The biology and treatment of chronic lymphocytic leukemia

M. Palma, P. Kokhaei, J. Lundin, A. Choudhury, H. Mellstedt & A. Österborg

Departments of Hematology and Oncology, and Immune and Gene Therapy Laboratory, Cancer Centre Karolinska, Karolinska University Hospital, Stockholm, Sweden

introduction

B-cell chronic lymphocytic leukemia (B-CLL) is characterized by the progressive accumulation of functionally incompetent, mature looking, monoclonal CD5+, CD23+ B lymphocytes in blood, bone marrow, lymph nodes and spleen/liver. CLL B cells express low levels of surface immunoglobulin (Ig)M, IgD (10% of those on normal B cells), CD21, CD22 and CD79b. Close to 99% of B-CLL lymphocytes in peripheral blood are in the G0 or early G1 phase of the cell cycle [1]. Defects in apoptosis have been associated with the accumulation of leukemic cells and disease progression, and presumably account for much of the chemotherapy-resistance of this disease. However recent data indicate that a relatively high proportion of cells are also dividing [2].

The diagnosis of B-CLL has usually been based on the classical criteria as outlined by the International Workshop on CLL (IWCLL) and the National Cancer Institute (NCI)-working group guidelines; however, current definitive criteria require an absolute blood lymphocytosis of >5 × 10^9/l consistent with the appearance of small mature looking lymphocytes, with an immunophenotype consistent with that described for B-CLL. The typical immunophenotype required for establishing the diagnosis is based on the identification of CD19+ B cells which also are CD5+, CD23+, FMC7-, and weak or negative for CD22/CD79b and surface Ig [3].

B-CLL is the most common adult leukemia in the Western world and accounts for about 40% of all leukemias in adults over the age of 65 years. The clinical course of B-CLL is heterogeneous. Some patients remain stable for a long time (even for the rest of their lives), without need of therapy, while others progress rapidly to a more advanced disease and die despite aggressive treatment.

prognostic factors

clinical stage

Clinical stage is still the most commonly used predictor of survival in B-CLL. The clinical staging systems proposed by Binet et al. [4] and Rai et al. [5], define early (Rai 0, Binet A), intermediate (Rai I/II, Binet B) and advanced (Rai III/IV, Binet C) stages of the disease on the basis of lymphoid area involvement and the presence of different levels of anemia and thrombocytopenia (Table 1). These conventional, clinical criteria, though, do not completely allow predicting the clinical outcome of early stage disease, even when compounded with other parameters reflecting the tumor burden or disease activity, such as serum lactate dehydrogenase (LDH) level, the lymphoid infiltration pattern in the bone marrow (BM) and the lymphocyte doubling time. Recently, more informative prognostic parameters have been identified, which may add to the classical assessments. These include serum and/or surface markers such as soluble CD23, β2-microglobulin or thymidine kinase, CD38, ZAP-70 and genetic markers of tumor cells, such as genomic aberrations, gene abnormalities (p53, ataxia telangiectasia mutated (ATM)) [6] and Ig variable region mutation status (see below).

immunoglobulin variable region mutation status and VH gene usage

B-CLL was traditionally regarded as a disease that occurs before naive B cells meet the antigen in the lymph nodes (pre-germinal centre disease). However, it was later found that half of the patients with B-CLL might have somatic hypermutations, i.e. an increased rate of mutations in the variable region of the immunoglobulin light and heavy chains [7]. These data suggested that B-CLL comprises two separate types of tumors arising at different stages of B-cell maturation: a pregerminal center naive (unmutated) B cell and a postgerminal center memory (mutated) B cell [8]. Two other studies, published simultaneously, confirmed the presence of somatic mutations in the variable heavy chain (VH) region of the Ig genes in approximately half of B-CLL patients and, most importantly, that the absence of mutations (unmutated CLL) had a major negative prognostic impact [9, 10]. Thus, B-CLL can be subdivided into two prognostic subgroups depending upon the presence or the absence of mutations in the VH genes of the Ig locus of the malignant B cells. A 2% difference from the corresponding germ-line gene has been accepted as a cut-off point to distinguish B-CLL patients with mutated Ig VH genes from those without mutations. This cut off was chosen to eliminate the potential influence of allelic variants and undiscovered polymorphisms of an individual’s germ-line VH genes in defining mutations [11].

Some characteristics of mutated versus unmutated VH genotypes are summarized in Table 2. Additional information came from analysis of VH family gene usage in B-CLL; the leukemic cells have a biased IgVH gene usage and the most
frequently used genes are VH3-21, VH3-07, VH4-34 and VH1-69 [12]. Close to 25% of unmutated B-CLL use VH1-69 while VH3-21 is more common in mutated CLL cells. Despite this, VH3-21 has been identified as a B-CLL subgroup with a poor prognosis [13]. The finding of preferential VH, D, and JH gene usage and specific CDR3s in VH1-69 \( ^+ \) B-CLL has led to the speculation of a possible antigenic epitope involved in the development of B-CLL with the hypothesis that specific Ig gene combinations could generate Ig molecules with affinity to certain antigenic epitopes [14]. Recent findings in patients with IgVH3-21 [12, 15, 16] further support this assumption [17]. However, the type and origin of such a ‘CLL antigen’ remains unclear; it has been speculated that it would most likely be an autoantigen [12].

### Table 1. Clinical staging systems and the prognostic impact

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td>Definition</td>
<td></td>
<td></td>
<td>Stage</td>
<td>Definition</td>
</tr>
<tr>
<td>A</td>
<td>&lt; 3 lymphoid areas*</td>
<td>60</td>
<td>Low</td>
<td>0</td>
<td>Lymphocytosis only</td>
</tr>
<tr>
<td>B</td>
<td>&lt; 3 lymphoid areas</td>
<td>30</td>
<td>Intermediate</td>
<td>1</td>
<td>Lymphadenopathy</td>
</tr>
<tr>
<td>C</td>
<td>Hemoglobin &lt; 10 g/dl or platelets &lt; 100 ( \times 10^3 ) /dl**</td>
<td>10</td>
<td>High</td>
<td>III</td>
<td>Haemoglobin &lt; 11 g/dl**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IV</td>
<td>Platelets &lt; 100 ( \times 10^3 ) /dl**</td>
</tr>
</tbody>
</table>

* Lymphoid areas considered are the following five: unilateral or bilateral cervical, axillary and inguinal lymph nodes, spleen and liver.

** With exclusion of hemolysis and unrelated causes of anemia or thrombocytopenia.

### Table 2. Molecular, phenotypic, and clinical characteristics of mutated and unmutated B-CLL subgroups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mutated B-CLL</th>
<th>Unmutated B-CLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>V gene mutations</td>
<td>Significant numbers (&gt;2%)</td>
<td>Few or none (&lt;2%)</td>
</tr>
<tr>
<td>Gender</td>
<td>M = F</td>
<td>More males</td>
</tr>
<tr>
<td>Age at presentation</td>
<td>No difference</td>
<td>No difference</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Treatment requirement</td>
<td>Uncommon</td>
<td>Common</td>
</tr>
<tr>
<td>Lymphocyte doubling time</td>
<td>Frequently &gt;12 months</td>
<td>Frequently &lt;12 months</td>
</tr>
<tr>
<td>Activation markers</td>
<td>CD71+/CD62L+</td>
<td>CD38+/CD69+</td>
</tr>
<tr>
<td>CD38 expression</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>ZAP70 expression</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Telomere length</td>
<td>Diverse lengths</td>
<td>Uniformly short</td>
</tr>
<tr>
<td>Telomerase activity</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Retention of BCR* signaling</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Frequency of 13q14 deletion</td>
<td>50%</td>
<td>26%</td>
</tr>
<tr>
<td>Frequency of 11q23 deletion</td>
<td>4%</td>
<td>27%</td>
</tr>
<tr>
<td>Frequency of 17p13 or 11q23 deletion</td>
<td>7%</td>
<td>35%</td>
</tr>
<tr>
<td>Frequency of trisomy 12</td>
<td>15%</td>
<td>19%</td>
</tr>
<tr>
<td>Frequent VH gene usage</td>
<td>VH3-21</td>
<td>VH1-69</td>
</tr>
</tbody>
</table>

* B cell receptor.

### zeta-associated protein 70 (ZAP-70)

As sequencing IgVH genes is technically difficult and time consuming, a surrogate marker was sought. ZAP-70 is a protein associated with the \( \zeta \) chain of the T-cell receptor (TCR) and transmits a signal to downstream pathways that is selectively expressed by T cells and NK cells. Normal B cells do not express ZAP-70 in significant quantities. However, elevated levels of ZAP-70 mRNA were noted in gene expression studies of B-CLL [18], and high levels of ZAP-70 protein were detected by Western blotting [19] and flow cytometry [20]. It was initially shown that ZAP-70 expression correlated well with unmutated CLL [21, 22] and that ZAP-70 was associated with a poor prognosis. More recent data however indicated discordant results in as much as 23% of patients [23]. A European collaborative group is currently trying to standardize flow cytometry techniques for ZAP-70 staining, the results of which must be awaited until this marker can be used in routine health care.

### CD38

The presence of CD38 on the surface of B-CLL cells represents a significant risk for the patient of succumbing earlier to B-CLL and CD38 was suggested as a surrogate marker for mutational status [9]. However, CD38 levels may change over time in about one quarter of cases [24] and the most useful cutoff (in the range of 5–30%) still remains unclear. Therefore, CD38 is not generally used outside clinical studies as a prognostic marker.

### Chromosomal aberrations

Approximately 20% of patients with B-CLL have normal karyotype [25]. Using interphase Fluorescence in situ Hybridization (FISH), recurrent abnormalities have been demonstrated in 80% of patients. In the 300 patients studied by Döhner et al. [26] the most frequent aberration was deletion \( (d) \) of (13q) which was found in 55% of the patients, followed by del (11q) (18%) and trisomy 12 (16%). Del (17p) was found in only 7% of patients at diagnosis but was associated with a particularly poor prognosis. Patients in the 17p and 11q deletion groups had more advanced disease than those in the...
Other three groups. Patients with 17p deletions had the shortest median treatment-free survival (9 months) and patients with 13q deletions had the longest (92 months). The median overall survival times for the groups with 17p deletion, 11q deletion, 12q trisomy, normal karyotype, and 13q deletion as the sole abnormality were 32, 79, 114, 111, and 133 months, respectively [26]. Genomic aberrations also provide insights into the pathogenesis of the disease since they point to loci of candidate genes (17p13: p53; 11q22-q23: ATM). Specific genomic aberrations have been associated with disease characteristics such as marked lymphadenopathy (11q deletion) [27]. p53 tumor suppressor gene, located at 17p13.1 is mutated in 10% of CLL patients and possibly plays a role in Richter’s transformation of B-CLL and may be associated with resistance to chemotherapy [28, 29] but apparently not to CD52-targeted monoclonal antibody therapy [30].

telomeres
Telomeres are repetitive TTAGGG (6–12kbp) sequences at the end of eukaryotic chromosomes, which protect the end of the chromosome from damage, and prevent the chromosomes from fusing into rings, or binding haphazardly to other DNA in the cell nucleus.

A correlation of telomere length and telomerase activity of circulating B cells from patients with B-CLL and their prognostically relevant Ig VH gene subgroups has been reported and the level of telomerase activity in unmutated cells was significantly higher than that of mutated cells and B cells from healthy donors [31]. This finding is supported by other studies that showed the association between VH gene mutation frequency and telomere length [32]. Bechter et al. showed that short telomeres and high telomerase activity was associated with a shorter median survival [33] whereas the study by Verstovsek showed that telomerase activity may not be a prognostic factor in B-CLL [34]. Mutated B-CLL could be divided into two prognostic subgroup based on telomere length [35]. Tchirkov et al. demonstrated that the level of expression of the human telomerase reverse transcriptase (hTERT) mRNA in mononuclear blood cells of B-CLL patients correlated with a significantly shorter survival. Expression of hTERT was a significant prognostic factor when evaluated in a univariate analysis and, together with IgVH mutations, also in a multivariate analysis [36].

T cells in B-CLL

The existence of immune dysfunctions in B-CLL patients taken together with the increased numbers of circulating T cells (2.5–4 fold) [37] has led to the long-standing hypothesis that T cells are involved in the pathobiology of B-CLL. It has been shown that intracellular levels of interferon gamma (IFN-γ) and interleukin-4 (IL-4) in both CD4 and CD8 T cells in B-CLL were significantly higher than in T cells from healthy donors. B-CLL cells purified from involved lymph nodes and BM but not from peripheral blood, constitutively express mRNA for the T-cell attracting chemokines CCL17 and CCL22 [38]. CD40-crosslinking of peripheral blood B-CLL cells induces the expression of both chemokines at the RNA level. CCL22 is also released and is capable of attracting CD4+/CD40L+ T-cells.

These findings indicate that the stimulation of malignant cells via a physiologic signal present in the tumor microenvironment endows B-CLL cells with the chemo-attracting capacity for activated CD4+ T-cells, which in turn can deliver survival signals to tumor cells [38]. A number of cytokines including IL-4, IFN-α, IL-8, IL-13 and tumor necrosis factor alpha (TNF-α) have been shown promote the B-CLL cells survival in vitro [39, 40] and exogenous IL-4 increase the leukemic cell number in vivo [41]. The proliferative B-CLL compartment [2], which continuously feeds the accumulation compartment, may be represented by aggregation of proliferating cells that form so-called pseudo-follicles in lymph nodes and BM [42]. Pseudo-follicles consist of survivin-positive CLL B cells, co-expressing Bcl-2 and proliferation markers (Ki67) surrounded by CD4+ CD154+(CD40L) T cells and some follicular dendritic cells, which might promote the survival and proliferation of the B-CLL cells [43].

Disease-related immunological disorders

hypogammaglobulinemia

The most frequent immunological disorders in B-CLL are hypogammaglobulinemia (8% of cases at diagnosis) and autoantibodies against histones, DNA, cytoskeleton proteins and red blood cells [44]. Hypogammaglobulinemia contributes to the risk of bacterial and viral infections which are the main cause of deaths in almost 60% of cases [45]. The prevalence of hypogammaglobulinemia increases to 40 % at 5 years after diagnosis of B-CLL and 65% at 10 years [44]. In such patients, high dose intravenous Ig therapy is important to minimize the risk of bacterial infections [46].

autoimmune cytopenias

Progressive B-CLL is frequently complicated by autoimmune complications, such as autoimmune hemolytic anemia (AIHA) and/or thrombocytopenia, which is estimated to occur in 10–20 % of the patients [47, 48]. Although the exact mechanism of autoimmunity remains unclear, it is thought that the imbalance between subsets of T cells, which is further aggravated by therapy, may contribute to the development of the autoimmune conditions [49, 50]. Therapy for AIHA and immune-mediated thrombocytopenia include corticosteroids, splenectomy, rituximab (anti CD20) therapy and, in refractory cases, also alemtuzumab [51, 52].

richter transformation

About 5–10% of patients will undergo a transformation of B-CLL to a more rapidly progressing condition either as prolymphocytic leukemia (PLL), in which there are increased numbers of prolymphocytes (blood prolymphocytes >55%); or as Richter’s syndrome (RS). The median survival duration ranges from 5 to 8 months [53]. At present there is no test available to predict which patients are likely to undergo transformation of their disease [44]. Long-term T cell depletion...
as induced by alemtuzumab therapy appeared not to increase the risk of RS [54]. RS may be triggered by virus reactivations, such as Epstein-Barr virus (EBV), which are common in immunosuppressed patients.

**response evaluation**

The NCI-sponsored working group formalized criteria for response to treatment. The fulfillment of these criteria is verified by a careful clinical examination and by the evaluation of the peripheral blood [and bone marrow, to confirm complete response (CR)]. Computed tomography is however not required for response evaluation according to NCI criteria: this is subject to ongoing discussions as it may have a substantial impact on CR rates in clinical trials. The NCI-WG definition of CR implies, in fact, that <30% lymphocytes are found in the bone marrow with a morphologically normal trephine biopsy. The eradication of minimal residual disease (MDR) verified at either flow-cytometry or polymerase chain reaction (PCR) was seen to correlate positively with improved overall survival in various studies [55–57], thus suggesting that achieving MDR negativity could be more relevant than achieving CR by current NCI criteria. The possible role of these evaluations as surrogate endpoint for long-term prognosis is still object of further investigation.

**early stage B-CLL: wait and watch**

B-CLL affects mainly subjects older than 55 years and often has an indolent course. Disease symptoms often appear only many years after diagnosis and the anticipated survival, even for the group of patients with the worst prognostic factors, is several years. In 1998, the French Co-operative Group on CLL reported the results of two trials comparing the impact on survival of patients with Binet A CLL receiving therapy (chlorambucil ± prednisone) either at the time of diagnosis or at the time of disease progression (symptomatic disease), showing that early therapeutic intervention gave no benefit in terms of survival [58].

It must be considered, then, that at present the only potentially curative treatment is by allogeneic bone or marrow transplantation (allo-BMT), which has a remarkable toxicity that may have a detrimental effect on survival. For all these reasons, in accordance with the recommendations of the NCI-sponsored working group on CLL [59] and the IWCLL, the decision of initiating treatment should be forwarded until the patient becomes symptomatic or there are signs of active disease. Whether early therapy may be beneficial in patients with poor-prognostic features is to be explored in prospective randomized trials.

**first-line treatment**

Chemotherapy is effective in B-CLL. Before the introduction of purine analogs, treatment was primarily based on alkylating agents. Chlorambucil achieves 60–70% partial response (PR) in previously untreated patients, with no significant CR rate, while combination chemotherapy (CAP, CVP, CHOP) may give slightly higher response rates, but still no survival benefit [60].

Purine analogs act by inhibiting the DNA polymerase and the ribonucleotide reductase, finally promoting apoptosis [61]. Fludarabine is the purine analog most extensively studied and employed in the treatment of B-CLL patients. Major fludarabine-containing trials are listed in Table 3. Its superiority to older chemotherapy regimens (chlorambucil or CAP or CHOP) in terms of achievement of complete remissions and prolongation of progression-free survival (PFS) was assessed by three large randomized studies [62–64], which, though, evidenced no statistically significant advantage in overall survival.

The activity of fludarabine was also studied in combination with cyclophosphamide (FC), based on *in vitro* data showing a synergetic cytotoxic effect of this combination on B-CLL cells [65, 66]. A number of phase II studies with FC were performed [67–71], which showed good response rates, and a phase III trial was initiated to compare the combination versus fludarabine alone [72] as primary treatment for B-CLL patients aged ≥66 years, in either stage C asymptomatic disease or stage A/B symptomatic disease. Overall response rates were 83% and 94% (P < 0.01) and CR rates were 7% and 24% for the fludarabine arm and the combination arm, respectively (P = 0.01).

Progression-free survival and treatment-free survival were significantly longer in the FC arm (48 versus 20 months, P = 0.001; and 37 versus 25 months, P > 0.001, respectively).

**Table 3.** Randomized phase III trials on fludarabine and cladribine as single agents or in combination as primary treatment for CLL

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment</th>
<th>No patients</th>
<th>ORR (%)</th>
<th>CR (%)</th>
<th>Median OS (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leporrier (2001) [63]</td>
<td>F vs 341</td>
<td>71</td>
<td>69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leporrier (2001) [63]</td>
<td>F vs 357</td>
<td>71</td>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eichhorst (2005) [72]</td>
<td>FC vs CHOP</td>
<td>185</td>
<td>94</td>
<td>24</td>
<td>not reached</td>
</tr>
<tr>
<td>Catovery (2005) [74]</td>
<td>F vs FC</td>
<td>172</td>
<td>81</td>
<td>15</td>
<td>not reached</td>
</tr>
<tr>
<td>Catovery (2005) [74]</td>
<td>F vs Clb</td>
<td>173</td>
<td>94</td>
<td>39</td>
<td>not reached</td>
</tr>
<tr>
<td>Robak (2000) [133]</td>
<td>GdA vs Clb</td>
<td>126</td>
<td>87</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>Robak (2005) [134]</td>
<td>CdA/C vs FC</td>
<td>63</td>
<td>90</td>
<td>32</td>
<td>not reached</td>
</tr>
</tbody>
</table>

ORR, Overall response rate; CR, complete response; OS, Overall survival; F, fludarabine; Clb, Chlorambucil; C, cyclophosphamide; GdA, cladribine; P, prednisone; CHOP, cyclophosphamide, adriamycin, vincristine, prednisolone.
but no survival benefit has been demonstrated so far, with a 3-year overall survival rate of 80% in both arms. The superiority of FC versus fludarabine alone was also seen in two other trials [73, 74]. Therefore, FC appears to become the new golden standard as first-line therapy for many patients with B-CLL.

Another purine analog, cladribine (2-CdA) was found to achieve similar responses as fludarabine in both previously treated and untreated patients, but no advantage was seen in overall survival compared to chlorambucil/prednisone. Detailed data are reported in Table 3 and suggest that cladribine has similar activity to that of fludarabine, but evidence is yet limited. At present, cladribine cannot be considered a standard alternative therapy for B-CLL. Moreover, sequential use of the two drugs is not meaningful due to the known cross-resistance between them suggested by both clinical and in vitro studies.

**first-line treatment with monoclonal antibodies as single agent or in combination with chemotherapy**

**Alemtuzumab**

Alemtuzumab is a CDR-grafted, human IgG, monoclonal antibody against the CD52 antigen expressed on normal and leukemic B and T lymphocytes, macrophages and monocytes. It is assumed to exert its anti-tumor activity through antibody-dependent cell-mediated cellular toxicity, complement activation and apoptosis induction [75]. Data from published alemtuzumab trials are listed in Table 4. When administered to previously untreated patients, either intravenously [76] or subcutaneously [77], it induced remarkably high response rates: 89% overall response (OR) with 33% CR in the first pilot study and 87% OR with 19% in the later phase II study. In this latter, median time to treatment failure was 32 months [54]. In this study, alemtuzumab was well tolerated, with cytomegalovirus (CMV) reactivations as the main side effect (see below). The study also made clear that subcutaneous administration of the antibody was better tolerated than intravenous infusion and also more practical [77].

Alemtuzumab has also been compared to chlorambucil in an FDA-requested, first-line phase III trial. An interim analysis revealed that the safety profile of alemtuzumab was acceptable [78]. Efficacy data are expected during 2006.

The efficacy of alemtuzumab as consolidation therapy after first-line chemotherapy was also investigated. In the CCL4 trial [90], alemtuzumab arms were randomized to receive consolidation alemtuzumab or no therapy after CR or PR had been achieved either with first-line fludarabine or FC. Progression-free survival was significantly longer and molecular remission rates were higher in the alemtuzumab arm [79], but the trial had to be prematurely closed due to a high infection rate. In a phase II trial by Rai et al. [80] 36 patients with stable disease or respondent to fludarabine were treated with alemtuzumab and a response rate of 92% (42% CR and 50% PR) was noted. In particular, out of 11 patients with stable disease (SD) after fludarabine, 3 achieved CR and 5 had PR following alemtuzumab. In a phase II study conducted by O'Brien et al. [81], alemtuzumab was administered to 41 patients following maximum response to induction chemotherapy. The OR rate was 46% and a molecular disease remission was achieved in 38% of the patients. Conversion from PR to CR or from CR to MDR negativity was also reported using low doses of subcutaneous alemtuzumab after debulking with chemotherapy [82]. These encouraging results indicate that the role of consolidation therapy with alemtuzumab has to be further evaluated in prospective randomized trials. Importantly, the latter studies reported less infectious problems than the CCL4 trial [79], which may be related to a lower dose of alemtuzumab or to a longer wash-out period between last dose of chemotherapy and initiation of alemtuzumab treatment.

**Rituximab**

Rituximab is a chimeric anti-CD20 monoclonal antibody, currently approved for the treatment of B-cell lymphomas. Its cytotoxic activity occurs through various mechanisms, encompassing complement-mediated lysis, antibody-dependent cellular cytotoxicity and direct induction of apoptosis [83]. Data from published trials in CLL are listed in Table 5.

In the only published phase II study [84] of rituximab given as monotherapy to previously untreated CLL patients, rituximab was administered to 44 CLL/SLL patients and a 51% OR rate with 4% CR rate was observed.

Evidence was provided by preclinical studies that rituximab sensitizes cells to both fludarabine and cyclophosphamide [85–87] and that fludarabine can down-regulate complement-resistance proteins on leukemic cells, thus enhancing their sensitivity to rituximab-induced complement-mediated lysis [88]. A randomized phase II study of the Cancer and Leukemia Group B (CALGB) reported a significantly higher CR rate for the concurrent administration of fludarabine and cyclophosphamide (FR) (47% CR) versus the sequential treatment (F followed by R) (28% CR) [89]. A tentative alternative advantage in terms of PFS and overall survival given by the FR combination compared to fludarabine alone has been shown by a retrospective comparison of the CALGB 9712 and 9011 trials [90]. Preliminary results from a single-institution trial combining fludarabine, cyclophosphamide and rituximab (FCR) as first-line therapy are encouraging, showing a 70%

**Table 4. Phase II trials on alemtuzumab as single agent or in combination for the treatment of untreated or previously treated CLL patients**

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment</th>
<th>No patients</th>
<th>Prior therapy</th>
<th>ORR (%)</th>
<th>CR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osterborg (1996) [76]</td>
<td>A</td>
<td>9</td>
<td>no</td>
<td>89</td>
<td>33</td>
</tr>
<tr>
<td>Lundin (2002) [77]</td>
<td>A</td>
<td>41</td>
<td>no</td>
<td>87</td>
<td>19</td>
</tr>
<tr>
<td>Osterborg (1997) [135]</td>
<td>A</td>
<td>29</td>
<td>yes</td>
<td>42</td>
<td>3</td>
</tr>
<tr>
<td>Keating (2002) [105]</td>
<td>A</td>
<td>93</td>
<td>yes</td>
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<td>2</td>
</tr>
<tr>
<td>Moreton (2005) [57]</td>
<td>A</td>
<td>91</td>
<td>yes</td>
<td>55</td>
<td>36</td>
</tr>
<tr>
<td>Elter (2005) [106]</td>
<td>A/F</td>
<td>36</td>
<td>yes</td>
<td>83</td>
<td>30</td>
</tr>
</tbody>
</table>

A, alemtuzumab; F, fludarabine; R, rituximab; ORR, overall response rate; CR, complete response.
pretreated B-CLL patients resulted in a reduction of the CR rates from 3% to 37% [92–95]. In an MD Anderson Cancer Center [103], the FCR combination induced an ORR of 73% (CR 23%, nPR 16%, PR 32%) in 177 pretreated patients. Notably, the response rate was significantly higher in the fludarabine-sensitive patients than in those fludarabine-resistant ones (OR 76% versus 58%, CR 31% versus 5%, respectively, P = 0.039), and toxicity was not increased compared to previous studies with the FC combination. Moreover, a trial is presently ongoing combining alemtuzumab with FCR and preliminary results showed a 65% ORR with 27% CR in 44 heavily pretreated patients [104]. Finally, the combination of rituximab with pentostatin and cyclophosphamide was also investigated (Table 5).

A phase III randomized study by the German CLL Study Group is presently ongoing to compare FCR with FC as first-line therapy.

**salvage therapy for B-CLL**

The indications for second-line and subsequent treatment are basically the same as for initial treatment, that is active, symptomatic disease [59]. Factors predictive of response to subsequent lines of treatment are clinical stage, presence of adverse prognostic factors, number of prior therapy lines and response to previous therapy.

**chemotherapy**

Fludarabine as second-line or subsequent treatment has been tested in a number of phase II trials, and showed an overall response rate (ORR) ranging from 32% to 57% with CR ranging from 3% to 37% [92–95]. In an MD Anderson trial [70], the FC combination achieved an ORR of 80% and 38% in non-fludarabine refractory and fludarabine-refractory patients, respectively, while another group [68] reported a 94% ORR in previously treated patients. Interestingly, a 78% ORR with 50% CR was found combining FC with mitoxantrone [35]. As expected, patients relapsing after a treatment with fludarabine are likely to respond to the same agent used alone or in combination if the response duration time exceeded 6 months.

Response rates to second-line cladribine [96, 97] seem to be similar to those achieved by fludarabine, with a similar toxicity profile.

**monoclonal antibodies as single agent or in combination with chemotherapy**

**rituximab.** Rituximab monotherapy in chemotherapy-pretreated B-CLL patients resulted in a reduction of the leukemic cell count in most patients, but the ORR in such patients was usually low (Table 5). This may be due to a modest effect of this antibody on tumor cells in the bone marrow [98]. In contrast, CLL lymph nodes tended to respond better [99]. The time to treatment failure was often short in these studies, 3–5 months [99, 100]. A higher ORR was reported when high rituximab doses were used [101, 102]. Thus, rituximab seems to be used better in B-CLL as part of combination therapy, rather than as single agent. In a study from the MD Anderson Cancer Center [103], the FCR combination induced an ORR of 73% (CR 23%, nPR 16%, PR 32%) in 177 pre-treated patients. Notably, the response rate was significantly higher in the fludarabine-sensitive patients than in the fludarabine-resistant ones (OR 76% versus 58%, CR 31% versus 5%, respectively, P = 0.039), and toxicity was not increased compared to previous studies with the FC combination. Moreover, a trial is presently ongoing combining alemtuzumab with FCR and preliminary results showed a 65% ORR with 27% CR in 44 heavily pretreated patients [104]. Finally, the combination of rituximab with pentostatin and cyclophosphamide was also investigated (Table 5).

*High dose rituximab used in these studies

<table>
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<tr>
<th>Author</th>
<th>Treatment</th>
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<th>CR (%)</th>
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<tr>
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</table>

F, fludarabine; R, rituximab; C, cyclophosphamide; Pn, pentostatin; D, dexamethasone. ORR, overall response rate; CR, complete response.

Table 5. Rituximab as single agent or in combination for the treatment of untreated and previously treated CLL patients

A number of trials showed that alemtuzumab as a single agent has clinically significant activity in fludarabine-refractory patients, with overall response rates ranging from 33% to 55% and response duration time of 9–12 months (Table 4). The median overall survival in responding patients was 32 months [105]. The drug is now approved for the treatment of fludarabine-refractory CLL. In one of these studies [57] the eradication of MDR assessed by four-color flow cytometry was seen to correlate positively with longer overall and treatment-free survival. Notably, in all these trials, tumor cells were more effectively cleared from blood and BM than in lymph nodes; the reason for this is still not understood.

The combination of alemtuzumab with fludarabine (FluCam) [106] has shown acceptable toxicity and promising results, with an OR rate of 83% (CR 30%) and a median overall survival of 36 months in 36 patients, of whom 41% were fludarabine-refractory. Combining alemtuzumab and rituximab may also be interesting [107]. Importantly, alemtuzumab appears to be equally effective in patients with p53 mutation/17p deletion, who are otherwise refractory to conventional therapy and have a very poor prognosis [30].

Immunosuppression and risk of infections are the most common complications associated with alemtuzumab therapy. Both nucleoside analogs and alemtuzumab may affect cellular immunity for an extended period. The occurrence of infections by Pneumocystis carinii and Herpes zoster has been substantially reduced by prophylactic treatment with antibiotics and antivirals. The risk of CMV reactivation after alemtuzumab monotherapy is also of concern, as reported data show it occurs in ±20% of patients, typically after 3–8 weeks of alemtuzumab therapy. At present, prophylaxis for CMV reactivation is not standardized and it is recommended that patients receiving alemtuzumab are carefully monitored for early symptoms of CMV reactivation (i.e. fever) during therapy. If verified by PCR, oral or intravenous ganciclovir therapy must be started without delay.
blood and bone marrow transplantation

The use of high-dose therapy followed by stem cell transplantation has been quite limited in B-CLL, mainly because in the majority of patients with B-CLL have an indolent course and it affects mainly patients older than 60 years. Moreover, by the time patients are referred to transplantation centers, most have already been heavily pretreated, which negatively affects the chemosensitivity of their disease and their stem cell reserve. While most patients do not have a suitable allogeneic donor, the use of autologous hematopoietic cell transplantation (HCT) is in many cases hampered by the disease-related extensive BM infiltration and peripheral blood involvement. All these reasons create reluctance in many centers to submit patients to such a procedure. Approximately 10–15% of patients, though, are younger than 50 years and there is a subgroup of patients in whom disease progresses rapidly despite intensive treatment. In particular, patients who have failed a purine analog-based regimen have a remarkably poor prognosis, with a median survival of approximately 8 months [105]. Selection based on prognostic factors is thus mandatory to identify poor prognosis patients eligible for more aggressive therapies with a potentially curative intent.

Autologous HCT is feasible in young patients, though available data up to now globally indicate that this procedure is not curative in CLL, with transplant-related mortality rates ranging from 4% to 10% and 4-year overall survival ranging from 65% to 94% [108–111]. The recently reported pilot study by Milligan et al. [112] showed an early transplantation-related mortality (TRM) rate of only 1.5% and 5-year overall survival and DFS rates of 77% and 51%, respectively.

Though these results are encouraging, no randomized trials are available that compare autologous transplantation with standard chemotherapeutic approaches and published trials differ substantially in patient selection criteria, type of conditioning therapy, use of in vitro or in vivo purging. Moreover, the optimal timing for this procedure and the optimal mobilization and conditioning therapies have to be defined.

Allogeneic HCT, on the other hand, is known to achieve better results in terms of overall survival and can be considered a potentially curative treatment in CLL, but this is at the cost of a TRM ranging from 10% to 52% [111, 113–116]. The recently published trials show a 2-year TRM ranging from 15 to 26% and 50–70% 2-year overall survival [118–120]. Hopefully, this approach will improve the outcome of transplanted patients, both in terms of responses and treatment tolerability, extending the applicability of this treatment to a wider population of CLL patients.

new perspectives

Several clinical trials are presently ongoing to investigate the activity of new drugs. These include, among others, the nucleoside analog clofarabine, the cyclin-dependent kinase inhibitor flavopiridol, the IL-2 receptor ligand immunotoxin Ontak, and the telomerase template antagonist GRN163L. A recently published phase I-II trial [123] tested the activity of the Bcl-2 antisense oligonucleotide Oblimersen sodium in fludarabine-pretreated patients and found a modest ORR. Recently reported data from a phase II trial of lenalidomide, a compound belonging to a new group of drugs with immunomodulatory properties, showed an ORR of 68% (15% CR) in 19 patients with relapsed or refractory CLL [124]. Some monoclonal antibodies, such as lumiliximab (anti-CD23), epratuzumab (anti-CD22), apolizumab (anti-MHC II) and galiximab (anti-CD80) have already been tested in phase I trials. The human monoclonal antibody HuMax-CD20 [125] was given in a phase I/II trial to patients with relapsed or refractory CLL and an ORR of 46% was seen in 33 patients at the highest dose level: further trials are warranted. Other monoclonal antibodies such as anti-CD40 antibodies, TRAIL receptor, DR4 and DR5 directed antibodies, and antibody-like molecules targeting CD37 have been tested in vitro.

A vaccination therapy for the treatment of CLL is an attractive option to standard treatments. Considering the indolent nature of the disease and the advanced age of most patients, in fact, the possibility of delivering a treatment with negligible toxicity is surely appealing. As a good immune function is a prerequisite for the success of this approach, a substantial period of time must have lapsed from previous therapy, as currently employed agents for CLL, including fludarabine and alemtuzumab, increase the disease-related immune deficiency by depleting T cells. In the few published studies, all phase I trials, modified autologous leukemic cells were used as the immunogen. Clinical responses were noted [126, 127], together with enhanced T-cell reactivity against autologous tumor cells [126, 128, 129]. Another approach currently under investigation exploits the antigen-presenting capacity of dendritic cells to prime a T-cell activation [130–132].

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