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1. Explain the basic principles of CLL pathogenesis
2. Explain how to establish the diagnosis and to use clinical and genetic information to predict the prognosis of a given patient with CLL
3. Identify when treatment is indicated and to design the appropriate therapy for different patients with CLL

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ANNUAL CLINICAL UPDATES IN HEMATOLOGICAL MALIGNANCIES: A CONTINUING MEDICAL EDUCATION SERIES

Chronic lymphocytic leukemia: 2013 update on diagnosis, risk stratification and treatment

Michael Hallek*

***Disease Overview*: Chronic lymphocytic leukemia (CLL) is the commonest leukemia in western countries. The disease typically occurs in elderly patients and has a highly variable clinical course. Leukemic transformation is initiated by specific genomic alterations that impair apoptosis of 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 B-cell markers.**

***Prognosis*: Two prognostic staging systems exist, the Rai and Binet staging systems, which are established by physical examination and blood counts. Various biological and genetic markers also have prognostic value. Deletions of the short arm of chromosome 17 (*del(17p)*) predict resistance to most available therapies.**

***Therapy*: Patients with active or symptomatic disease or with advanced Binet or Rai stages require therapy. For physical fit patients, chemoimmunotherapy with fludarabine, cyclophosphamide and rituximab represents the current standard therapy. For unfit patients, treatment with an anti-CD20 antibody plus a milder chemotherapy (chlorambucil) is currently established as standard treatment. At relapse, the initial treatment may be repeated, if the treatment-free interval exceeds two years. If the disease relapses earlier, alternative therapies such as bendamustine alone or with rituximab, alemtuzumab, lenalidomide, or ofatumumab should be used. Patients with a del(17p) or TP53 should be considered for an allogeneic SCT.**

***Future Challenges*: Several new agents (e.g., ibrutinib, obinutuzumab) hold the potential to change standard of CLL treatment in the next 6–12 months. Therefore, CLL patients should be included into current clinical trials whenever possible. Am. J. Hematol. 88:804–816, 2013. © 2013 Wiley Periodicals, Inc.**

Introduction and Disease Overview

With an age-adjusted incidence of 4.1/100,000 inhabitants in the United States, chronic lymphocytic leukemia (CLL) is the most common type of leukemia in western countries. More than 15,000 newly diagnosed cases and ~4,500 deaths are currently estimated [1,2]. The median age at diagnosis lies between 67 and 72 years. More male than female patients (1.7:1) are affected [3–5]. As the incidence rate rises with age, the prevalence and mortality of CLL are likely to increase further due to the demographic changes in society in the forthcoming decades. Moreover, the proportion of younger patients with early stage CLL and minimal symptoms seems to increase due to more frequent blood testing [6].

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 [7]. Very recently, it has been reported that in CLL the capacity to generate clonal B cells might be acquired at the hematopoietic stem cell (HSC) stage [8], suggesting that the primary leukemogenic event in CLL might involve multipotent, self-renewing HSCs. The leukemic transformation is initiated by specific genomic alterations causing the deletion of specific micro-RNA genes and increasing the resistance of B cells towards apoptosis [9,10].

Deletions on the long arm of chromosome 13, specifically involving band 13q14 (*del(13q14)*) represent the single most frequently observed cytogenetic aberration in CLL, occurring in approx. 55% of all cases. An isolated *del(13q14)* is typically characterized by a benign course of the disease. The miRNAs, miR-15a and 16-1, were recently identified to be located in the critical region of *del(13q14)* [9]. The pathophysiologic role of these miRNAs is further underscored by the phenotype of genetically engineered mice carrying a targeted deletion of the *mir-15a/16-1* locus in combination with a deletion of the non-coding RNA gene *DLEU2*. These animals develop a monoclonal B-cell lymphocytosis-like disorder, CLL, and lymphoma, suggesting that the miRNAs 15a and 16-1 indeed play a role in CLL leukemogenesis [11].

Deletions of the long arm of chromosome 11 (*del(11q)*) can be found in ~25% of chemotherapy-naïve patients with advanced disease stages and 10% of patients with early stage disease [12,13]. 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 overall survival [14]. Interestingly, some of the poor prognostic features of *del(11q)* seem to be overcome by the use of chemoimmunotherapy [15].

Trisomy 12 is observed in 10–20% of CLL patients. However, the genes involved in the pathogenesis of CLLs carrying a trisomy 12 are largely unknown. Furthermore, the

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prognostic relevance of trisomy 12 remains a matter of debate [16].

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. CLL patients carrying a *del(17p)* clone show marked resistance against genotoxic chemotherapies that cannot be overcome by the addition of anti-CD20 antibodies in the context of state of the art chemo-immunotherapy [15]. Mutations of *TP53* are found in 4–37% of patients with CLL, and have been associated with very poor prognosis (ultra-high risk) in a number of studies [17]. Among cases with confirmed *del(17p)*, the majority show 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 [16]. *TP53* mutations are also associated with higher genomic complexity in CLL, indicating that a crippled DDR promotes a "mutator phenotype" in CLL [16].

The recently reported whole genome sequencing projects in CLL have revealed a number of recurrent somatic gene mutations that occur in parallel to the above-mentioned structural genomic aberrations. These include the genes *NOTCH1, MYD88, TP53, ATM, SF3B1, FBXW7, POT1, CHD2,* and others [13,18]. Of note, *TP53, ATM, POT1,* and *CHD2* encode for proteins critically involved in DNA damage signaling and DNA repair [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 [13,18]. This observation underscores the critical importance of the ATM-Chk2-p53 signaling axis in mediating apoptosis in response to DNA damage in CLL.

Survival of CLL cells strictly depends on a permissive microenvironment composed of cellular components like macrophages, T cells, or stromal follicular dendritic cells [20–22] providing stimuli for activation of crucial survival and proproliferative 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 [10,22,23].

In light of these important advances it is not surprising that the management of this leukemia is constantly undergoing important changes that have started ~20 years ago and still gain in dynamics [24]. Several new drugs have been approved (fludarabine, bendamustine as well as three monoclonal antibodies, alemtuzumab, rituximab, and ofatumumab). Chemoimmunotherapies composed of fludarabine and rituximab (with our without cyclophosphamide), or of fludarabine and alemtuzumab have shown to improve overall survival when used as therapy for CLL patients. In addition, several specific inhibitors interrupting important pathways for CLL cell survival are currently in the final phases of clinical development. It is the purpose of this educational review to integrate the latest knowledge on CLL therapy and diagnostic tools into a simple algorithm to guide the diagnostic and therapeutic decisions in daily practice.

Diagnosis

This section uses the recently updated iwCLL guidelines [25] (with minor modifications), which give very clear and concise recommendations on how to establish the diagnosis of CLL. In most cases the diagnosis of CLL is established by blood counts, differential counts, a blood smear, and immunophenotyping. The World Health Organization (WHO) classification of hematopoietic neoplasias describes CLL as leukemic, lymphocytic lymphoma, being only distinguishable from SLL (small lymphocytic lymphoma) by its leukemic appearance [26]. 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) [27].

The diagnosis of CLL requires the presence of $\geq$5,000 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 [28]. Finding prolymphocytes in excess of this percentage would favor a diagnosis of prolymphocytic leukemia (B-cell PLL). Gumprecht nuclear shadows, or smudge cells, found as cell debris, are other characteristic morphologic features found in CLL.

Monoclonal B lymphocytosis

In the absence of lymphadenopathy or organomegaly (as defined by physical examination or CT scans) [25], cytopenias, or disease-related symptoms, the presence of fewer than 5,000 B-lymphocytes per μL blood is defined as "monoclonal B-lymphocytosis" (MBL) [29]. 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 [30].

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 5,000/μL. In SLL, the diagnosis should be confirmed by histopathological evaluation of a lymph node biopsy whenever possible.

Immunophenotyping

CLL cells co-express the T-cell antigen CD5 and B-cell surface antigens CD19, CD20, and CD23 [25]. The levels of surface immunoglobulin (Ig), CD20, and CD79b are characteristically low compared to those found on normal B cells [31,32]. Each clone of leukemia cells is restricted to expression of either kappa or lambda immunoglobulin light chains [31]. Variations of the intensity of expression of these markers may exist and do not prevent inclusion of a patient in clinical trials for CLL. In contrast, B-cell PLL cells do not express CD5 in half of the cases, and typically express high levels of CD20 and surface Ig [33]. Also, the leukemia cells of mantle cell lymphoma, despite also expressing B cell surface antigens and CD5, generally do not express CD23.

Risk Stratification, Staging, and Indication for Treatment

Again, very concise definitions for the initiation of therapy have been proposed by the updated iwCLL guidelines [25] and are used here. Two widely accepted staging methods co-exist, the Rai [34] and the Binet system [35]. The original Rai classification was modified to reduce the number of prognostic groups from five to three [36]. Both systems describe three major prognostic groups with discrete clinical outcomes. These two staging systems are simple, inexpensive, and solely rely on a physical examination and standard laboratory tests. They do not require ultrasound, computed tomography, or magnetic resonance imaging.

The modified 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 Stages I or 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. (5) Palpable liver (clinically enlarged). Binet stages are defined as follows:

Stage A. Hb $\geq$ 10 g/dL and platelets $\geq 100 \times 10^9$/L and up to two of the above involved.

Stage B. Hb $\geq$ 10 g/dL and platelets $\geq 100 \times 10^9$/L and organomegaly greater than that defined for Stage A (i.e., three or more areas of nodal or organ enlargement).

Stage C. All patients who have Hb of less than 10 g/dL and/or a platelet count of less than 100×10^9/L, irrespective of organomegaly.

Criteria for initiating treatment may vary depending on whether or not the patient is treated in a clinical trial. In general practice, newly diagnosed patients with asymptomatic early-stage disease (Rai 0, Binet A), should be monitored without therapy unless they have evidence of disease progression. Studies from both the French Cooperative Group on CLL [37], the Cancer and Leukemia Group B (CALGB) [38], and the Spanish Group Pethema [39] in patients with early-stage disease showed that the use of alkylating agents did not prolong survival in this specific situation. This result was confirmed by a meta-analysis [40]. In one study, treated patients with early-stage disease had an increased frequency of fatal epithelial cancers compared with untreated patients [37]. Therefore, the potential benefit of an early-intervention therapy with anti-leukemia drugs remains to be proven.

Whereas patients at intermediate (Stages I and II) and high risk (Stages III and IV) according to the modified Rai classification or at Binet Stage B or C usually benefit from the initiation of treatment, some of these patients (in particular Rai intermediate risk or Binet stage B) can be monitored without therapy until they have evidence for progressive or symptomatic/active disease as defined by iwCLL guidelines [25]:

- evidence of progressive marrow failure (anemia and/or thrombocytopenia);
- massive (i.e., $\geq$ 6 cm below the left costal margin) or progressive or symptomatic splenomegaly;
- massive nodes (i.e., $\geq$ 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy;
- progressive lymphocytosis with an increase of $>$ 50% over a 2-month period;
- lymphocyte doubling time (LDT) of less than 6 months;
- autoimmune anemia and/or thrombocytopenia that is poorly responsive to corticosteroids or other standard therapy;
- disease-related symptoms such as unintentional weight loss $\geq$ 10% within the previous 6 months, significant fatigue, fevers of greater than 100.5°F or 38.0°C for 2 or more weeks without other evidence of infection; or night sweats for more than 1 month without evidence of infection.

Patients with initial blood lymphocyte counts of less than 30.000/μL may require a longer observation period to determine the LDT. Also, factors contributing to lymphocytosis or lymphadenopathy other than CLL (e.g., infections) should be excluded.

Patients with CLL may present with a markedly elevated leukocyte count; however, the symptoms associated with leukocyte aggregates that develop in patients with acute leukemia rarely occur in patients with CLL. Therefore, the absolute lymphocyte count should not be used as the sole indicator for treatment.

Several genetic markers, in particular some of the above defined genetic and chromosomal aberrations, have been described to add prognostic information to these two staging systems, but so far there is no consensus or evidence that the use of additional markers is needed in general practice to define a treatment indication [41]. It is anticipated that the prognostic classification might change in the near future (Bahlo et al., submitted).

Response Assessment

The update 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 [25]: complete remission, partial remission, stable disease, and progression, as well as refractory disease. In addition, the assessment of minimal residual disease (MRD) has been introduced as an additional and increasingly important category of response assessment. This results in four categories of response (Fig. 1), CR+MRD−, CR+MRD+, PR+MRD−, PR+MRD+.

Eradicating MRD

The assessment of minimal residual disease (MRD) recently has become a very important endpoint with prognostic impact in clinical trials [42]. Detectable MRD after therapy following alemtuzumab consolidation therapy predicts relapse and shorter (progression-free) survival [43–47]. Results of a small Phase III trial by the GCLLSG showed improved PFS with alemtuzumab consolidation therapy compared to the observation arm (no progression vs. 24.7 months, $P = 0.036$) when calculated from the start of fludarabine-based treatment [43]. In a similar approach, an OR of 53% was achieved by an alemtuzumab consolidation therapy (39% at a 10-mg dose and 65% at a 30-mg dose ($P = 0.066$)) [47]. MRD was efficiently cleared from the bone marrow in most patients, with 38% of the patients achieving a molecular remission. Median time to disease progression had not yet been reached for patients who achieved MRD negativity, compared with 15 months for patients who still had residual disease after alemtuzumab consolidation treatment [47]. However, this approach may cause considerable myelotoxicity, lymphocytopenia, and sometimes life threatening infections, in particular if conventional doses of alemtuzumab are administered within 3–6 months after the last chemotherapy in patients with a low tumor load [48,49].

The quantitative assessment of MRD in 471 patients in the CLL8 trial receiving FC or FCR has provided additional insight into the clinical significance of MRD as assessed by four color flow cytometry [50]. MRD levels below 10^{-4} were correlated with longer PFS. The FCR regimen produced lower median MRD levels compared with FC, resulting in longer PFS [50].

Therefore, MRD assessment is recommended in clinical trials using standardized protocols of either four-color flow cytometry or allele-specific oligonucleotide PCR (one CLL cell in 10,000 leukocyte sensitivity) [51], although evaluation

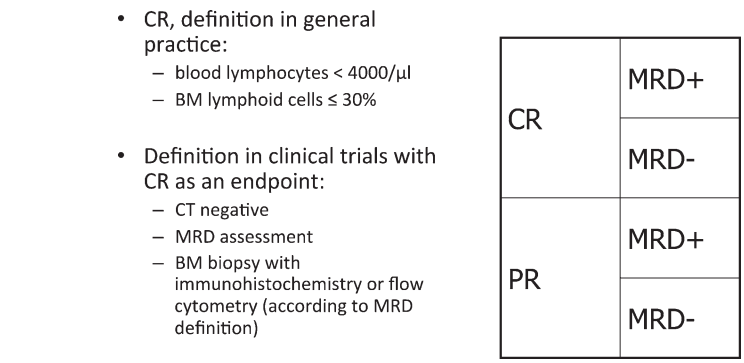

Figure 1. Definition of response in CLL. MRD, minimal residual disease; CR, complete remission; PR, partial remission.

of MRD is currently not recommended for routine clinical practice [25].

Treatment Components

Single Agents

Cytostatic agents. Monotherapy with alkylating agents has served as initial, front-line therapy for CLL, and chlorambucil has been considered the "gold standard" for several decades [40]. Even today, this drug remains an appropriate option, particularly in frail elderly or unfit patients. The advantages of chlorambucil 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). Novel results indicate that chlorambucil monotherapy may be used less frequently, since the combination with anti-CD20 antibodies has proven more effective (see below).

Three purine analogues are currently 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 (OR) rates compared with other treatment regimens containing alkylating agents or corticosteroids [52–54]. Fludarabine induced more remissions and more complete remissions (CR) (7–40%) than other conventional chemotherapies, like CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), CAP (cyclophosphamide, doxorubicin, prednisone), or chlorambucil, but did not improve overall survival when used as single agent [54–57]. Similarly, cladribine monotherapy was shown to produce a higher CR rate than chlorambucil plus prednisone (47% vs. 12%) without resulting in a longer survival [58].

More recently, bendamustine, 4-[5-[Bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid, which has been used in Germany for more than 30 years, was compared with chlorambucil in a randomized trial. Bendamustine produced improved responses but greater toxicity and no OS benefit [59]. The overall response (OR) and median PFS rates were 67% and 22 months respectively for bendamustine versus 30% and 8 months for chlorambucil (both $P < 0.0001$).

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 [60] 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 [61]. Some newer CD20-antibodies challenge rituximab [62–64].

Rituximab. In CLL, Rituximab is less active as a single agent than in follicular lymphoma, unless very high doses are used [65,66]. 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 CDC activity and similar ADCC activity compared with Rituximab, especially in cells expressing low levels of CD20 [67]. The American FDA and the European EMA recently licensed Ofatumumab as monotherapy in patients that are fludarabine- and alemtuzumab-refractory. This decision was based on an analysis of 201 patients that were either fludarabine- and alemtuzumab-refractory (FA-refractory) or only fludarabine-refractory and suffered from bulky disease (>5 cm) [68]. The overall response rate was 51% in the FA-refractory group and 44% in the bulky disease group. The definitive value of Ofatumumab for the treatment of B-cell lymphoma and CLL will require further investigations.

Obinutuzumab (GA101). The humanized and glyco-engineered monoclonal antibody Obinutuzumab showed impressive results in vitro with higher rates of apoptosis in B-cells in comparison to Rituximab [69]. The humanization of the parental B-Ly1 mouse antibody and subsequent glycol-engineering lead to higher affinity binding to CD20 type II epitope, increased antibody-dependent cellular cytotoxicity (ADCC), low complement-dependent cytotoxicity (CDC) activity, and increased direct cell death induction [70]. A phase-I study with Obinutuzumab showed promising results in 13 CLL patients [71] but also in other indolent lymphoma [72]. Major side effects included infections, neutropenia, thrombocytopenia, and tumor lysis syndrome, which all resolved. There were no dose-limiting toxicities. Encouraging results were reported in the run-in phase of the CLL11 trial on CLL patients with increased comorbidity [73]. Six subjects older than 70 years (median age 76 years; cumulative illness rating score of 8) received 6 × 28-day cycles of obinutuzumab (1,000 mg on Days 1, 8, and 15 of cycle 1 and Day 1 of cycles 2–6) plus chlorambucil (0.5 mg/kg on Day 1 and 15 of each cycle). Infusion-related reactions occurred in 5 patients, but were mild. Grade 3–4 neutropenias were seen in five patients. No febrile neutropenias or Grades 3–4 infections were observed. All subjects completed therapy. Complete responses were documented in 2 patients while 4 responded partially. After a median follow-up of 23 months from start of treatment, none of the patients had progressed with CLL or died. These results suggest that chemoimmunotherapy with obinutuzumab and chlorambucil is feasible and promisingly active in CLL patients with increased comorbidity. The results of the CLL11 will be available soon.

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 who were previously treated with alkylating agents and had failed or relapsed after second-line fludarabine therapy [74–76]. In addition, alemtuzumab has proven effective in patients with high-risk genetic markers such as deletions of chromosome 11 or 17 (*del(11q)* and *del(17p)*) and *TP53* mutations [77,78]. Therefore, alemtuzumab is considered a reasonable therapeutic option for patients with these poor

TABLE I. Selected New, Nonapproved Drugs in CLL

Class	Agent (References)	Target	Route	Stage of development
Antibodies	Obinutuzumab (GA101) [69,73]	CD20	i.v.	Phase III
	Blinatumomab [82,83]	CD3/CD19	i.v.	Phase I
	Dacetuzumab [84–86]	CD40	i.v.	Phase I
	Lucatumumab [87]	CD40	i.v.	Phase II
	Mapatumumab [88]	TRAIL-R1	i.v.	Phase Ib/II
	mAb 37.1 [89,90]	CD37	i.v.	Phase I
Immune gene therapy	ISF35 (Adenovirus-CD154) [91–93]	CD40	i.v., intranodal injection	Phase I
SMIP	TRU-016 [94,95]	CD37	i.v.	Phase II
Bcl2 antagonists	ABT-263 (Navitoclax) [96]	Bcl-2	p.o.	Phase I/IIa
	Obatoclax [97–99]	Pan-Bcl2 family	i.v.	Phase II
	ABT-199 [100–102]	BH3 mimetic	p.o.	Phase II
Tyrosine kinase inhibitors	Fostamatinib [103]	Syk	p.o.	Phase I/II
	Idelalisib (CAL-101) [104,105]	PI3K p110δ	p.o.	Phase I
	Ibrutinib (PCI-32765) [106]	BTK	p.o.	Phase I
Cyclin-dependent kinase inhibitors	Flavopiridol [107,108]	CDK	i.v.	Phase II
	Dinaciclib [109]	CDK 1,2,5,9	i.v.	Phase I
	SNS-032 [110]	CDK 2,7,9	i.v.	Phase I
mTOR inhibitors	Everolimus (RAD001) [111,112]	mTOR	i.v./p.o.	Phase I/II
IMiD	Lenalidomide [113,114]	multiple	p.o.	Phase III

prognostic features. In a recent prospective randomized study alemtuzumab was tested against chlorambucil [79]. Alemtuzumab led to a greater OR and CR ($P < 0.0001$), superior PFS with a 42% reduction in risk of progression or death ($P < 0.0001$) and significantly longer median time to progression (TTP) ($P = 0.0001$). Therefore, the drug has been approved by the US FDA as front-line therapy for CLL.

New Drugs in Clinical Development

In addition to the approved drugs described in the previous section, there are an increasing number of very hopeful new compounds in clinical development (for reviews see [80,81]). Since a detailed description of these fascinating agents is beyond the scope of this review, Table I shows a summary of the most promising agents. This article will only describe a selected number of agents that are now being tested in advanced (Phase III) clinical trials.

Agents targeting B-cell receptor signaling. B-cell receptor signaling seems to play an important role for the survival of CLL cells [115,116]. Different aspects of the B-cell-receptor have been recognized as a prognostic marker in chronic lymphocytic leukemia, such as immunoglobulin heavy chain variable gene (IGHV) or stereotypy. Continuous or repetitive BCR signaling supports CLL cell survival (reviewed in [116]). This might explain why inhibition of BCR signaling is a new and potent strategy to treat CLL [80]. The B-cell receptor signaling in CLL cells is supported by different tyrosine kinases, such as Bruton's tyrosine kinase (BTK), Spleen tyrosine kinase (Syk), ZAP70, Src family kinases (in particular Lyn kinase) as well as PI3K [80]. The following section will describe selected inhibitors of these kinases, which have entered testing in Phase III trials (see Table I).

Fostamatinib. Spleen tyrosine kinase, so called Syk, transfers and enhances the signal of the B-cell receptor. Activation of Syk results in cell survival through activation of phosphatidylinositol 3-kinases and AKT [117]. The expression of Syk is upregulated in chronic lymphocytic leukemia cells turning it into a promising target [118]. Fostamatinib disodium, the first clinically available oral Syk inhibitor, induces apoptosis through disruption of B-cell receptor (BCL) signaling. A Phase I/II trial in recurrent B-cell malignancies demonstrated an overall response rate of 21% (5 of 23) for DLBCL, 10% (2 of 21) for FL, 54% (6 of 11) for SLL/CLL, and 11% (1/9) for MCL [103]. All 6 responses among the CLL patients were partial remissions. Median progression-free survival was 4.5 months for all entities and 6.4 months for the CLL patients [103]. Dose-limiting toxicity in the phase I population were neutropenia, diarrhea, and thrombocytopenia.

Idelalisib (CAL-101). Class I phosphatidylinositol 3-kinases (PI3Ks) regulate cellular functions relevant to oncogenesis [119]. 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 [120]. CAL-101 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 antibody-dependent cellular cytotoxicity. CAL-101 inhibits CLL cell chemotaxis toward CXCL12 and CXCL13 and migration beneath stromal cells (pseudoemperipolesis). CAL-101 also down-regulates secretion of chemokines in stromal cocultures and after BCR triggering [120]. CAL-101 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 [120].

In a Phase I clinical trial in heavily pre-treated CLL patients, CAL-101 showed acceptable toxicity, positive pharmacodynamic effects and favorable clinical activity (high level of lymph node regression and prolonged duration of symptomatic tumor control) [104]. Totally, 22 of the 37 patients that were enrolled so far had high risk cytogenetic aberrations like *del(17p)* or *del(11q)*. Of the 32 patients CAL-101 reduced lymphadenopathy in all 32 (100%) patients. 29/32 (91%) achieved a lymph node response (≥50% reduction in target lesions). An initial increase of peripheral lymphocyte counts could be observed in 21 of the 37 patients, reaching its peak during the first 2 cycles and decreasing afterwards. This was interpreted as a phenomenon of lymphocyte-redistribution between lymph nodes and peripheral blood.

Ibrutinib. Bruton tyrosine kinase (Btk) leads to downstream activation of cell survival pathways such as NF-κB and MAP kinases via Src family kinases [121]. Ibrutinib (formerly called PCI-32765) is an orally active small molecule inhibiting Bruton's tyrosine kinase (BTK) that plays a role in the signal transduction of the B-cell receptor (BCR). Inhibition of BTK might induce apoptosis in B-cell lymphomas and CLL-cells [121]. Fifty-six patients with relapsed or refractory B-cell lymphoma and CLL received escalating oral doses of ibrutinib at two schedules were evaluated: one, 28 days on, 7 days off; and two, once-daily continuous dosing. Objective response rate in 50 evaluable patients

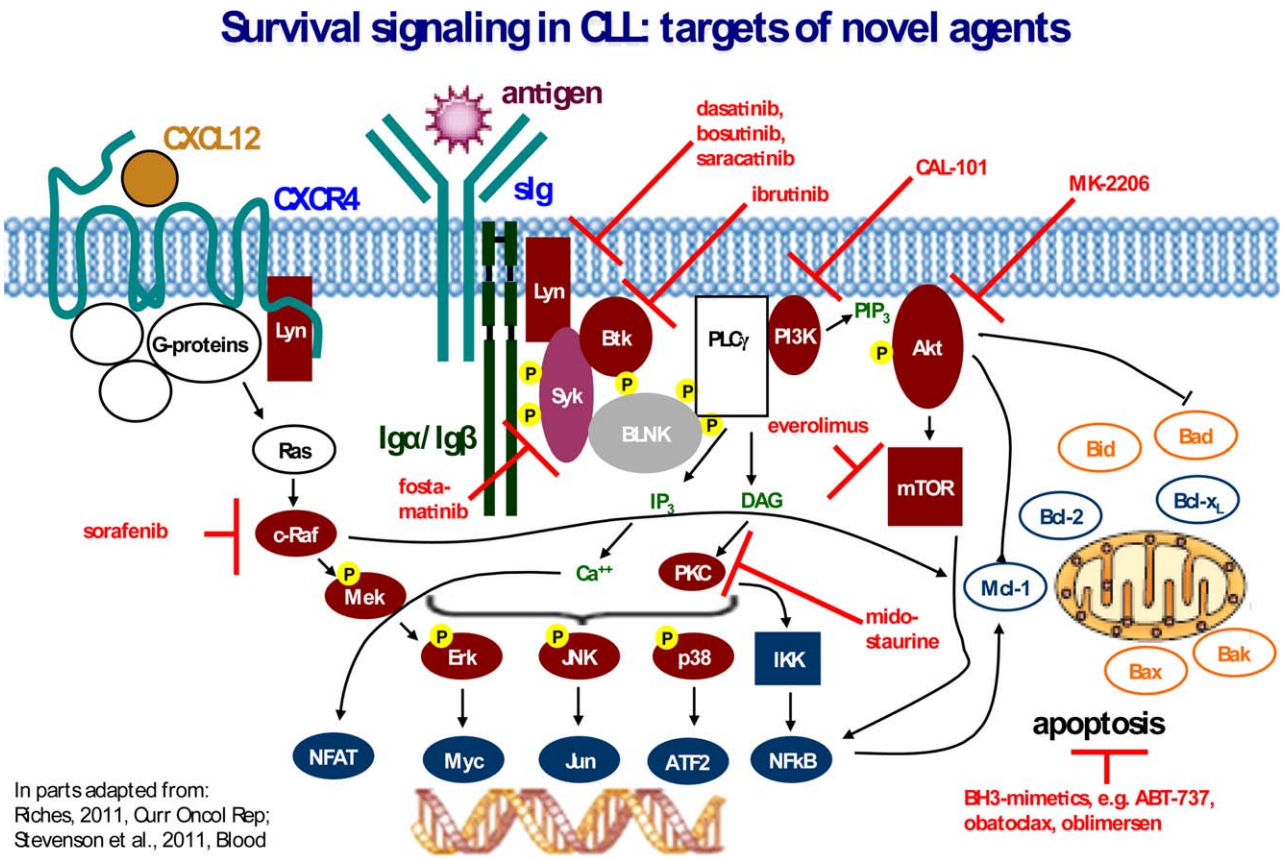

Figure 2. Targeting of the BCR signaling as a therapeutic strategy in CLL. Red symbols and letters indicate new therapeutics as discussed in the text. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

was 60%, including complete response of 16%. Median progression-free survival in all patients was 13.6 months [106]. The most relevant treatment-related side effects iere viral infections. All patients had no changes in NK- or T-cell counts.

Dasatinib. Dasatinib is a Src- and Abl- kinase inhibitor that induces apoptosis in primary CLL cells [122]. In addition Dasatinib seems to increase the apoptotic effects of various agents like fludarabine, chlorambucil, sorafenib, the HSP90 inhibitor 17-DMAG, dexamethasone, or the BH3-mimetic ABT-737 [122–127]. In a Phase II study, 6 of 15 patients in a Phase II study showed nodal remissions lacking a decrease of more than 50% in lymphocyte count, only 2 patients showed a partial remission [128]. In summary, dasatinib seems effective in reduction of nodular tumor masses, but seems to lack efficacy on peripheral blood lymphocytes.

Bcl-2 inhibitors. Proteins in the B cell CLL/lymphoma 2 (Bcl-2) family are key regulators of the apoptotic process [129]. 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 which was initially described in follicular lymphoma as a protein in translocations involving chromosomes 14 and 18 [130].

The Bcl-2 inhibitor ABT-263 (Navitoclax) and ABT-199. ABT-263 is a small molecule Bcl-2 family protein inhibitor that binds with high affinity (Ki $\leq$ 1 nM) to multiple anti-apoptotic Bcl-2 family proteins including Bcl-XL, Bcl-2, Bcl-w, as well as Bcl-B and has a high oral bioavailability [131]. Initial studies showed very promising results for this drug as a single agent [96]. However, its therapeutic use seemed somewhat limited by severe thrombocytopenias being a prominent side effect. Therefore, the compound was re-engineered to create a highly potent, orally bioavailable and Bcl-2-selective inhibitor, ABT-199 [101]. This compound inhibits the growth of BCL-2 dependent tumors in vivo and spares human platelets. A single dose of ABT-199 in three patients with refractory chronic lymphocytic leukemia resulted in tumor lysis within 24 hr [101]. Together, these data indicate that selective pharmacological inhibition of BCL-2 shows promise for the treatment of BCL-2-dependent hematological cancers, including CLL.

The BH3-mimetic AT-101. AT101 is an orally active BH3-mimetic, which inhibits the anti-apoptotic activity of Bcl-2, Bcl-XL and Mcl-1 and might be an active agent for the treatment of CLL, as the resistance to apoptosis in CLL cells is associated with high levels of Bcl-2 protein expression. AT101 was found to induce apoptosis in CLL cells in vitro and to overcome drug resistance mediated by the microenvironment [132]. It showed a good tolerability and satisfactory efficacy in combination with weekly infusions of rituximab in previously treated CLL patients [133,134].

Immunomodulatory drugs

Lenalidomide is a second generation thalidomide analogue and an immunomodulatory agent with antiangiogenic properties that is used in treatment of myelodysplastic syndrome and multiple myeloma and is currently investigated in the treatment of CLL. It showed encouraging results in the treatment of high risk patients including carriers of a *del(17p)* [135]. In 58% of the patients lenalidomide causes a so called tumor flare reaction, which leads to a sensation of heat and burning in the lymph nodes and occurs only in

CLL patients [113,136]. The overall response rate of lenalidomide monotherapy varied between 32% and 54% in different clinical trials [113,114,137].

The combination of lenalidomide and rituximab seems to increase the response rate without a higher risk of toxicity, even in patients with *del(17p)* and/or unmutated IGHV-status. In a Phase II trial, 59 patients with relapsed or refractory CLL received a combination of lenalidomide and rituximab [138]. Lenalidomide was started on Day 9 of cycle one at 10 mg orally and administered daily continuously. Each cycle was 28 days. Rituximab was administered for 12 cycles; lenalidomide could continue indefinitely if patients benefitted clinically. The overall response rate was 66%, including 12% complete responses and 12% nodular partial remissions. Time to treatment failure was 17.4 months. The most common grade 3 or 4 toxicity was neutropenia (73% of patients). Fourteen patients (24%) experienced a grade 3 to 4 infection or febrile episode. In essence, this combination seems a helpful alternative for patients with refractory CLL and warrants further investigation.

In contrast to these encouraging results a trial testing the combination of lenalidomide, rituximab and fludarabine in previously untreated CLL patients was stopped prematurely due to severe side [139]. The high toxicity rate observed in this trial is likely to be explained by the parallel start of all drugs. Flinn et al. [140] started with a very similar treatment schedule of fludarabine, rituximab and lenalidomide, and 3 out of 4 patients who received all three drugs on Day 1 experienced severe side effects. After changing the administration schedule and starting with lenalidomide on Day 8 of the first cycle, the regimen was better tolerated. This observation was confirmed by Egle et al. [141]. The German CLL study group is currently investigating combination of Bendamustine, Rituximab, and Lenalidomide in previously treated physically fit patients (CLL2P protocol of the GCLLSG).

Blum et al. [142] presented preliminary, encouraging results on the addition of flavopiridol to lenalidomide which lead to a response in 7 of 15 patients (among them 4 with a *del(17p)* and 3 with a *del(11q)*). Lenalidomide is also being tested in combination with other agents like ofatumumab [143].

Everolimus (RAD001). Everolimus has shown good efficacy in hematological malignancies [144], in particular T-cell lymphoma, and was therefore tested in CLL as well. So far, the results in CLL have been disappointing with low response rates, and the enthusiasm for this drug has been further reduced by severe infectious complications [111,112].

Combination chemotherapy

A major advance in CLL treatment was achieved by the combined use of different treatment modalities, in particular for patients that had a good fitness.

Since purine analogs and alkylating agents have different mechanisms of action and partially non-overlapping 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 [145]. 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 chlorambucil or prednisone increased hematological toxicity without improving the response rate compared with fludarabine alone (response rates 27%–79%) [54,146]. The most thoroughly studied combination chemotherapy for CLL is fludarabine plus cyclophosphamide (FC) [146]. In noncomparative trials, the overall response rates did not appear to be better than with fludarabine alone, but the addition of cyclophosphamide (with or without a third drug, mitoxantrone) appeared to improve the CR rate up to 50% [146]. The addition of mitoxantrone to FC in 37 patients with relapsed/refractory CLL produced a high CR rate (50%), including 10 cases of MRD negativity, with a median duration of response of 19 months [45]. All MRD-negative patients were alive at analysis; the median duration of response had not been reached in the CR patients compared to 25 months in non-CR patients. A Phase II study of cladribine in combination with cyclophosphamide has also demonstrated activity in advanced CLL, but the results seemed inferior to FC [147].

Three randomized trials have shown that FC combination chemotherapy improves the CR and OR rate and PFS as compared to fludarabine monotherapy [148–150]. The rate of severe infections was not significantly increased by the FC combination despite a higher frequency of neutropenias. A reanalysis of the CLL4 trial of the GCLLSG suggested that the first-line treatment of CLL patients with FC combination may improve the OS of the non-high risk CLL patients (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 [151]. Surprisingly, the CC combination therapy did not produce any benefit in terms of progression free survival or response rates when compared with 2-CdA alone. Compared with 2-CdA, CMC induced a higher CR rate (36% vs. 21%, $P = 0.004$), and a trend for a higher CR rate with CC was observed (29% vs. 21%, $P = 0.08$). The percentage of patients who were in CR and were MRD negative was higher in the CMC arm compared with 2-CdA (23% vs. 14%, $P = 0.042$). There were no differences in overall response, progression-free survival, and overall survival among treatment groups. Grade 3/4 neutropenia occurred more frequently in CC (32%) and CMC (38%) than in 2-CdA (20%) ($P = 0.01$ and $P = 0.004$, respectively). Infections were more frequent in CMC compared with 2-CdA (40% vs. 27%, $P = 0.02$). On the basis of these results, cladribine combination therapies do not seem to offer a major advantage when used as first line treatment for CLL.

Chemoimmunotherapy

Combinations using rituximab. Since preclinical studies showed evidence for a synergy between rituximab and fludarabine [152], rituximab combinations with fludarabine or fludarabine-based regimens were investigated in Phase II trials. A GCLLSG trial on 31 previously treated or untreated CLL patients showed 27 (87%) responses and 10 (32%) CRs [153]. 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 [154]. 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 [155]. The patients receiving fludarabine and rituximab had a better progression-free survival (PFS) and overall survival (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 large Phase II trial conducted at the MD Anderson Cancer Center on 300 patients with previously untreated CLL, rituximab plus

fludarabine/cyclophosphamide (FC) achieved an overall response rate of 95%, with CR in 72%, nPR in 10%, PR due to cytopenia in 7%, and partial remission due to residual disease in 6% [156]. Six-year overall and failure-free survival were 77% and 51%, respectively. Median time to progression was 80 months.

These results led the GCLLSG to conduct a randomized trial, the CLL8 protocol [15]. 817 patients (median age 61 years) with good physical fitness were randomly assigned to receive 6 courses of FC ($n = 409$) or FCR ($n = 408$). 64% were at Binet Stage B, 32% Binet C and 5% Binet A. FCR induced a higher OR rate than FC (92.8 vs. 85.4%) and more CR (44.5 vs. 22.9) ($P < 0.001$). PFS at 2 years was 76.6% in the FCR arm and 62.3% in the FC arm ($P < 0.01$). FCR treatment was more frequently associated with CTC Grades 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 [157]. 272 patients were treated with FC and 274 with FCR. Overall response rates were 58% and 70% for FC and FCR, respectively, with 13% and 24.3% CR. TTF was 20.6 versus 30.6 months. Taken together, these results suggest that rituximab plus fludarabine-based therapies represent a significant advance in therapy for CLL.

Since CLL often occurs in elderly patients with relevant comorbidity, a dose-modified FCR-Lite regimen was designed to maintain the efficacy but decrease the toxicity of the FCR regimen [158]. This regimen reduced the dose of the two cytostatic agents, (fludarabine to 20 mg/m^2 and cyclophosphamide 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 CLL patients with an OR rate 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.

Several variations have been tested to further improve the efficacy of the FCR regimen: Alemtuzumab (A) was added to FCR (CFAR) in a phase 2 trial on 60 high-risk untreated patients < 70 years with serum beta$_2$-microglobulin >/= 4 mg/L [159]. Complete remission (CR) was achieved in 70%, partial remission (PR) in 18%, nodular PR in 3%, for an overall response of 92%. Of 14 patients with 17p deletion, CR was achieved by 8 (57%). Grade 3–4 neutropenia and thrombocytopenia occurred with 33% and 13% courses, respectively. The median progression-free survival was 38 months and median OS was not reached. In conclusion, CFAR seems an active frontline regimen for high-risk CLL that might be helpful for cytoreductive therapies before an allogeneic stem cell transplant.

In another study on seventy-two untreated CLL patients </= 70 years, mitoxantrone was combined at 6 mg/m^2 on Day 1 of each cycle with FCR [160]. The overall response, minimal residual disease (MRD)-negative complete response (CR), MRD-positive CR, and partial response rates were 93%, 46%, 36%, and 11%, respectively. Severe neutropenia developed in 13% of patients. These results do not justify the broad use of this regimen outside of clinical trials.

An alternative idea was to substitute fludarabine in the FCR regimen with pentostatin (PCR) in order to reduce myelotoxicity. In a Phase III randomized trial comparing FCR to PCR in previously untreated or minimally treated CLL patients, there were no statistical differences between treatments in OS or response [161]. Moreover, this trial did not demonstrate a lower infection rate with PCR.

Bendamustine has been combined with rituximab (BR) in 81 patients with relapsed CLL [162]. 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 overall response rate was 59.0% (95% CI, 47.3% to 70.0%). Complete response, partial response, and nodular partial response were achieved in 9.0%, 47.4%, and 2.6% of patients, respectively. Overall response rate 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 CLL patients [163]. Bendamustine was administered at a dose of 90 mg/m^2 on Days 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. Overall response rate was 88.0% (95% CI, 80.7% to 100.0%) with a complete response rate of 23.1% and a partial response 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 adverse events for neutropenia, thrombocytopenia, and anemia were documented in 19.7%, 22.2%, and 19.7% of patients, respectively.

Overall, these results compared favorably with the FCR regimen in that BR achieved similar response rates, albeit lower CR rates, but induced less neutropenias than FCR. Therefore, GCLLSG currently compares BR to FCR in a randomized phase III trial, the CLL10 protocol.

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 been proven to result in higher efficacy as compared with FCR.

Combinations 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 [164]. The combination of fludarabine and alemtuzumab (FA) was investigated in a Phase II trial enrolling patients with relapsed CLL using a four-weekly dosing protocol [165]. This combination has proven feasible, safe, and very

Stage	Fitness	del(17p) p53mut	Therapy
Binet A-B, Rai 0-II, inactive	Irrelevant	Irrelevant	None
Active disease or Binet C or Rai III-IV	Go go	No	FCR
		Yes	Allo-SCT
	Slow go	No	CLB + anti-CD20-Mab
		Yes	Al, HD R or O

Response to First-Line Therapy	Fitness	Therapy	
		Standard	Alternatives (trials)
Refractory or progress within 2 years	Go go	Al-Dex, FA, FCR→Allo SCT	Lenalidomide, BR, BR² Combination with kinase inhibitors
	Slow go	Change therapy (if possible, include in trial)	Al for del(17p), FCRlite, BR, bendamustine, lenalidomide, ofatumumab, HD rituximab, kinase inhibitors
Progress after 2 years	All	Repeat first-line therapy	

Figure 3. (a,b) Treatment algorithm for CLL patients, in first and second line indications. Al, alemtuzumab; R, rituximab; O, ofatumumab; F, fludarabine; C, cyclophosphamide; CLB, chlorambucil. Figure created by Dr. Günter Krause, Cologne; used with permission of the author. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

effective. Among the 36 patients, the ORR was 83% (30/36 patients), which included 11 CRs (30%) and 19 PRs (53%) and one stable disease (SD). Sixteen of 31 evaluated patients (53%) achieved MRD negativity in the peripheral blood by 3 months' follow-up. Resolution of disease was observed in all disease sites, particularly in the blood, bone marrow and spleen. The FA therapy was well tolerated. Infusion reactions (fever, chills, and skin reactions) occurred primarily during the first infusions of alemtuzumab, and were mild in the majority of patients. While 80% of patients were cytomegalovirus immunoglobulin G (CMV IgG)-positive before treatment, there were only two subclinical CMV reactivations. The primary grade 3/4 hematological events were transient, including leukocytopenia (44%) and thrombocytopenia (30%). Stable $CD4^+$ T-cell counts ($>$ 200/μl) were seen after 1 year.

Two phase III trials tested alemtuzumab in combination with FC (FCA) with or fludarabine (FA). The trial of the French study group, which compared FCA to FCR in first line therapy was closed prematurely due to the higher toxicity and treatment-related mortality observed in the FCA arm [166]. In this trial, alemtuzumab was given subcutaneously. The therapeutic efficacy FCR was clearly superior to FCA in this trial. A second randomized trial compared FA to fludarabine monotherapy in previously treated patients with relapsed or refractory CLL [167]. In this trial, alemtuzumab was given intravenously. FA ($n = 168$) resulted in better PFS than fludarabine monotherapy ($n = 167$; median 23.7 months vs. 16.5 months; hazard ratio 0.61; $P = 0.0003$) and overall survival (median not reached vs. 52.9 months; HR 0.65; $P = 0.021$) compared with fludarabine alone. All-cause adverse events occurred in 161 (98%) of 164 patients in the combination treatment group and 149 (90%) of 165 in the fludarabine alone group. Patients in the FA group had more cytomegalovirus events (14% vs. $<$1%) and grade 1 or 2 infusion-related adverse reactions (62% vs. 13%). Major Grade 3 or 4 toxicities in the combination treatment and monotherapy groups were leucopenia (74% vs. 34%), lymphopenia (94% vs. 33%), neutropenia (59% vs. 68%), thrombocytopenia (11% vs. 17%), and anemia (9% vs. 17%). The incidence of serious adverse events was higher in the combination treatment group (33% vs. 25%); deaths due to adverse events were similar between the two groups (6% vs. 12%).

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) [168]. These results need to be confirmed by larger trials.

Combinations using novel agents. The effect of oblimersen, an anti-Bcl2 antagonist, when added to the FC regimen was investigated in 241 patients. Fludarabine 25 mg/m^2/d plus cyclophosphamide 250 mg/m^2/d were administered intravenously for 3 days with or without oblimersen 3 mg/kg/d as a 7-day continuous intravenous infusion (beginning 4 days before chemotherapy) for up to six cycles. CR/nPR was achieved in 20 (17%) of 120 patients in the oblimersen group and eight (7%) of 121 patients in the chemotherapy-only group ($P = 0.025$). Achievement of CR/nPR was correlated with both an extended time to progression and survival ($P < 0.0001$). The overall survival and the progression-free survival were improved in patients that achieved at least a partial response. This study already announced the potential of Bcl-2 targeted treatment strategies, but the more relevant combination studies using novel Bcl-2 antagonists (see Table I and above) are currently being done.

Selecting of the Right Treatment: How to Treat CLL?

Parameters to be considered

Given the impressive choice of options, the right choice of treatment of a given CLL a patient becomes a task that requires experience, a good clinical judgment and an appropriate use of diagnostic tools. The following parameters should be considered before recommending a treatment for CLL [41]:

1. The clinical stage of disease.
2. The fitness of the patient.
3. The genetic risk of the leukemia.
4. The treatment situation (first versus second line, response versus non-response of the last treatment).

Using the assessment of these 4 parameters, the following recommendations can be given:

First line treatment

In a patient with advanced (Binet C, Rai III-IV) or active, symptomatic disease, treatment should be initiated. In this situation, patients need to 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" (CIRS) [169], patients should be offered more combination therapies such as FC or FR or FCR.

Patients with a somewhat impaired physical condition ("slow go") may be offered either chlorambucil in combination with an anti-CD20 antibody or a dose-reduced fludarabine containing regimen with a CD20 antibody. The aim of therapy in this situation is symptom control.

Patients with symptomatic disease and with *del(17p)* or *TP53* mutations man receive FCR or BR or an alemtuzumab-containing regimen as first-line treatment. In

general, these regimen all yield response rates above 50%. These patients respond poorly to fludarabine or fludarabine-cyclophosphamide. Since none of these therapies promises long-lasting responses (except, maybe, ibrutinib), an allogeneic stem cell transplantation should be offered and discussed.

Second-line treatment

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 treatment may be repeated, if the duration of the first remission exceeds 12 months (or with the modern chemo-immunotherapies 24 months).

The choice becomes more difficult and limited in treatment-refractory CLL (as defined by an early relapse within 6 months after the last treatment) or in cases with the chromosomal aberration *del(17p)*. In principle, the initial regimen should be changed. There are the following options that can be applied:

- Alemtuzumab alone or in combination [75,165].
- Allogeneic stem cell transplantation with curative intent [170].
- Experimental protocols using the new drugs described above (Table 1)

The choice of one of these options depends on the fitness of the patient, the availability of the drugs and the molecular cytogenetics. According to recommendations of an EBMT consensus group, physically fit patients with refractory CLL or with a *del(17p)* should be offered an allogeneic transplantation, since their prognosis so far has remained extremely poor with conventional therapies [170]. Finally, it is important to emphasize that patients with refractory disease should be treated within clinical trials whenever possible.

Summary and Outlook

Like in other hematologic malignancies, the management of CLL is currently undergoing a dynamic and rapid change. While writing this article, I am fully aware that in 6–12 months from now there will be several very potent, targeted and nonchemotherapeutic drugs in development or even on the market that have the potential of completely changing the current treatment recommendations. Therefore, it is important that all hematologists and oncologists contribute to the impressive and historically unique chance by offering their time and commitment to include their patients into the current clinical trials.

Moreover, it has never been as important as now to have a format of annually updated treatment recommendations, in a format like the one offered by this Journal.

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