safe and tolerable (Table 2).

The following parameters should be considered before recommending a treatment for CLL:

1. The clinical stage of the disease
2. The symptoms of the patient.
3. The fitness and concomitant diseases of the patient, particular with regard to the potential organ toxicity of the newer, targeted agents.
4. The genetic risk of the leukemia.
5. The treatment situation (first versus second line, response versus nonresponse to the last treatment).

Using these five parameters, the following recommendations can be given:

6.2 | First-line treatment

In a patient with advanced (Binet C, Rai III-IV) or active, symptomatic disease (Table 3), treatment should be initiated. In this situation, patients should be evaluated for their physical condition (or comorbidity). For patients in good physical condition ("go go") as defined by a normal creatinine clearance and a low score at the "cumulative illness rating scale,"[227] chemoimmunotherapy with FCR can still be debated when long-term remissions or cure are the desired endpoint for the patient and the leukemic clone shows a mutated IGVH gene (Figure 2).

Patients with an impaired physical condition ("slow go") may be offered either venetoclax plus obinutuzumab or ibrutinib monotherapy or CLB plus obinutuzumab. Currently, there is still no clear evidence to favor one of these options, as no survival benefit has been documented for any of them. The potential side effects (e.g., AF, tumor lysis, and autoimmune disorders) or the desire to use a fixed-duration therapy should be discussed with the patient. The aim of therapy in this situation is symptom control.

Patients with *del(17p)* or *TP53* mutations represent a somewhat different category. No chemoimmunotherapy should be applied as other options (venetoclax and obinutuzumab, ibrutinib, and I-R; Figure 2) usually offer a good, although not definitive disease control.

In these patients, an allogeneic stem cell transplantation should be discussed at the first or second relapse.[228]

6.3 | Second-line treatment: intensification or optimal sequencing?

Figure 3 summarizes the principles of managing of patients at relapse according to the duration of remission and the physical fitness. As a general rule, the first-line therapy may be repeated, if the duration of the first remission exceeds 36 months.

The choice is entirely different in treatment-refractory CLL (as defined by an early relapse within 6 months after the last treatment), similar to relapsed cases with a chromosomal aberration *del (17p)*. By principle, the initial regimen should be changed since the second remission tends to be shorter and one of the potent second-line regimen should be selected. The following options exist:

1. Venetoclax in combination with rituximab for up to 2 years (or alone as a continuous therapy).
2. BTK inhibitors (ibrutinib, acalabrutinib) alone or combined with venetoclax.
3. Acalabrutinib combined with obinutuzumab.
4. PI3K inhibitors (idelalisib and rituximab, duvelisib, umbralisib, etc.).
5. Cellular therapies like CART cell therapy[229] or allogeneic stem cell transplantation with curative intent.[228]
6. Alemtuzumab alone or in combination.[93,193]

The choice of one of these options may depend on the molecular risk profile of the leukemia, the fitness and comorbidity of the patient, in particular, with regard to the potential side effects of the new drugs (cardiovascular disorders as a potential risk factor for ibrutinib; pulmonary disease and prior [CMV] infections as a potential risk factor for idelalisib, etc.), and the availability of the drugs in a given region of the world. According to recommendations of a European consensus group, physically fit patients with refractory CLL or with a *del(17p)* may be offered an allogeneic transplantation, if they relapse to one kinase inhibitor and respond to a second regimen.[228] Finally, it is important to emphasize that patients with refractory disease should be treated within clinical trials whenever possible.

7 | CURRENT CHALLENGES AND UNCERTAINTIES

As novel agents have emerged for the treatment of CLL, the **optimal sequencing and combination strategies** remain to be established for these agents. So-called "real-world" observations suggest that ibrutinib appears superior to idelalisib when used as first kinase inhibitor.[230] In the setting of ibrutinib failure, venetoclax therapy appears superior to both idelalisib and chemoimmunotherapy,[230,231] while patients refractory to venetoclax showed best outcomes when consequently treated with ibrutinib.[232,233] These data are largely derived from registries or retrospective cohort studies, lending support for randomized studies that test different sequencing strategies.

The sequenced application of single agents rarely leads to MRD-negative responses. In contrast, their combined application may induce deep and durable remissions with long-treatment-free intervals. One of these trial concepts uses sequential, targeted therapies to eradicate residual disease.[69,234] Moreover, combinations of all available drugs, as well as novel strategies to prevent clonal evolution of CLL need to be investigated[235,236] in order to achieve long-lasting remissions or even cure for patients with CLL. So far, results obtained by these combination therapies appear promising, in particular, when combining anti-CD20

CLL first line treatment

Stage	del(17p) or *TP53*mut	Fitness	IGHV	Therapy
Inactive disease, Binet A-B, Rai 0-II	Irrelevant	Irrelevant	Irrelevant	None
Active disease or Binet C or Rai III-IV	Yes	Irrelevant	Irrelevant	Ibrutinib/Acalabrutinib[1] or Venetoclax + Obinutuzumab or Idelalisib-Rituximab (if contraindications for other options)
	No	Go go	M	FCR (BR above 65 years) or Ibrutinib/Acalabrutinib[1] or Venetoclax + Obinutuzumab[2]
			U	Ibrutinib/Acalabrutinib[1] or Venetoclax+Obinutuzumab or FCR (BR above 65 years)
		Slow go	M	Venetoclax + Obinutuzumab or Ibrutinib/Acalabrutinib[1,2] or Chlorambucil-Obinutuzumab
			U	Venetoclax + Obinutuzumab or Ibrutinib/Acalabrutinib[1,2] + Chlorambucil-Obinutuzumab

1) Addition of obinutuzumab to acalabrutinib may be considered.
2) Consider and discuss with patient: Continuous versus fixed-duration therapy, specific side effects of drug classes (myelosuppression, infections, secondary malignancies for CIT; cardiac toxicity and bleeding for BTKi (Acalabrutinib < Ibrutinib); TLS and infections for Ven-Obi; autoimmune disease and opportunistic infections for Idelalisib.

FIGURE 2 Updated treatment algorithm for patients with chronic lymphocytic leukemia in first-line indications. M, mutated; U, unmutated

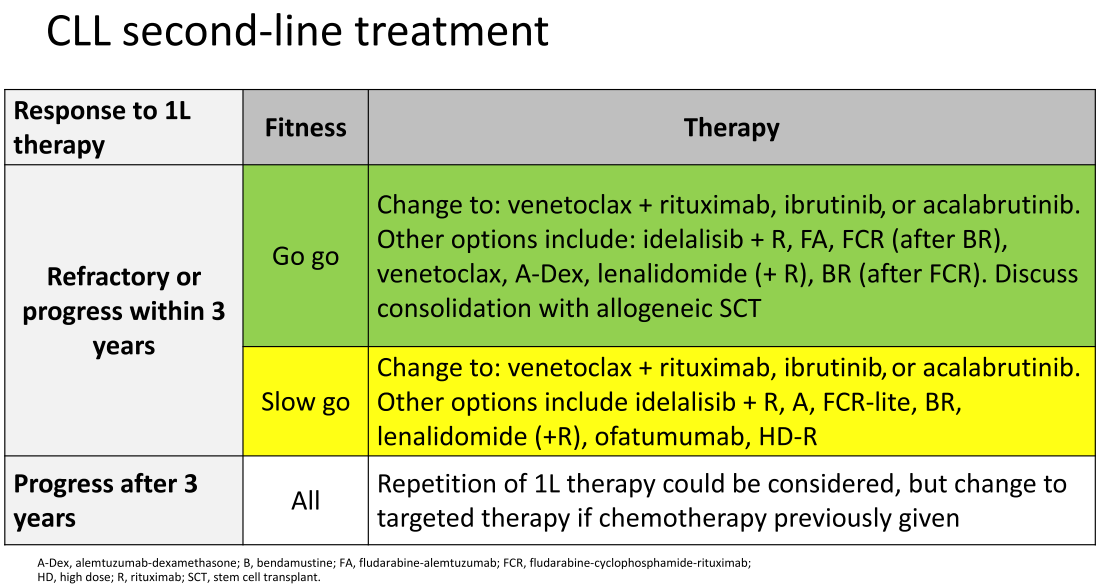

FIGURE 3 Updated treatment algorithm for patients with chronic lymphocytic leukemia in second-line indications. BR, Bendamustine + Rituximab; FCR, fludarabine, cyclophosphamide, rituximab. R, Ritxumab

antibodies with targeted agents.[138,212,215,216,218,221,222,237,238] While ibrutinib has been tested in combination with anti-CD20 antibodies and yielded high response rates, the choice of the antibody clearly has an impact on the efficacy. The time-limited combination treatments of ibrutinib and obinutuzumab showed an MRD-negativity rate of 48%, while ibrutinib and ofatumumab only yielded 14%. The CLL2-BAG protocol (bendamustine, venetoclax, and obinutuzumab) yielded excellent OR and MRD-negative response rates around 90% both in treatment naïve and pre-treated patients.[239] Similarly, the Murano trial produced MRD negative responses in 64% of the 130 patients who completed the 24-month venetoclax plus rituximab treatment, translating into significantly longer PFS.[216] Most importantly, these studies demonstrated that the majority of MRD negative remissions were sustained for more than 1 year after the end of study treatment.[239,240] Venetoclax and ibrutinib also appear to achieve deep remissions. Two phase 2 studies evaluating the use of this combination have been described above.[221,222] Another trial combined the three most promising, approved agents (obinutuzumab, ibrutinib, and venetoclax) yielding a rate of MRD-negative responses of 67% in the treatment-naïve cohort of the study.[241]

The biologically informed combination of targeted agents has paved the way for the development of regimens that induce **deep, MRD-negative remissions with the possibility to discontinue therapy**. This limited-duration treatment concept is different to continuous targeted therapies, in particular, with BTK inhibitors that rarely induce MRD-negative remissions, but achieve substantial disease control. It is so far unclear, which of the two paradigms creates the greatest benefit for patients with CLL or for specific subgroups, for example, patients with high-risk disease. The ongoing CLL17 study of the GCLLSG (NCT04608318) addresses this very important question by randomizing patients with previously untreated CLL to either ibrutinib continuous monotherapy, fixed-duration venetoclax-obinutuzumab, or fixed-duration venetoclax-ibrutinib.

When comparing the different trials for relapsed patients with CLL (Table 3), it becomes evident that all combinations using targeted agents (idelalisib, venetoclax, obinutuzumab, ibrutinib) are more potent than chemoimmunotherapy with regard to key variables of efficacy such ORR, CRs, MRD negative remissions, PFS, and OS. These results justify the broad use of targeted agents, alone or in combination for second-line therapy of CLL. It remains questionable, whether the addition of chemoimmunotherapy with BR to ibrutinib or idelalisib is of any substantial benefit.

Another use of kinase inhibitors may allow to enhance the function of T cells.[242] It was shown that ≥5 cycles of ibrutinib therapy improved the expansion of CD19-directed CAR T cells (CTL019), in association with a decreased expression of the immunosuppressive molecule programmed cell death 1 on T cells and of CD200 on B-CLL cells.[243] Two clinical studies recently showed that this effect can be translated into higher efficacy of CAR-T cells when combined with ibrutinib, yielding high response rates and a trend toward deeper remissions compared to CAR-T cell infusions alone.[244,245]

Finally, despite the tremendous progress in our understanding and treatment of CLL, new challenges are emerging. As the majority of patients treated with targeted agents are not cured, disease relapses will eventually occur after exposure to BTK, PI3K, or BCL2 inhibitors. In particular, salvage options for disease that is **refractory to BTK and BCL2 inhibitors** are limited, and the outcome of patients with double-refractory disease is quite poor.[246] For this group of patients, alternative therapeutic concepts that go beyond BCR or Bcl2 signaling pathways are highly needed.

In any case, the management of CLL will continue to undergo a very dynamic development. Therefore, it is important that we continue to work toward the long-term control of this disease by

including our patients in current clinical trials. Moreover, in such a fast-developing era of medicine bi-annually updated recommendations offer the possibility to constantly monitor and summarize the clinically relevant progress in CLL management.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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