

# Chronic Lymphocytic Leukemia

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## ABSTRACT

The diagnosis and management of chronic lymphocytic leukemia (CLL) is reviewed, including the basic aspects of epidemiology, molecular biology, and cytogenetics with clinical relevance. The importance of

immunophenotype in the differential diagnosis of other lymphoproliferative disorders related to CLL, staging, prognostic factors, promising new drugs, and approaches is summarized. *The Oncologist* 1999;4:352-369

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## INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most frequent form of leukemia in adults in western countries, accounting for 25% of all leukemias [1], but fewer than 5% of the cases in the eastern hemisphere. In the USA, 95% of CLL cases are B-cell phenotype, while in Asia, T-cell CLL predominates [1, 2]. The true incidence of CLL is difficult to assess. Patients may be asymptomatic prior to an incidental diagnosis. In the last 20 years, the number of cases of CLL presenting in asymptomatic stages has doubled from 30% to 60%, likely due to an increasing number of blood tests performed for other medical or surgical reasons. Moreover, sensitive techniques to diagnose and differentiate CLL from other chronic lymphoproliferative disorders have only recently become routinely available [3, 4]. The estimated current annual incidence in the USA varies from 7,300 to 12,500 new cases, with an overall incidence rate of 2.3/100,00 [5-8].

## ETIOLOGY

The etiology of CLL is unknown [1]. Several lines of evidence suggest a genetic component, such as the increased prevalence of CLL among first-degree relatives, the phenomenon of anticipation, where there is an increased severity and earlier age of onset with each generation and the increased frequency of autoimmune disorders in relatives of CLL patients [9-15].

Environmental factors, such as ionizing radiation, chemicals (benzene and solvents from the rubber industry), and drugs have shown no apparent relationship [16-19].

## MOLECULAR BIOLOGY

CLL is a model for failed programmed cell death or apoptosis. The BCL-2 family proteins, key regulators of programmed cell death (PCD), are overexpressed in 90% of B-CLL cells, although in the vast majority of the cases (96%-99%), no translocation involving the BCL-2 gene has been detected. These slow-growing B-CLL cells accumulate in the body, predominantly in the G<sub>0</sub> phase of the cell cycle. One consequence is an acquired resistance to cell-cycle-active agents. The imbalance in the ratio of major pro- and anti-apoptotic BCL-2 family proteins, such as BAX and BAK (induction of apoptosis), BCL-2 (anti-apoptotic), BAD, BIK, and HRK (anti-apoptotic inhibitors) seems to play an important role in the behavior and treatment response of CLL, although convincing clinical evidence is not yet available [20-22].

Mutations of the tumor suppressor gene p53 and increased expression levels of the cyclin-dependent kinase inhibitor p27 have been shown to correlate with disease progression and overall poor prognosis. Poor response to therapy has been associated with p53 mutations [23-26].

Cytokines produced and released directly by CLL cells, such as tumor necrosis factor (TNF) and interleukin 8 (IL-8), as well as IL-2, which is produced by T lymphocytes and absorbed by CLL cells through specific receptors, participate in autocrine or paracrine loops and affect CLL cell survival and proliferation [27-31]. IL-4 production is associated with increased expression of CD30 by expanded CD8<sup>+</sup> T cells. Since there is evidence that most CLL cells express the

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CD30 ligand, this interaction may influence the CLL cells environment and its immune functions [32].

One critical step for the immune response to antigen is the expression of the ligand for CD40 (CD40L or CD154), produced by activated T cells. Downregulation of this ligand induced by leukemic CLL lymphocytes results in severe immunodeficiency states. Gene therapy trials involving transfer of a functional CD40 ligand into CLL cells to generate cytotoxic T lymphocytes against autologous nontransduced leukemic cells are ongoing [33, 34].

### CYTOGENETICS

There is no single specific cytogenetic abnormality in CLL [1]. The development of new techniques, such as fluorescent in situ hybridization (FISH), has increased the detection of numerical and structural chromosome abnormalities. The most common cytogenetic alteration is deletion 13q14 (51%), followed by deletion 11q22-q23 (17%-20%), trisomy 12 (15%), and deletion 17p13 [20, 35-39]. Complex abnormalities may be present; for example, CLL patients with trisomy 12 may have simultaneous 13q14 deletions [10, 39-41].

A number of cytogenetic abnormalities, including trisomy 12 and the presence of 14q, 11q, and 17p chromosome abnormalities, have been related to poor outcome. Trisomy 12 is associated with atypical lymphocyte morphology and immunophenotype (CD5<sup>-</sup>, FMC7<sup>+</sup>, strong immunoglobulin expression) and disease progression. Patients with 11q deletions tend to be younger, with an advanced clinical stage at presentation associated with extensive peripheral, abdominal, and mediastinal lymphadenopathy, and a treatment-free interval of nine months in contrast to 43 months for those without the deletion. Chromosome 17 abnormalities have been associated with p53 mutation, fludarabine-resistance therapy, and therapy failure in patients with Richter's syndrome, atypical CLL morphology, and prolymphocytic leukemia (PLL). It is unclear whether these cytogenetic abnormalities are primary or secondary events [35, 36, 38, 39, 40, 42-47].

The presence of IgV gene mutations have been associated with the lack of CD38 expression in a group of patients with good clinical outcome and better survival. Therefore, CD38 may be useful both as a surrogate for IgV gene mutations and as a prognostic factor [48, 49].

### CLINICAL FEATURES

About 40%-60% of patients with CLL are diagnosed in the absence of disease-related symptoms, even with very high numbers of circulating lymphocytes  $>100 \times 10^9/l$ . Frequently, the presence of lymphadenopathy or an abnormal CBC performed during a routine medical examination is the only reason to consider the diagnosis [1, 50]. The remaining patients may present with weakness, fatigue, night sweats, fever, and

may be with or without infections or autoimmune diseases [51-56]. Physical examination generally reveals nontender, painless, and mobile lymphadenopathy [1], splenomegaly, or hepatomegaly. Metabolic abnormalities (e.g., hyperuricemia) or mechanical disorders (e.g., airway obstruction) related to the tumor burden, may also be present. Any part of the body, including skin and meninges may be infiltrated by CLL cells [50]; however, such findings are uncommon. Manifestations of bone marrow (BM) involvement, particularly significant anemia (hemoglobin  $<11$ g/dl) or thrombocytopenia (platelets count  $<100 \times 10^9/l$ ), are noted at presentation in 15% of CLL patients. A positive direct antiglobulin test (DAT) is present in about 20% of patients at diagnosis but is not commonly associated with hemolytic anemia [57].

### DIAGNOSIS

The National Cancer Institute-Sponsored Working Group (NCI-WG) published guidelines for the diagnosis and criteria for response for CLL (Table 1) [58]. The peripheral blood should exhibit an increase in the number of small mature-appearing lymphocytes to  $>5,000/\mu l$  [58]. The BM aspirate smear must show  $>30\%$  of all nucleated cells to be lymphoid. Although a BM examination is rarely required to make the diagnosis of CLL in general practice, it may be valuable prior to the start of treatment in order to define prognostic factors (Table 2). Subsequently, a BM examination is indicated primarily to evaluate response to treatment or to assess normal elements if there is an unexplained anemia or thrombocytopenia [58].

### DIFFERENTIAL DIAGNOSIS

CLL can be distinguished from other closely related chronic lymphoproliferative disorders on the basis of its morphology and immunophenotype (Table 3), such as the leukemic phase of mantle-cell lymphoma (MCL-L) and follicular lymphoma (FL-L), PLL, hairy cell leukemia (HCL), and splenic lymphoma with villous lymphocytes (SLVL) [48, 58, 59]. Flow cytometry has been very helpful in differentiating between MCL-L and CLL. The morphology of MCL is small cleaved lymphocytes, and the immunophenotype is that of B-cell neoplasms (CD5<sup>+</sup>, CD19<sup>+</sup>, CD20<sup>+</sup>), but MCL can be distinguished from CLL based on its expression of cyclin D1 and the lack of CD23 expression. In addition, MCL has a brighter or more intense surface immunoglobulin and CD20 expression than CLL. t(11;14), involving *BCL-1*, the locus of PRAD-1 or cyclin D1 gene, is typically present.

Three main phenotypic features present on B-cell CLL cells differentiate CLL from PLL and HCL. CLL cells are positive for B-cell antigens, CD19, CD20, and CD23, and they coexpress CD5 in the absence of T-cell

**Table 1.** NCI-WG guidelines for the diagnosis and response criteria for CLL [58]

<b>Diagnosis</b>	
Lymphocytosis ( $\times 10^9/l$ )	$>5$ ; $\geq 1$ B-cell marker (CD19, CD20, CD23) + CD5
Apyctical cells (%) (eg. prolymphocytes)	$<55$
Duration of lymphocytosis	None required
BM lymphocytes (%)	$\geq 30$
Staging	Modified Rai, correlate with Binet
Eligibility for trials	Active disease
<b>Response criteria</b>	
<b>CR</b>	
Physical exam	Normal
Symptoms	None
Lymphocytes ( $\times 10^9/l$ )	$\leq 4$
Neutrophils ( $\times 10^9/l$ )	$\geq 1.5$
Platelets ( $\times 10^9/l$ )	$>100$
Hemoglobin (g/dl)	$>11$ (untransfused)
BM lymphocytes (%)	$<30$ ; no nodule
<b>PR</b>	
Physical exam (nodes, and/or liver/spleen)	$\geq 50\%$ decrease
<b>Plus <math>\geq 1</math> of:</b>	
Neutrophils ( $\times 10^9/l$ )	$\geq 1.5$
Hemoglobin (g/dl)	$\geq 11$ or 50% improvement
Platelets ( $\times 10^9/l$ )	$>100$
Duration of CR or PR	$\geq 2$ months
<b>Progressive disease</b>	
Physical exam (nodes, liver, spleen)	$\geq 50\%$ increase or new
Circulating lymphocytes	$\geq 50\%$ increase
Other	Richter's syndrome
<b>Stable disease</b>	
	All others

markers. The cells are monoclonal with respect to their expression of either kappa or lambda light chains. Surface immunoglobulin (sIg) is of low density. Other markers more characteristic of NHL or HCL, such as CD10 and CD103, are absent.

### STAGING AND PROGNOSTIC FACTORS

Rai and Binet staging systems (Table 4) are the most commonly used staging systems in CLL [60-62]. However, neither system accurately identifies those patients in early stages who will progress from those who will remain indolent. Lymphocytosis alone is not classified by the Binet system, and neither system includes splenomegaly alone.

Unfavorable prognostic factors, independent of clinical stage, are age ( $>55$  years), male sex, black race, and poor performance status. There are no differences between older and younger CLL patients in presenting features, response rates, or duration of response.

Laboratory prognostic factors which may predict outcome include lymphocyte doubling time, with a 12-month cut-off,  $\beta 2$ -microglobulin, soluble CD23, and LDH. Whether the pattern of BM infiltration and the degree of lymphocyte infiltration are independent factors is controversial. The prognostic value of bcl-2, fas, and multidrug resistance gene expression is unclear [10, 39, 40, 62-78].

### TREATMENT

Over the past few decades, there has been little progress in prolonging survival of patients with CLL, and it remains an incurable disorder.

#### Indication for Treatment

Since no treatment to date has made a significant impact on the outcome of patients with early-stage CLL, when to initiate therapy becomes an important decision. Factors which should prompt the initiation of therapy include the presence of disease-related symptoms, massive and/or progressive lymphadenopathy or hepatosplenomegaly, BM failure, or recurrent infections. The lymphocyte doubling time should be considered in the total clinical picture but not used as the primary criterion [58].

Nevertheless, about 30% of patients have indolent or smoldering CLL characterized by Binet stage A, non-diffuse BM histology, hemoglobin  $\geq 13$  g/dl, blood lymphocytes  $\leq 30 \times 10^9/l$ , and lymphocyte doubling time  $>12$  months [59]. Their life expectancy is similar to that of a population without CLL. Only 15% of these patients are likely to exhibit progressive CLL, and careful observation is the standard of care (Tables 2, 3) [58, 79, 80].

#### Previously Untreated Patients

##### Early Versus Delayed Treatment

Whether early therapeutic intervention should be used in patients with limited-stage disease has been addressed by several randomized trials [81, 82]. The French Cooperative Group's recently updated studies included 1,535 previously untreated patients with Binet A disease. In the CLL-80 trial, 609 patients were randomly assigned to receive either treatment with daily chlorambucil (CLB) (0.1 mg/kg/day) until clinical resistance to the drug was observed or no initial therapy. The response rate in the treated group was 76%. After follow-up of more than 11 years, 49% of patients had still not progressed; however, 27% of the stage A CLL patients died of

disease-related causes. In the CLL-85 trial, 926 patients were randomly assigned to receive intermittent CLB (0.3 mg/kg/day for five days every month), plus prednisone (40 mg/m<sup>2</sup>/day for five days every month) for three years or no treatment. The response to therapy in the treated group was 69%.

Although CLB slowed progression to stage B and C in both trials, there was no advantage to early intervention on the overall survival. The reason for the survival differences

between the untreated and treated groups for those patients who progressed to stage B (54% versus 21%) is unknown. Disease progression and infections, followed by second malignancies—mostly epithelial malignancies—were the most frequent CLL-related causes of deaths in the treated group in both trials. Although there was an apparent increase in second malignancies in the treated group from the earlier trial, this observation was not made in the subsequent study. One possible explanation

for this discrepancy is the difference in drug administration schedules (daily versus intermittent).

In another study, Cancer and Leukemia Group B (CALGB) investigators [82] randomized 51 patients to intermittent CLB once a month, or to no initial treatment. At five years from randomization, the proportion of patients exhibiting active disease was 70% in the untreated group and 55% in the treated group; however, there was no significant difference in survival between the two groups. Other U.S. and European trials with alkylating-agent-based protocols have also failed to show benefit for early treatment in patients with indolent CLL [83].

**Table 2.** NCI-WG recommendations for evaluation and monitoring CLL patients [58]

Recommendation	General practice	Clinical trial
<b>Pretreatment evaluation</b>		
History and physical	✓	✓
Examination of peripheral blood smear	✓	✓
Immunophenotyping of peripheral blood lymphocytes	✓	✓
BM at diagnosis	+	✓
BM prior to therapy	✓/✗	✓
Cytogenetic/molecular studies	×	*
CT scans, MRI, lymphangiogram, gallium scan	×	×
<b>Indications for treatment</b>		
Treat with stage 0-1	×	*
Treat for active/progressive disease (newly diagnosed)	✓	✓
Treat without active/progressive disease (newly diagnosed)	×	*
Treat without active/progressive disease (relapsed/refractory)	×	*
<b>Response assessment</b>		
CBC, differential	✓	✓
BM	+	✓
Phenotype	×	+
Cytogenetics/FISH	×	*

General practice is defined as the accepted treatment options for CLL patient not enrolled in a clinical trial.

Abbreviations: ✓ = always; × = not generally indicated; + = desirable; \* = if a research question; ✗ = if a study not performed recently; e.g. = at diagnosis; MRI = magnetic resonance imaging; FISH = fluorescence in situ hybridization.

**Table 3.** Phenotype of chronic B-cell lymphoid leukemias [59, 71]

Disease	sIg	CD5	CD23	CD10	CD11c	CD43	CD103	FMC7	CD25
CLL	-/+	+	+	-	-/+	+	-	-	-
PLL	++	-/+	-/+	-	+/-	-/+	-	+	-
HCL/v	++	-	-	-	+	+/-	+	+	++
MZL	+	-	+/-	-	+	+	-	+	-/+
MCL-L	+	+	-	-/+	-	+	-	+/-	-
FL-L	+	-	-	+/-	-	-	-	+	-
LPL	+	-	-	-/+	+	-/+	-	+	-/+
SLVL	+	-/+	+/-	-/+	+/-	+	-/+	+	-/+

All express pan B-cell-associated antigens (e.g., CD19, CD20) and HLA-DR class II antigens. -/+, -, +, ++, +/- symbols refer to frequency a marker is expressed.

Abbreviations: CLL = chronic lymphocytic leukemia; PLL = prolymphocytic leukemia; HCL/v = hairy cell leukemia/hairy cell leukemia variant; MZL = marginal zone lymphoma; MCL = mantle cell lymphoma-leukemic phase; FL-L = follicular lymphoma-leukemic phase; LPL = lymphoplasmacytoid lymphoma; sIg = surface immunoglobulin; FL = follicular lymphoma; LPL = lymphoplasmacytoid lymphoma (immunocytoma, Waldenström's macroglobulinemia); SLVL = splenic lymphoma with villous lymphocytes.

**Table 4.** The Rai and Binet Staging Systems; main features and median survival [62]

Staging system	Stage	Modified three-stage-system	Clinical features	Median survival (years)
<i>Rai</i>	0	Low-risk	Lymphocytes only (in blood and marrow)	>10
	I	Intermediate-risk	Lymphocytosis + lymphadenopathy	7
	II		Lymphocytosis + splenomegaly and/or hepatomegaly ± lymphadenopathy	7
	III	High-risk	Lymphocytosis + anemia (hemoglobin <110 g/l) ± lymphadenopathy ± splenomegaly ± hepatomegaly	1.5
	IV		Lymphocytosis + thrombocytopenia (platelets <100 × 10 <sup>9</sup> /l) ± anemia ± lymphadenopathy ± splenomegaly ± hepatomegaly	
<i>Binet</i>	A		<3 node-bearing areas	>10
	B		≥3 node-bearing areas	5
	C		Anemia and/or thrombocytopenia	2

### Front-Line Treatment

Alkylating agents and purine analogs (PAs) are the most important agents available for the treatment of CLL. CLB, has been the most commonly used alkylating agent in CLL.

Cyclophosphamide has been used for patients unresponsive to or intolerant of CLB. Early trials used different criteria to start treatment and different doses of drug and schedules of administration. Definition of clinical response varied widely, making it difficult to interpret response rates [72, 84-87]. In general, the overall response rate with CLB-based regimens has been between 40% and 60%, with complete responses (CRs) in fewer than 20% of patients.

PAs, fludarabine (2-fluoro-ara-AMP) and cladribine (2-chlorodeoxyadenosine, 2-CdA), and the irreversible inhibitor of adenosine deaminase, pentostatin (2'-deoxycoformycin, 2-DCF) [88], altered dramatically the approach to CLL patients. Their basic chemical structure is listed in Figure 1. The algorithm for CLL patient approach is suggested in Figure 2 [89].

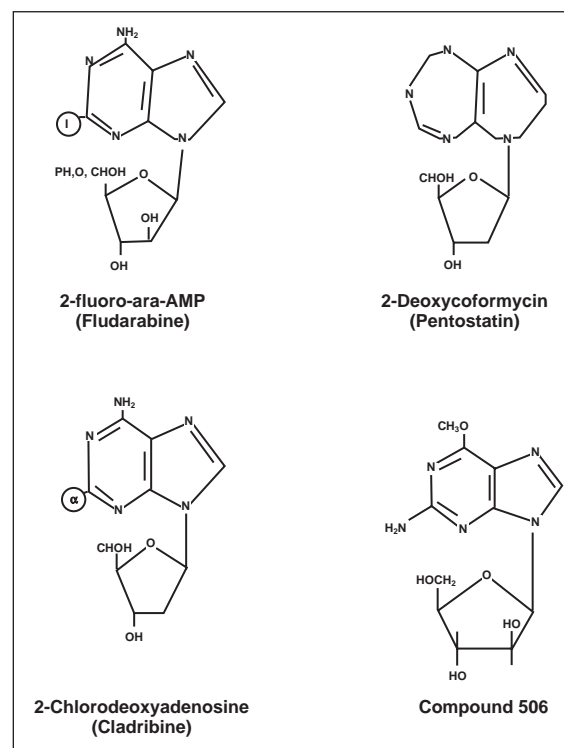
### CLB

CLB is available in tablet form, and its absorption by the gastrointestinal tract is almost complete. The most common and least myelosuppressive schedule is 20-30 mg/m<sup>2</sup> every two weeks. Regimens of 20-40 mg/m<sup>2</sup> every four weeks or the daily schedule of 4-8 mg/m<sup>2</sup> are also used. There is no indication for maintenance therapy following maximal response [91-93]. In previously untreated patients, combination chemotherapy, including such drugs as prednisone, vincristine, or anthracyclines, has produced responses between 10% and 80%, with very few CRs. The heterogeneous population enrolled in these trials does not allow accurate comparisons. No combination has been

proven to be superior either to another combination or to a single agent [71, 81, 83, 91-93].

### Corticosteroids

The use of corticosteroids should be reserved for patients with CLL and concurrent autoimmune phenomena [53, 71, 85, 90, 93-95]. These agents have limited single-agent activity and are associated with substantial toxicity, including opportunistic infections and osteopenia.



**Figure 1.** Chemical structures of the purine nucleoside analogs.

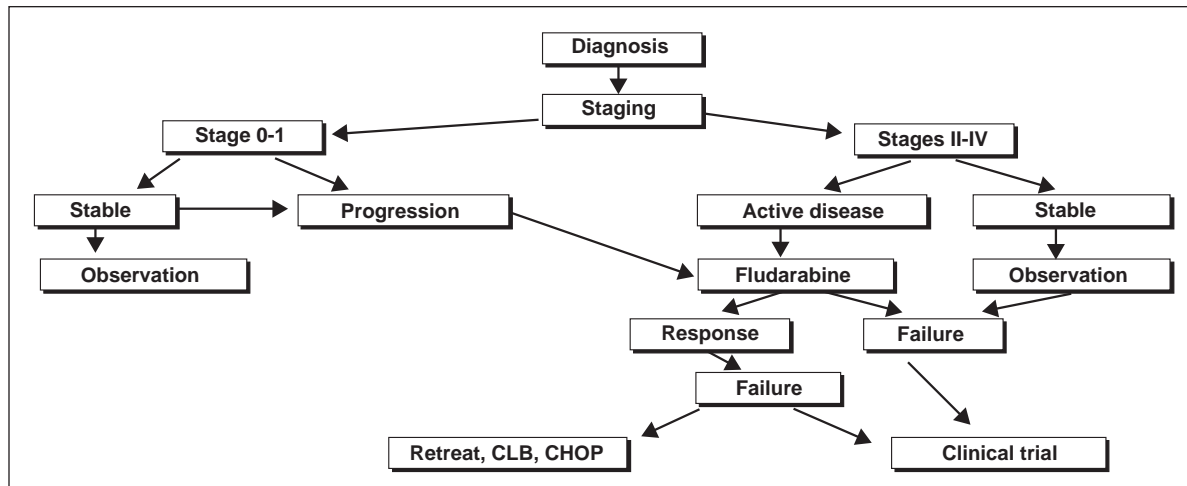


Figure 2. Suggested algorithm for approaching CLL in clinical practice [89].

### Fludarabine

Fludarabine (2-fluoro-ara-AMP), is an adenosine deaminase (ADA)-resistant nucleoside analog. It is initially phosphorylated intracellularly by DCK to 2-fluoroadenosine monophosphate (2-fluoro-ara-AMP) and sequentially phosphorylated to diphosphate by adenylic kinase, then by nucleoside diphosphate kinase to the active metabolite 2-fluoro-ara-ATP, which inhibits DNA synthesis and repairs RNA synthesis. Apoptosis, resulting from the accumulation of oligonucleosomal fragments, may be one of the main anti-tumor mechanisms in dividing and nondividing cells [73].

The initial data with fludarabine as front-line agent in the CLL therapy were reported and recently updated by *Keating et al.* [95-102]. Their experience included 191 CLL patients treated with fludarabine or fludarabine with prednisone [100-101]. There were 30% complete remissions (by NCI-WG criteria), with an overall response rate (partial response [PR] + CR) of 78%, whereas 22% of patients failed to respond to fludarabine. Three percent of patients died during the first three months. The average time to progression was 30 months, with patients reaching CR (37%) having the longest time to progression, regardless of the CLL stage. Survival was the same for CR and nodular partial remission (nPR). With an average follow-up close to three years, 10%-15% of these patients are still in remission. The results of these trials and the randomized ones indicate that fludarabine is the most active agent for front-line therapy of CLL.

### Fludarabine and Randomized Clinical Trials

Two major randomized trials have compared the efficacy of fludarabine with alkylating-agent-based treatment as front-line treatment of CLL. CALGB, Southwest Oncology Group (SWOG), Cancer Therapy Group/ National Cancer Institute of Canada (CTG/NCI), and Eastern Cooperative

Oncology Group (ECOG) intergroup study [102] allocated 544 untreated patients with active disease to receive fludarabine (25 mg/m<sup>2</sup>/day for five days) or CLB (40mg/m<sup>2</sup>/day 1) or a combination of fludarabine and CLB (20mg/m<sup>2</sup> of each drug, CLB on day 1, fludarabine days 1-5) every four weeks for up to 12 months. Patients who failed to respond or who experienced progressive disease with the original treatment in the first six months, were crossed over to the other arm. The 167 patients from the fludarabine group had an overall response rate of 70% (27% CR and 43% PR), which was significantly higher than the 173 patients from the CLB group who had an overall response rate of 43% (3% CR and 40% PR),  $p < 0.001$ . The duration of response for those patients who reached CR and PR was also significantly longer in the fludarabine group, 32 months versus 18 months for those in the CLB group ( $p = 0.0002$ ). Survival analysis for 385 patients was available. The median progression-free survival was 27 months for the fludarabine group and 17 months for the CLB group ( $p < 0.0001$ ). However, no difference in the overall survival was seen at a median follow-up of 30 months.

The French Cooperative Group on CLL [103, 104] evaluated 695 previously untreated stage B (486) and C (209) patients; 225 received fludarabine (25 mg/m<sup>2</sup>/day), or one of two anthracycline-based standard combination regimens: 238 patients received CAP (cyclophosphamide 750 mg/m<sup>2</sup>/day 1; doxorubicin 50 mg/m<sup>2</sup>/day 1, and prednisone 40 mg/m<sup>2</sup>/days 1-5), and 232 patients received CHOP (cyclophosphamide 300 mg/m<sup>2</sup>/day 1, doxorubicin 25 mg/m<sup>2</sup>/day 1, vincristine 1 mg/m<sup>2</sup>/day 1, and prednisone 40 mg/m<sup>2</sup>/days 1-5). Complete histological remission (CHR) and immunological remission (IR) were defined as normal BM biopsy and <100 CD5<sup>+</sup>CD19<sup>+</sup> cells/μl in the peripheral blood. CHR was 28%, 13%, 37%; partial remission, defined as greater than 50% reduction of measurable disease manifestations and a more than 50%

improvement of all abnormal blood counts, was 49%, 53%, 44%, respectively; and failure was 23%, 35%, and 19%, respectively, for the CHOP, CAP, and fludarabine group. CHR was observed in 37% of fludarabine, 28% of CHOP, and 13% in the CAP group. In the fludarabine, CHOP and CAP groups, IR was 55%, 35%, and 29%, respectively, and the number of reported deaths were 38, 51, and 62, respectively.

While nausea and vomiting were more frequent in the CAP group, anemia and treatment-related thrombocytopenia were higher in the fludarabine group. Eleven treatment-related deaths were reported, three in the CHOP, five in the CAP, and three in the fludarabine group. To date, no treatment combination has given better results than fludarabine alone. Nevertheless, no chemotherapy regimen has clearly prolonged survival in CLL.

**Cladribine**

Varying schedules, routes of administration, small numbers of patients, different response criteria, and short follow-up of these trials have made the comparison among various studies difficult (Table 5). Pooling together 174 previously untreated patients from these trials, and using the NCI criteria,

the overall response (PR + CR) is estimated to be similar to fludarabine (75%-85%), with CRs between 10% and 26%. The duration of response, between three and eight months, is shorter than with fludarabine. Based on the trials above, 2-CdA induces fewer CRs with a shorter duration of response than fludarabine [89, 90, 95, 105-113]. Myelosuppression and infections, including opportunistic agents, were the most common side effects [89].

**Pentostatin**

The majority of trials (Table 5) with pentostatin (2-DCF) were conducted in previously treated patients. Only one trial involving 13 previously untreated patients has been published, with six PRs, no CRs, and an overall response of 46%. The response rate with pentostatin in CLL patients appears to be lower than in patients treated with fludarabine.

**Second-Line Treatment**

Therapy for patients who have relapsed after initial therapy or who were refractory is palliative in intent. Therefore, patients without disease-related symptoms or deterioration of blood counts, infections, or autoimmune

**Table 5.** Fludarabine, cladribine, and pentostatin activity in previously treated and untreated CLL patients

Fludarabine					Cladribine					Pentostatin				
Trial	Patients (n)	RR (%)	CR (%)	Duration (months)*	Trial	Patients (n)	RR (%)	CR (%)	Duration (months)*	Trial	Patients (n)	RR (%)	CR (%)	Duration (months)*
<b>Previously treated patients</b>														
Grever [184]	32	13	3†	NA	Saven [105]	90	44	4†	4	Grever [96]	20	20	5	10.9
Keating [101]	78	58	14	21	Tallman [106]	26	31	0	16	Dillman [97]	26	16	5	NA
O'Brien [198]	169	52	37†	22	Robak [107]	92	36	5†	NA	Ho [190]	26	27	0	7.5
Hiddemann [185]	20	55	20†	NA	Julliusson [112]	52	58	31†	20‡	Dearden [98]	17	35	0	6
Puccio [186]	42	31	0	6	Rondelli [109]	19	68	11†	CR, 9+§	Dearden [98]	29	30	NA	NA
Bergmann [187]	18	67	NA	6						O'Dwyer [99]	29	24	3	NA
Spriano [188]	21	48	5	NA										
Montserrat [189]	68	28	4†	NA										
Sorensen [123]	703	32	3†	13										
French cooperative Group on CLL [103]	48	48	13†	11										
<b>Previously untreated patients</b>														
Keating [101]	35	80	37	33	Robak [107]	18	56	17†	NA	Dillman [97]	13	46	0	NA
O'Brien [198]	95	79	63†	CR,	Saven [110]	20	85	10	8+					
French cooperative Group on CLL [103]	52	71	23†	24+§	Delannoy [111]	19	74	47†	NA					
Rai [102]	166	70	27	PR 30	Julliusson [108]	63	75	16	14					
				34+§	Tallman [113]	54	81	26	NA					
				33										

Abbreviations: n = number of assessable patients in trial; RR = percentage of patients responding to treatment (CR + PR); CR = complete response by the current NCI guidelines (including absence of BM nodules); PR = partial response; NA = information not available.

\*Median duration of remission.

†Percentage of patients achieving a CR with or without lymphoid nodules in the BM.

‡Actuarial estimate.

§Median duration not reached at time indicated.

disease, may be followed until therapy is indicated, using the same criteria as with initial treatment [58]. When treatment is indicated, patients should be considered for clinical research trials.

For patients who were initially treated with fludarabine and experienced a response that lasted a year or longer, retreatment with fludarabine is associated with an overall response of more than 50% [100-102, 114]. Patients with shorter durations of response should be considered for alternate treatment options. Combinations with chemotherapy agents such as cyclophosphamide [114] and biologic agents such as rituximab are currently being evaluated [88, 100, 101, 115-122]. Alkylating agents alone or in combination with anthracyclines have limited effect as second-line treatment [88, 90, 100, 101, 118]. In fludarabine failures, CLB achieves only a 17% PR rate [102].

For patients whose disease was refractory to fludarabine, a clinical trial of a novel agent is the best treatment option.

Fludarabine is the drug of choice for patients whose disease relapsed after or was refractory to an alkylating agent as initial treatment.

Several trials (Table 5) have shown overall response rates between 28% and 67%. The discrepancies among these trials reflect not only the small number of patients with different prognostic factors but also different schedules. Duration of response and overall survival are longer for patients who relapse than for those patients who were refractory to their initial therapy [101].

The largest single-center experience with fludarabine in CLL was from the M.D. Anderson Cancer Center, in which there were 57% complete remissions and 36% partial remissions of 28 relapsed patients and 28% complete remissions and 10% partial remissions of 50 refractory patients. However, the complete remission rate in this trial is artificially high because in 63% of those cases there were residual lymphoid nodules in the BM, which would now be considered nodular PRs [100]. The median time to progression was 18 months for patients who were refractory to alkylating agents and 17 months for patients who had relapsed after previous response; however, the median survival was 29 months for the relapsed patients and nine months for the refractory patients. The NCI group C study [123] included 791 patients with advanced CLL who failed to respond to alkylating agents. Of the 724 patients entered, 703 were evaluated for response. Fludarabine was to be delivered at 25 mg/m<sup>2</sup>/day for five consecutive days every four weeks. The median age was 65 years. The overall response rate was 32%, with CR in 3%, and PR in 29%. With a follow-up duration of 59 months, the median duration of response was 13.1 months, median survival time from registration was 12.6 months, median time to respond 4 months, and time to progression was 7.5 months. Age, performance

status (PS), and Rai stage correlated with survival ( $p < 0.01$ ). Toxicity was similar to that noted in other trials, with grade 4 hematologic toxicity reported in 43% and infection in 22%. Neurotoxicity correlated with age [124]. High risk, older age, and extensive prior treatment may explain, in part, the low CR and overall response rate in this study.

In summary, the challenging decision regarding when and how to treat CLL patients should be based on the evaluation of all prognostic and risk factors involved for each particular patient, including molecular (e.g., p53 abnormalities) and cytogenetic factors (e.g., 11q deletions regardless of the symptoms) and severity of CLL-related signs and symptoms (Table 6).

Based on the trial results above, fludarabine is clearly the first-line therapeutic option in CLL and for patients refractory to alkylating agents. Due to PA toxicity, CLB should be considered in patients older than 90 years with severe comorbid disease such as renal insufficiency who will likely live less than one year regardless of the CLL therapy.

In fludarabine failures, the best option is to enroll patients in a clinical trial. Cladribine, analogs, pentostatin, and alkylating agents induce few PRs of short duration and are associated with significant toxicity; therefore, they cannot be recommended in this setting.

The role of alkylating agents in combination with PAs (e.g., fludarabine and cyclophosphamide) or pentostatin (e.g., pentostatin and CLB) for previously untreated and treated CLL patients is promising, but its indication must be restricted to clinical trials. Preliminary results of phase II trials involving a small number of patients have shown a comparable

**Table 6.** NCI-WG criteria for initiating therapy in CLL patients [58]

- ▲ ≥1 of the following disease-related symptoms:
  - Weight loss ≥10% within the previous 6 months;
  - Extreme fatigue (i.e., ECOG PS ≥ two, cannot work or unable to perform usual activities)
  - Fevers of ≥100.5°F for ≥ two weeks without evidence of infection.
- ▲ Evidence of progressive marrow failure (development of or worsening anemia and/or thrombocytopenia).
- ▲ Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroid therapy.
- ▲ Massive (i.e., >6 cm below the left costal margin) or progressive splenomegaly.
- ▲ Massive nodes or clusters (i.e., >10 cm in longest diameter) or progressive lymphadenopathy.
- ▲ Progressive lymphocytosis with an increase of >50% over two-month period, or an anticipated doubling time of <six months.
- ▲ Marked hypogammaglobulinemia or the development of a monoclonal protein in the absence of any of the above criteria for active is not sufficient for protocol therapy.



frequency of CRs with fludarabine as the single agent [125-133].

The recommendations above will likely change with a better definition of new prognostic factors being evaluated and development of more effective treatment options for CLL with curative intent.

#### **Toxicities of Purine Analogs (PAs)**

Toxicities of PAs include myelosuppression, immunosuppression, neurotoxicity, and tumor lysis syndrome (<3%). Gastrointestinal toxicities, such as nausea and vomiting, hepatic toxicity, and fever have also been reported [88, 89, 90, 95-99].

#### **PA**

As general rule, CLL patients should be treated with the fewest number of cycles for maximal responses. Therapy for more than six to nine months and maintenance therapy with a PA should be avoided, as well as combinations with steroids, since administration of two agents in temporal proximity only adds unnecessary toxicity and increases life-threatening opportunistic infections and myelosuppression [95, 134]. Infections occur in about one-third to one-half of patients receiving a PA at any time during the treatment [95]. The role of infection prophylaxis is unknown. Profound, prolonged lymphopenia occurs within weeks of administration, with a disproportionate decrease in CD4 cells for several years after completion of cladribine or pentostatin. As a consequence of these effects, opportunistic infections, such as *Candida*, *herpes*, and *Pneumocystis carinii* pneumonia (PCP), as well as unusual infections, such as *Listeria monocytogenes* meningitis, disseminated *Cryptococcus neoformans*, *Mycobacterium*, *Legionella*, and reactivation of hepatitis A are being encountered with increased frequency in CLL patients [95].

The major predisposing risk factors to myelosuppression and treatment-related infections include older age (>65 years), poor PS, advanced disease (Rai stage III or IV), BM involvement, previous refractory cytotoxic chemotherapy and extensive prior therapy, pancytopenia from previous treatment, administration of higher-than-recommended doses, a combination or sequence of PAs, a repeated course of PA, poor/slow response to PA therapy, concurrent corticosteroids, absolute granulocyte count <1,000 cells/ $\mu$ l, CD4 count <50 cell/ $\mu$ l, hypogammaglobulinemia (IgG titer <400 mg/dl), and renal dysfunction [95, 104].

#### **PAs and Autoimmune Reactions**

Autoimmune phenomena may be related to CLL and/or to CLL therapy. Autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), and pure red cell aplasia (PRCA) have been reported at any time during therapy. Although PAs may be associated with the development

of AIHA, the frequency is unknown and probably low. Whether the PA has to be discontinued in the setting of AIHA is controversial. Although most patients can be treated with no further complication, others appear to experience a worsening of the hemolysis. Splenectomy may ameliorate prolonged cytopenias [53, 95, 135].

#### **Growth Factors**

The use of myeloid growth factors should follow the American Society of Clinical Oncology (ASCO) guidelines [136]. Recombinant human erythropoietin (rh-Epo), administered s.c., at an initial dose of 150 U/kg, up to a maximum of 300 U/kg three times a week has been shown to be effective for some patients with CLL-related anemia, independent of the pretreatment endogenous serum levels of erythropoietin [137-139].

#### **BM and Stem Cell Transplantation**

The data for autologous stem-cell transplantation in CLL are limited, and the role of this treatment approach has yet to be defined. Although some investigators have suggested that the best time for autologous BM transplant is earlier in the disease course [140, 141], this approach is still considered investigational and should be restricted to the clinical trial setting.

Allogenic transplant has been performed on a limited number of younger patients (<50 years), generally those who failed initial therapy or who relapsed after fludarabine-based therapy and had an HLA-compatible donor. Graft-versus-host disease (GVHD) has been a limiting factor for this selected population. Conditioning regimens using fludarabine may be associated with attenuated acute GVHD, without increasing the relapse rate [140].

The major clinical trials with autologous and allogenic transplants are summarized in Table 7.

Recent reports suggest that submyeloablative regimens (“mini-transplant” or “transplant-lite”) may achieve successful engraftment. Donor leukocyte infusions are delivered in the presence of residual disease or mixed chimerism. Although acute GVHD may be reduced, chronic GVHD remains a major problem. This procedure is being explored in older patients and those with significant medical comorbidity who are usually not eligible for a traditional allogenic BMT.

In summary, in the clinical trial context, when donors are available, myeloablative allogeneic stem cell transplantation should be considered for young patients who are refractory to fludarabine therapy. Nonmyeloablative allotransplant should be considered for older patients or young patients with poor prognostic features who are not eligible for myeloablative protocols (e.g., significant medical comorbidity).

**Table 7.** Allogenic and autologous BM transplant in CLL patients

Trial	Patient (n)	Age (years)	No. of prior therapy	Interval from diagnosis (months)	Remission	Event-free survival (months)	Overall survival (follow-up in months)
<b>Allogenic</b>							
<i>Michallet</i> [191]	54	41 (21-58)	NA	37 (5-30)	38/54 HR (70%)	23/54 (43%) (27.5-80)	24/54 (44%) (NA)
<i>Khoury</i> [192]	15	43 (25-55)	3 (1-4)	40 (13-119)	13/15 CR (86.6%) 1/15 PR (6%)	8/15 (53%) (3-60)	8/15 (53%) (3-60)
<i>Rabinowe</i> [191]	8	40 (31-54)	2.5 (1-6)	41 (117-85)	7/8 CR (88%)	6/8 (75%) (12.6-18)	6/8 (75%) (12.6-18)
<i>Khoury*</i> [193]	6	59.5 (51-71)	4 (2-6)	NA	2/6 CR (33%) 2/6 PR (33%)	1/6 (16.6%) (NA)	3/6 (50%) (NA)
<i>Esteve</i> [197]	7	47 (29-51)	2	41 (12-94)	5/7 (71%)	5/7 (71%) (4-68)	5/7 (71%) (4-68)
<b>Autologous</b>							
<i>Khoury</i> [192]	11	59 (37-66)	3 (2-5)	49.5 (15-147)	6/11 CR (55%) 4/11 nCR, (36%) 1/11 PR, (9%)	3/11 (27%) (4-29)	6/11 (55%) (10)
<i>Rabinowe</i> [191]	12	45 (27-54)	2 (1-4)	25 (12-115)	10/11 CR 1 NE	10/12 (83%) (6.2-31)	11/12 (92%) (6-31)
<i>Pavlectic</i> [194]	16	9 (44-60)	2	13 (5-58)	16/16 (100%)	5/16 (31%) (22-125)	10/16 (62%) (41)
<i>Dreger</i> [195]	18	49 (29-61)	0-2	NA	6/18 (33%)	13/18 (72%) (12-48)	13/18 (72%) (12-48)
<i>Esteve</i> [197]	5	47 (29-51)	2	41 (12-94)	4/5 (80%)	4/5 (80%) (1-23)	4/5 (80%) (1-23)
<i>Sutton</i> [196]	20	55 (38-66)	3 (2-5)	53 (7-108)	10/20 (50%)	7/20 (35%) (5-63)	11/20 (55%) (1-63)

Abbreviations: HR = hematologic remissions; CR = complete remission; nCR = nodular complete remission; PR = partial remission; NA = not available; NE = not evaluable.

\*Nonmyeloablative regimen. One patient reached CR after a second transplant.

### New Drugs

Compound GW506U78 is a new PA (Fig. 1). This pro-drug is converted to arabinosylguanine (ara-G) by ADA. GW506U78 has produced exiting preliminary data in refractory T-cell and B-cell hematopoietic malignancies, including patients with B-CLL refractory to CLB and fludarabine regimens [142-144]. A national multicenter trial is currently accruing refractory CLL patients.

Other new agents include kinase inhibitor flavopiridol and the protein kinase C inhibitors UCN-01 and bryostatins [90, 145].

### BIOLOGIC AGENTS

#### Interferon-alpha

IFN- $\alpha$  has been the most widely studied biological agent in CLL. In previously untreated patient, IFN- $\alpha$  as single agent achieves a few brief responses. No improvement in the

disease-free survival or overall survival has been observed when this agent has been used as maintenance therapy for patients who reached PR and CR after fludarabine treatment [115-119].

### MONOCLONAL ANTIBODIES (mAbs)

#### CAMPATH 1H

CAMPATH-1 is a humanized IgG1 antiCD52 antibody that binds to B and T cells as well as to most B- and T-cell leukemias and lymphomas. This agent was almost abandoned because of excessive toxicity, myelosuppression, and infections. Less intense schedules have evaluated previously untreated and treated patients. Tumor regression is seen most notably in the peripheral blood, less in the BM and spleen, and rarely in lymph nodes [120-122]. Major responses (CR + PR) were achieved in eight of nine previously untreated patients [146]. In a phase II study trial with

refractory patients, CAMPATH 1H produced an overall response of 42%, including several prolonged remissions [147]. Complete remissions in previously fludarabine-treated B-PLL patients have been reported [148]. This compound is also being investigated as a “purging agent” for BM transplantation. Primary side effects include moderate myelosuppression with opportunistic infections. A few patients have become aplastic. The skin rash, fever, and hypotension are associated with i.v. administration of the antibody but may be less prominent with s.c. administration [121, 122]. The pivotal trial of this agent has been completed and is undergoing analysis.

### Rituximab

Rituximab is a chimeric anti-CD20 mAb therapy which is approved for the treatment of relapsed follicular low-grade lymphoma patients. Approximately half of treated patients respond, including CRs in 6%. In contrast, limited activity has been observed in previously treated CLL patients; in one series, a single PR was noted in a cohort of 15 patients [149]. The discrepancy between follicular non-Hodgkin’s lymphoma (NHL) and CLL likely reflects the reduced surface density of CD20 on the small lymphocytes. Rapid tumor lysis has been reported in CLL patients with lymphocytosis [150]. Ongoing studies are evaluating fludarabine in combination with rituximab as primary therapy for CLL [151-153].

Radioimmunoconjugates have not been actively pursued to date in CLL because of the concern that the extensive BM infiltration will predispose to significant and prolonged myelotoxicity. Preliminary studies in patients with BM involvement are in development.

### Gene Therapy

A goal of a genetic approach to CLL would be to modify the B-CLL phenotype so that it is capable of stimulating T cells to respond to presented antigens. The interaction of CD40 and its ligand CD40L (or CD154) expression on activated T cells plays a key role in the B-cell activation, survival, and differentiation [154, 155]. A preliminary report of a phase I study with three B-cell patients showed no immediate toxicity. Anorexia, fatigue, malaise and fever were the major side effects and subsided in three days without specific treatment [156]. Two chemotherapy-naïve patients experienced a greater than 50% decrease in peripheral blood lymphocytosis.

### Infections and CLL

Infections as the result of the disease and/or its treatment are the major cause of morbidity and mortality in CLL. The pathogenesis of this increased susceptibility is multifactorial

and involves both humoral and cellular defects, including hypogammaglobulinemia, defective complement activity, and cell-mediated abnormalities [94, 95, 157, 158]. Prior to treatment, the most frequent pathogens include organisms that require opsonization, such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Escherichia coli*, and *Klebsiella pneumoniae*. Fungal and viral infections are mostly encountered in late-stage CLL patients receiving alkylating agents with glucocorticosteroids and notably with PA therapy [95]. Trimetopim-sulfamethoxazole (TMP-SMX) in patients requiring concurrent corticosteroids reduces the risks of PCP [95, 134]. No studies have shown benefits for prophylactic antimicrobial therapy to patients on PA therapy. Prophylactic i.v. immunoglobulin therapy is neither cost-effective nor does it improve the quality of life of CLL patients. It has no effect on life-threatening infection [158-161].

### Immune Diseases and CLL

CLL cells produce polyreactive, low-affinity autoantibodies, which may explain the high incidence of hypogammaglobulinemia and other autoimmune-associated diseases [162-168]. Warm antibody autoimmune hemolytic anemia (WIHA) (10%-25%), immune thrombocytopenic purpura (ITP) (2%), and pure red cell aplasia (PRCA) (0.5%-6%) are most frequently reported. Autoimmune hemolysis may present simultaneously with ITP in CLL patients (Evans’ syndrome) [1, 51-53, 58, 166]. The primary approach to autoimmune diseases in CLL is to treat the underlying disorder, generally with regimens including prednisone. Alternative treatments for patients unresponsive to steroids include i.v. immunoglobulin (IVIg), splenectomy, or cyclosporine A for WIHA. For PRCA [53-56, 169-174], a regimen of cyclosporine A, alkylating agents, and antithymocyte globulin has been used with variable success. Secondary ITP is managed as autoimmune hemolytic anemia and does not confer a poor prognosis [53, 121, 174].

### Aggressive Transformation

About 15% of patients with CLL undergo a transformation to a more aggressive disorder, most often prolymphocytic leukemia (PLL) or Richter’s syndrome (RS).

PLL can occur de novo or as aggressive transformation from CLL (CLL/PLL). The main clinical features and differences between de novo PLL and CLL/PLL are listed in Table 8.

CLL/PLL occurs at a median of 52 months from the diagnosis of CLL; 80% are B-cell type and may present with a high density of surface immunoglobulin (sIg), usually IgM, with kappa or lambda light chains [63-69, 175-183]. The presence of greater than 55% and/or 15,000/μl prolymphocytes

**Table 8.** Differences between de novo PLL and CLL/PLL

	CLL transformation (CLL/PLL)	de novo PLL
Age	60s	70s
Lymphocytosis	Moderate	Marked
Splenomegaly	Moderate	Marked
Lymphadenopathy	Moderate	Rare
p53 mutations	>50% (substitutions)	>50% (deletions/insertions)
Response to therapy	Fair	Poor
Cytogenetics	Trisomy 12, 11q	t(6;12)

Substitutions = refers to a single base-pair substitution ("point mutation").

establishes a diagnosis of PLL [58]. The main differential diagnosis (Table 3) is with other CD5<sup>+</sup> B-cell disorders which include CLL and the leukemic phase of mantle-cell lymphoma [58].

PL patients are usually refractory to therapy, which may reflect frequent p53 abnormalities [63, 67, 69, 178-183]. Transient clinical responses lasting six to nine months have been reported with fludarabine, pentostatin, CAMPATH-1, and BMT [63-69]. The median survival with conventional treatments has been reported to be 7.5 months for T-cell PLL. Survival figures are more variable with B-cell PLL patients; differences among series reflect variability of de novo and transformed PLL cases as other prognostic factors [68, 180, 182].

RS has been reported to occur in 3%-5% of CLL patients; however, this figure is likely an underestimate [70]. RS represents the transformation from CLL to an aggressive non-Hodgkin's lymphoma, usually diffuse, large-cell lymphoma. The diagnosis requires biopsy. Gene rearrangement studies suggest the same clonal origin for CLL and RS [70, 71, 74]. Patients usually present with progressive clinical deterioration with a wasting picture, lymphadenopathy, and systemic symptoms, often with an abdominal mass or extranodal involvement and a rising LDH. RS responds poorly to therapy, with a median survival of only four to five months [70-73]. Rare cases of transformation from CLL to Hodgkin's disease, multiple myeloma, and acute lymphocytic leukemia have been reported [74-78].

## CONCLUSION

CLL continues to be an incurable disease. Fludarabine has changed the traditional approach for CLL patients and now is considered a therapeutic option for initial treatment. More important, however, will be to use this agent as a building block on which to base more effective treatment strategies. A better understanding of the molecular biology and immunology of this disease will lead to the rational development of strategies targeted at the underlying mechanisms of this disease. By integrating these new approaches, we may finally realize the goal of curing this disease.

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