CHRONIC LYMPHOCYTIC LEUKEMIA

RECENT OBSERVATIONS ON BIOLOGY AND THERAPY

INTERNAL MEDICINE GRAND ROUNDS

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October 3, 1991

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INTRODUCTION

Chronic lymphocytic leukemia is the most common form of leukemia in the Western world. More than 10,000 cases are diagnosed in this country annually (1). Due to the limitations of purely morphologic evaluation, earlier definitions of CLL included a somewhat heterogeneous group of lymphoid leukemias (table 1). Advances in molecular biology have provided more clear separations of these disorders (2). This discussion will be limited to those patients who fulfill the proposed criteria for the diagnosis of B-cell CLL (3,4) (page 6).

Table 1 Chronic Lymphoid Leukemias

B-Cell CLL
T-Cell CLL (Tγ lymphocytosis)
Hairy Cell Leukemia
Prolymphocytic Leukemia
Spillover Leukemia with Non-Hodgkin's Lymphoma
Splenic Lymphoma with Villous Lymphocytes
Plasma Cell Leukemia
Adult T-Cell Leukemia/Lymphoma

PATHOGENESIS OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

Immunologic and molecular biologic characterization of patients with the clinical picture of CLL have delineated that the vast majority represent a clonal disorder typified by an accumulation of a unique subset of B lymphocytes. This population of cells has an immunophenotype (with some variation) that separates it from the usual pattern seen with other lymphoid malignancies with the exception of some small lymphocytic and mantle zone lymphomas (table 2). The most salient features are 1) the presence of pan-B cell antigens;

Table 2 B CLL Phenotype

Surface Antigen	% of Patients
Weak Surface Ig	100*
B Cell (CD19, CD20,CD21, CD23 or CD24) 1 or more	100*
CD5	95
Other Pan T-Cell	0*
CD25	50
CD22	25
FMC7	15
Mouse RBC rosette receptor	> 50
Myelomonocytic (eg CD14, CD11b, CD11c)	85

*Required for diagnosis of B CLL

2) the presence of **weakly staining** surface immunoglobulin (sIg) with a single light chain type. The heavy chain type is usually μ (16%), δ (14%) or both (52%), though a few express γ (5%) and 13% express no heavy chain; 3) the presence of CD5 antigen (2,5,6,7). Of patients with clinical B-cell CLL, 94% are CD5+ (5). This latter observation was initially thought to be an aberration since the CD5 antigen was believed to be a specific pan-T cell marker. It is now clear that there is a specific subset (or stage of differentiation) of B cells that is associated with the expression of the CD5 antigen. It is only a rare case of other B cell leukemias that stain for CD5.

The CD5+ B Cell: Normal and Neoplastic. With recognition that the B cells in CLL expressed the T cell antigen CD5, a search for, and subsequent characterization of the normal counterpart in humans and mice (Ly1+) was investigated (8). Some characteristics of this unusual cell are summarized in table 3.

This is the predominant B cell in fetal and newborn periods. The proportion in blood declines after birth as a consequence of dilution by CD5- B cells. It appears that they again increase later in life in a pattern similar to the incidence of CLL. The location of this population in tissues appears to be in the cuff or mantle zone of the lymphoid follicle (9,10). The immunophenotype of these cells is essentially identical to the cells in B CLL (11). Although, under certain conditions, CD5- B cells can be induced in vitro to express the CD5 antigen (12), the bulk of data would support the concept that there is a distinct CD5+ B cell lineage rather than just a population of activated CD5- B cells (8). They may be capable of self-renewal under the influence of certain cytokines as well as being capable of differentiating to a cell with characteristics of germinal center blast cells (8,11). They may share developmental and functional properties with the monocyte/macrophage lineage as indicated by certain phenotypic features and the ability to produce IL-1 (7,8). B CLL cells synthesize and release TNF-α and this cytokine has also been proposed to have an autocrine regulatory role on the proliferation of CLL cells (13-15). CD5+ B cells from mice and humans produce IgM antibodies with specificity for self-antigens. A number of studies have attempted to implicate this subset of B cells in murine and human autoimmune disease (8). In normal individuals and patients with rheumatoid arthritis, CD5+ B cells are enriched for IgM autoantibodies, particularly anti-IgG (rheumatoid factor). Their is no clear evidence, however, that the auto-antibodies produced by these cells are pathogenic. Likewise, the lymphocytes from a proportion of patients with CLL secrete IgM antibodies that have reactivity with IgG and single or double stranded DNA (16,17). A recent study demonstrated that the antibodies eluted from the RBC's of 2 patients with CLL and autoimmune hemolytic anemia were monotypic with light chain restriction that correlated exactly with the light chain expressed on the surface of the CLL cells (18). It appears that the antibodies synthesized by CD5+ CLL

Table 3 Features of the CD5 B Cell

- The major B cell population in fetal life and cord blood
- Proportion declines after birth
  - 1-30% circulating B cells
  - < 30% lymph node and tonsilar B cells
  - < 10% splenic B cells
  - May increase again late in life
- Sub-population of B cells in mantle zone of lymphoid follicles
- Form rosettes with mouse erythrocytes
- Phenotype: CD19,20 + ; > 70% CD21 + ; 30% CD23 + ; 20% CD25 +
- Express myelomonocytic antigens
- Not activated CD5- B cells
- Probably distinct B cell lineage
- May be self-renewing B cell sub-population
  - 10% in cell cycle (cord blood)
  - Proliferate in response to IL-2, ImwBCGF or IL-4
  - Differentiate to sIg-,CD5-,CD10 + ,CD38 + lymphocytes in response to IL-1 plus IL-2 with features of germinal center B cells
- Associated with autoantibody production
  - Enriched for cells that produce IgM anti-IgG (rheumatoid factor) in normal persons and patients with rheumatoid arthritis
  - Increased proportion in patients with rheumatoid arthritis and Sjoegren's syndrome
- May have immunoregulatory activity (helper B cells)
- May share developmental and functional properties with monocyte/macrophages (eg. phenotypic features, IL-1, TNF-α production)
- Produce antibodies encoded by restricted set of highly conserved Ig V genes
- May play a major role in the ontogeny and homeostasis of the humoral immune system

cells utilize a limited library of V region genes both for light and heavy chains, cross reactive idiotypes (CRI). In one study, 25% of CLL patients with a κ Ig expressed the same light chain V gene idiotype. In another study, 20% of CLL sIg shared the same heavy chain V region idiotype (19). Furthermore there is a biased frequency of coexpression of both light and heavy chain CRI's in CLL patients. In addition, these particular V region genes appear to be highly conserved structurally. Finally, there is evidence that these same CRI's are found in high frequency on IgM rheumatoid factors as well as Ig expressed during early B cell ontogeny (8,20). These observations may have relevance to the presently unknown pathogenesis of CLL as well as possibly indicating the physiologic role of the normal CD5 B cell in the humoral immune system (8,21).

The CD5 Antigen. The structure and function of the CD5 molecule has been studied primarily in T cells (8). The molecule on B cells appears to be identical (21). Table 4 summarizes some of the features of this surface antigen. Its similarities to the IL-1 receptor are of interest. There is evidence that it may modulate the function of other surface molecules on both T and B cells.

Table 4 The CD5 Antigen

- 67 kDa glycoprotein
- Encoded by Ig Supergene Family
- May be Cytokine Receptor
- Not IL-1 Receptor
- May Involve Signal Transduction
- May Modulate Function of other Membrane Antigens

Other characteristics of CLL cells. In the majority of CLL patients, the proliferative activity is low. Utilizing flow cytometry, the majority of cells in most patients are found in G_0, and where a significant number of cells are in cell cycle, essentially all are in G_1 without detectable numbers in S or G_2/M phase (22). With special conditions, CLL cells can be grown in cell culture. The requirements for growth are more stringent for higher stages of disease, and clonogenic efficiency as well as colony numbers and size are greater in low stage disease. However, there is no correlation of clonogenicity with any identifiable biologic, phenotypic or cytogenetic properties (22). CLL cells do proliferate, as measured by ^{3}HTdr uptake, to IL-2 (23,24), IL-7 (25) and TNF-α (13-15). Responsiveness to IL-4 is not consistent between studies (23,26) but it does induce or enhance CD-23 expression (27). Clear differences in patterns of response to cytokines have not been shown to correlate with disease activity.

Therefore, despite these extensive studies of the B CLL cells as well as their normal CD5+ counterpart in addition to considerable cytogenetic data and searches for oncogene

activation (see page 9) it is clear that the pathogenesis of CLL is unknown. One recently proposed hypothesis suggests that there is an initial stimulation of a polyclonal expansion of the CD5+ B cell subset and that subsequently one or more transforming events occurs in one of these CD5 B cells resulting in a monoclonal expansion that is the clinical entity B CLL (1,21).

Also, studies to date have not revealed clues to the explanation of the remarkable clinical heterogeneity. What determines the development of lymph node, spleen and liver involvement, sometimes isolated and other times disseminated? What determines the degree and pattern of bone marrow infiltration and the development of bone marrow failure? Clearly, these features are major determinants of prognosis (see below). Some recent observations in other lymphoid malignancies and lymphocyte biology may have relevance to these questions. Lymphocyte homing receptors, as defined by CD44, and LFA-1 adhesion molecules are involved in lymphocyte binding to endothelial cells of high endothelial venules in tissues. It has been shown that the degree of expression of these molecules by cells from non-Hodgkins lymphomas correlates with the tendency to disseminate hematogenously. Their expression also was shown to be an independent predictor of outcome in addition to stage and histologic type (28). There is also evidence that cell proliferation is dependent upon adhesion to substratum in addition to growth factor requirements (29). Perhaps the capacity to "home" to lymphoid tissues and bone marrow and to subsequently proliferate in these sites may be a function of the degree of expression of certain surface molecules on CLL cells. Limited data has been published on the patterns of expression of homing and adhesion molecules on the cells from this disease. It has been demonstrated that LAM-1 is expressed on most CLL cells (29a). It has also been suggested that the presence of the myeloid and/or adhesion molecules CD13, CD11c and CD11b are associated with more advanced stages and the diffuse pattern of bone marrow involvement (29b).

DIAGNOSIS OF B CHRONIC LYMPHOCYTIC LEUKEMIA

Based upon phenotypic and morphologic studies as well as previously characterized clinical features, two working groups have proposed criteria for the diagnosis of B cell CLL (3,4). These criteria are summarized in table 5.

Table 5 Criteria for the Diagnosis of B CLL

- **Phenotypically Characterized B CLL**
  - **Prominent lymphocyte population that shares B-cell markers and CD-5 antigen with absence of other pan-T-cell markers**
  - **Expression of κ or λ light chains**
  - **Expression of sIg with low cell-surface density**
- **Absolute blood lymphocyte count $\geq$5,000/μL at least 4 weeks**
- **Morphologically mature appearing lymphocytes with no more than 55% atypical lymphocytes, prolymphocytes or lymphoblasts**
- **$\geq$30% of bone marrow nucleated cells lymphoid on smear**
  - **Diffuse or non-diffuse (nodular, interstitial, mixed) lymphocytic infiltration**
  - **Normocellular or hypercellular bone marrow**

NATURAL HISTORY OF CLL

The natural history of CLL is remarkably heterogeneous. It varies from a rapidly progressive disease with death within a year to a disorder which may last 10-20 years without any apparent impact upon age corrected survival. This has confounded therapy decisions and attempted clinical trials. The primary clinical manifestations consist of lymphocytosis, "mass" disease consisting of lymphadenopathy, hepatosplenomegaly or any combination, bone marrow failure, autoimmune cytopenias and

Table 6 Rai Staging System for CLL

Stage	Modified (Risk)	Enlarged nodes	Enlarged Liver/Spleen	Hgb < 11gm	Platelets < 100 K
0	Low	-	-	-	-
I	Intermed	+	-	-	-
II	Intermed	+/-	+	-	-
III	High	+/-	+/-	+	-
IV	High	+/-	+/-	+/-	+

Table 7 Binet Staging System for CLL

Stage	≥3 Lymphoid Sites	Hgb < 10 gm or Platelets < 100 K
A	-	-
B	+	-
C	+/-	+

Lymphoid sites = cervical, axillary, inguinal LN (unilateral or bilateral), liver, spleen (ie 5 potential sites maximum)

propensity for infections. The development or progression of any one or more of these features may occur independently of changes in other aspects. Exhaustive studies have been performed to delineate factors which may predict outcome.

Staging Systems. Two separate systems have been developed to predict survival in CLL based on simple clinical criteria (30,31).

They both are widely employed for clinical and investigative purposes. The Rai system has fewer patients in the low risk group (30%) and a somewhat better outcome than the Binet stage A patients (60%). (Tables 6 and 7 and figures 1 and 2). These proportions are reversed in the intermediate risk and stage B groups and the Rai group also has a slightly better outcome . The high risk and stage C patients are essentially identical patients with similar survival predictions.

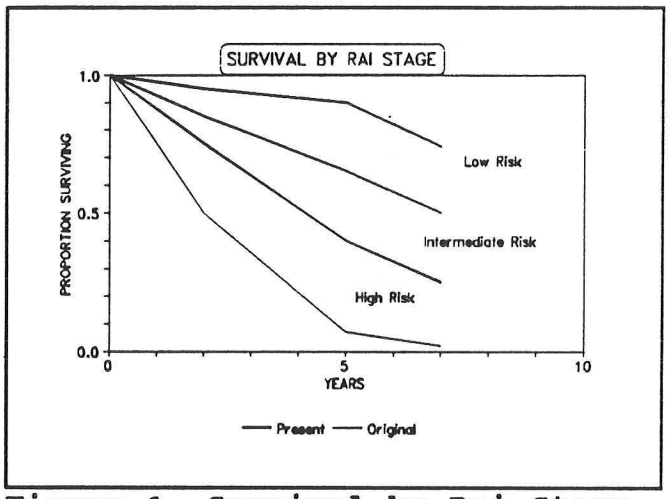

Figure 1 Survival by Rai Stage

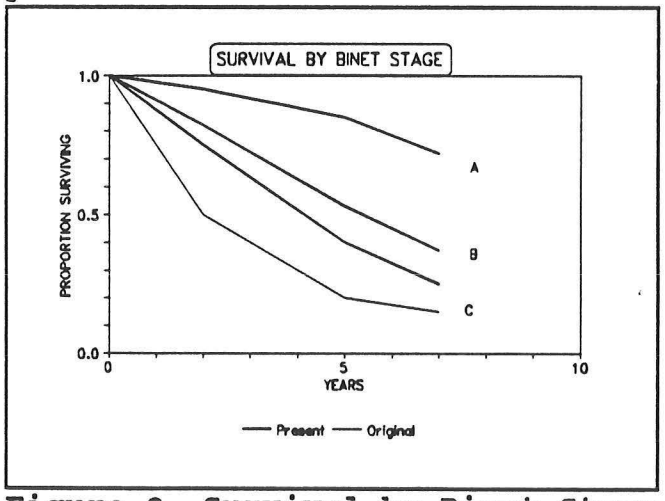

Figure 2 Survival by Binet Stage

The survivals shown are the first prospective evaluation of the staging systems (32). Survival for stage C or high risk patients is better than initially reported. Both systems suffer from significant heterogeneity remaining within groups. This has led to attempts to delineate other factors that might "fine tune" the predictive power of the basic staging systems. Many of these have not been confirmed in independent studies and remain in question as predictive determinants. Others, however, appear to be definitely helpful in separating further subgroups of patients in the Rai and Binet stages. (Table 8).

Lymphocyte Doubling Time. At the time of diagnosis, if patients remain untreated, the time required to double the absolute blood lymphocyte count strongly correlates with both the time to requirement for therapy as well as survival (33).

Although there is a correlation with both stage (shorter in more advanced stages) and bone marrow pattern (shorter with diffuse type), nevertheless, it is an independent predictive factor for time to treatment and survival with these two variables (Fig 3). The primary limitation to this determination is that it requires a period of observation without treatment.

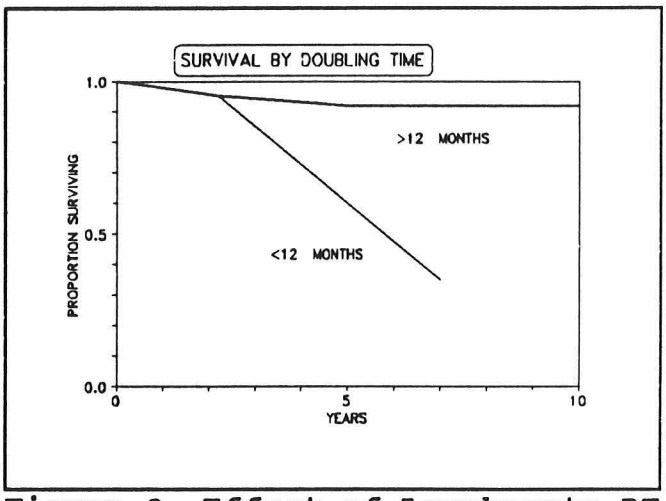

Figure 3 Effect of Lymphocyte DT

Bone Marrow Biopsy. The bone marrow infiltration of lymphocytes in CLL assumes several patterns (34). They have been characterized as interstitial, nodular, mixture of these two and diffuse. The latter shows obliteration of the fat and normal marrow elements by the CLL. There is a major difference in survival of patients with the diffuse as opposed to non-diffuse involvement either at diagnosis or when performed later in the course (figure 4). Although there is some correlation of the frequency of diffuse or non-diffuse patterns and the clinical stages, the bone marrow pattern is nevertheless a strong independent variable for predicting survival in all stages of the disease. It is particularly so for the intermediate risk or stage B patients.

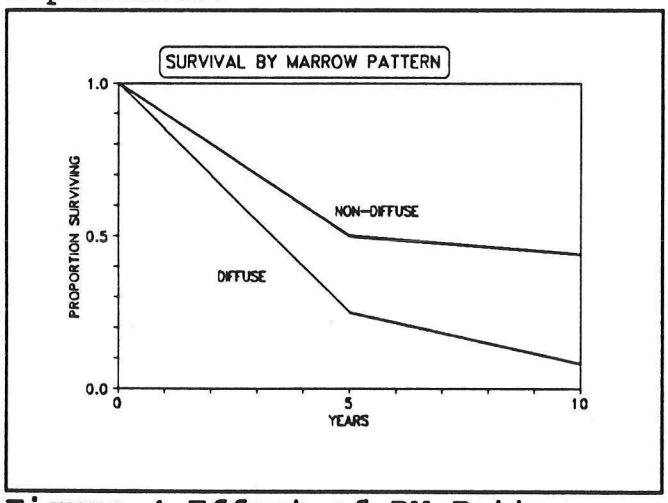

Figure 4 Effect of BM Pattern

Cytogenetics. Chromosomal aberrations can be detected in CLL (35,36). Because of their low inherent proliferative capacity, cytogenetic studies of these lymphocytes require stimulation with B cell mitogens. In a significant number of cases, inadequate numbers of karyotypes are obtained. In the largest published series (a cooperative effort from five institutions), 10% had non-evaluable karyotypes (35). Of the remaining 391 patients, 56% had non-random, clonal aberrations.

The most common abnormality is trisomy 12. Various abnormalities of the long arms of chromosomes 13 and 14 are the next most frequent findings. The presence of chromosomal abnormalities confers an adverse survival probability as compared to patients in which the karyotype is normal. In patients with a

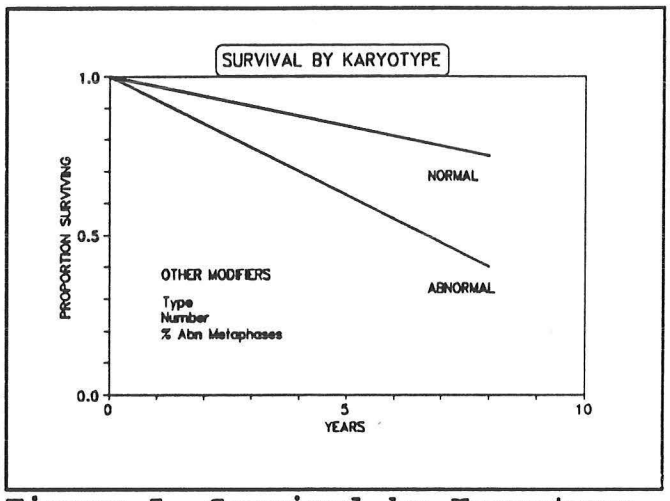

Figure 5 Survival by Karyotype

normal karyotype, it is not certain whether these are leukemic or normal cells in mitosis. In some cases, interphase cytogenetics reveal chromosomal abnormalities in non-dividing neoplastic B cells (37). In addition, the specific type of anomaly has a lesser or greater impact upon outcome as well as the number of different aberrations and the percentage of abnormal karyotypes. Figure 5 shows a schematic comparison of survival by karyotypic findings. It should be noted that low risk or stage A patients were disproportionately over-represented in this series. The % of abnormal karyotypes was an independent predictor of survival along with age, stage and sex in this series. Unfortunately, careful studies of the specific karyotypic abnormalities utilizing a variety of probes have not yet demonstrated any gene rearrangements unique to CLL that might point to potential mechanisms for the malignant transformation (36), with the possible exception of a putative oncogene, bcl-3, rearranged in a small number of CLL patients (37a). The p53 gene is mutated in 15% of CLL cases studied and is very frequent in patients undergoing Richter's conversion (37b). Translocations involving chromosome 14 as well as rearrangements or translocations involving the putative oncogenes bcl-1 and bcl-2 are seen frequently in certain B cell malignancies. It appears that bcl-1 or bcl-2 rearrangements occur infrequently in typical CLL and that translocations of chromosome 14 said to occur in CLL may actually represent leukemic phases of other B cell neoplasms (38,38a). Bcl-2 protein may be expressed in CLL as is true in several hematopoietic malignancies (38b).

Age. Age has been demonstrated to be an independent prognostic factor by multivariate analyses that include Rai or Binet stages as a variable (32,35). Also, younger adults, under age 50, present more frequently with advanced stage disease but survive longer than older adults with the same stage (39).

Sex. Females, for any given stage of disease, survive longer than males. They also tend to present with lower stages of disease and later in life (32,40).

Response to Treatment. Comparing the survival of treatment responders to non-responders is not statistically sound. However, if response to treatment is included as a variable in a multiple regression analysis, the effect of treatment on survival, corrected for other prognostic factors, is a valid measurement.

In a recent prospective trial, response to treatment was shown to have independent prognostic significance (32). Although this can not be used as a prospective parameter, it does give promise that if effective therapy with higher response rates is developed, survival will be beneficially effected.

Best Risk CLL. From both retrospective and prospective studies, distinct subgroups of patients have been delineated that, without treatment, will have a low likelihood of progression and survival not different for the non-CLL population when corrected from age and sex. (table 9) (41,42). Two of these definitions have the advantage of including more patients than Rai 0 (low risk). The French classification has the further advantage of not requiring followup to determine lymphocyte doubling time (42). Nevertheless, nearly 15% of these patients will eventually progress to more advanced stages of CLL and up to 5% may succumb to the disease. Confirmation of the predictive value of each of these systems for defining "smoldering" CLL has been published (42a).

Table 8 Prognostic Factors in CLL

Prognostic Parameter	Ref
*Rai or Binet Stage	30-32
*Bone Marrow Biopsy Pattern	34
*Lymphocyte Doubling Time	33
*Karyotype Abnormalities	35
*Age	32
*Sex	32
Absolute Lymphocyte Count	32
Morphology	43a
Autoantibodies	44
Labeling Index	45
Immunophenotype	46
Hypogammaglobulinemia	47
CD4/CD8 Ratio	47
Natural Killer Cell Numbers	47
Serum Thymidine Kinase	48
Serum Alkaline Phosphatase	40
Serum LDH	40
Serum β_2 Microglobulin	49

*Confirmed in more than one study

Causes of Death. The prospective trial of the British MRC prospectively followed the causes of death of patients entered (32,43). There were 660 patients evaluated, 392 of whom expired. The causes of death are listed in table 5. Two-thirds die of problems related to the CLL. One half of these are infectious events of which 80% were pneumonia. 50% of the Binet stage A patients expired due to CLL unrelated reasons while 80% of the stage B and C patients died due to CLL and its complications. There were no significant differences in the distribution of causes between males and females. The French Cooperative Group found a similar distribution of causes of death in stage A

Table 9 Definitions of Best Risk (Smoldering) CLL

	Rai	Spanish	French
Stage	Rai 0	Binet A	Binet A
Hemoglobin	≥ 11	≥ 13	≥ 12
Lymphocyte count	NA	< 30,000	< 30,000
Bone Marrow	NA	Non-diffuse	< 80% lymphs
No. Lymphatic sites	0	NA	< 2
Lymphocyte Doubling Time	NA	> 12 months	NA

patients. 60% died of problems unrelated to CLL (42).

Table 10 Causes of Death in CLL

CAUSE	%
CLL	32
CLL + Infection	36
Cardiovascular	16
Carcinomas	12
Other	1
Unknown	3

ISSUES IN THERAPY OF CLL

The impact of therapy on the natural history of CLL is unknown, particularly in those stages of disease in which death related to CLL or its complications is most likely to occur. Even the likelihood of response to "standard" therapeutic regimens is poorly documented. Several problems with reported studies account for this circumstance. They include 1) inconsistent and poorly defined criteria for response; 2) inclusion of both previously treated and untreated patients; 3) a failure to clearly compare the results between patients of the same clinical stage, let alone control for other prognostic factors; 4) significant differences in dose intensity and drug schedules between studies; 5) a paucity of well designed prospective randomized trials. These flaws have led both our National Cancer Institute and the International Workshop to delineate criteria for designing and interpreting future therapeutic studies in CLL (3,4) (see appendix). It has also been suggested that immunohistochemical, cytogenetic and molecular techniques be employed to determine whether minimal residual disease exists in patients classified as complete remission.

Standard Therapy. Initial treatment approaches in CLL have not changed significantly in over 2 decades. Standard chemotherapy consists of alkylating agents such as chlorambucil or cyclophosphamide with or without the addition of prednisone. Use of these agents was predicated on their action as non-cycle active drugs which was believed to be appropriate due to the apparent low proportion of cells found to be in cell cycle in CLL. Until recently, the introduction of other drugs has been mostly empirical due to the paucity of studies of single agent activity in this disease. Most commonly employed have been vincristine and doxorubicin (Adriamycin). Studies of the impact of chemotherapy in CLL have generally followed the direction of stage oriented evaluation since this is the most easily assessed and widely demonstrated prognostic parameter in CLL. Tables 11 and 12 summarize response and survival data from selected studies carried out in previously untreated patients. As noted above, response criteria have not been uniform, especially in terms of bone marrow evaluation and cause some difficulty in direct comparisons except between arms of randomized trials. Likewise, survival data are inexact due to the inherent chronicity of the disease. Usually, survival times are measured from the initiation of the therapy. Many patients have had their disease for varying periods of time prior to therapy and the stage at treatment may reflect progression from the initial presentation. These details are usually not documented in the publication. Certain points can be made, however. **Response** to therapy is better for lower stages of disease and progressively declines with more advanced stages. **Survival** continues to be significantly different for the Rai or

Table 11 Response to Therapy in Chronic Lymphocytic Leukemia

Study	Rx	Stage	# Pts	%CR (90%CI)	%CR + PR (90%CI)	Ref
SEG[1]	CbP	0	11	54 (31-76)	91 (68-98)	50
French[2]	Cb	A	303	41 (36-46)	70 (65-74)	51
SEG[1]	CbP	Int	84	37 (29-46)	81 (73-87)	50
French[2]	Cb	B	151	13 (9-18)	59 (52-65)	52
French[2]	COP	B	140	20 (15-26)	61 (54-67)	52
Spanish[2]	CbP	B	62	24 (16-34)	69 (59-78)	53
MDA[3]	CHP or POACH	B	36	33 (22-47)	64 (50-76)	54, 55
French[2]	CbP	B	72	15 (10-23)	53 (43-62)	56
French[2]	CHOP	B	81	27 (20-36)	76	56
CALGB[1]	CbP	High	58	14 (8-23)	47 (36-57)	57
SEG[1]	CbP	High	43	14 (7-25)	56 (43-68)	50
Spanish[3]	CbP or COP	C	61	7 (3-14)	54 (44-46)	58
Spanish[2]	CbP	C	34	12 (5-24)	56 (42-69)	53
French[2]	COP	C	34	6 (2-16)	38 (26-52)	59
French[2]	CHOP	C	36	19 (11-32)	83 (71-91)	59
MDA[3]	CHP or POACH	C	26	35 (21-51)	65 (49-79)	54, 55

CR = complete response; PR = partial reponse; A = Ara-C; C = cyclophosphamide; Cb = chlorambucil; O = vincristine; H = doxorubicin; P = prednisone; [1] CR required < 30% lymphocytes in BM; [2] CR did not require BM examination; [3] CR required normal BM

Table 12 Survival Following Initiation of Therapy in CLL

Study	Rx	Stage	# PTS	Survival 2yr %	Survival 5yr %	Survival Median (mos)	Ref
French	None	A	309	93	82	NR	51
French	Cb	A	303	93	75	NR	51
BMRC	2 Arms	A	305	95	85	NR	32
French	Cb	B	151	78	44	58	52
French	COP	B	140	78	43	57	52
BMRC	3 Arms	B	189	82	53	55	32
French	COP	C	34	44	14	22	59
French	CHOP	C	36	75	52	62	59
BMRC	3 Arms	C	166	72	37	47	32
ECOG	COP	High	44	66	37	48	60
ECOG	CbP	High	43	73	50	54	60
Spanish	COP or CbP	C	61	52	38	30	58
SEG	CbP	High	43	53	18	30	50

Abbreviations as in table 11

Binet stages. However, in these prospective studies, the overall survival in stage C or high risk patients is better than that originally published for these staging systems (30,31) which were predicated on retrospective data. It is not known whether this represents an improvement in survival where therapy is standardized and carried out systematically or reflects other differences in the patient populations.

Stage A and Low Risk Patients. As noted previously, it is clear that the majority of patients in this category will survive as if the CLL was not present (page 11). This is the only group of patients in which large, well designed, prospective randomized trials utilizing a control arm of no therapy until progression or symptoms occurred have been carried out (51,42,43). Results of the French trial are summarized in table 13. It is clear that routine therapeutic intervention is unnecessary. Therapy is generally reserved for indications listed in table 15.

Stage B and Intermediate Risk Patients. These represent the most heterogeneous group of patients as defined by the staging

Table 13 Results of French Cooperative Trial in Stage A CLL

DESIGN

- Binet stage A
- No prior therapy
- Daily chlorambucil versus no treatment until progression
  - Post progression treatment standardized
  - 303 patients treated - 309 untreated

RESULTS

- No difference in overall, 3 year or 5 year survival ($p = 0.21$)
  - Adjusted for pre-treatment variables - trend in favor of no treatment ($p = 0.09$)
- Chlorambucil delayed progression to stage B ($p = 0.021$)
  - Survival shorter for treated group after progression
- Response rate for chlorambucil (not evaluated with bone marrow)
  - Clinical remission 40% (Would be maximum for CR + nodular PR)
  - Partial response 28%
- Causes of death similar except 21% versus 6% carcinoma deaths with chlorambucil
- Defined a subset (A') with normal age-sex corrected survival
  - Hemoglobin ≥12 grams and Lymphocyte count <30,000
  - 77% of stage A - 50% of all CLL
  - 70% would qualify as "smoldering" CLL
  - 5 year survival 83% versus 62% for A" ($p < 0.0001$)
  - Trend for survival of A' favors untreated ($p = 0.08$)

CONCLUSIONS

- No indication for routine treatment of stage A CLL
- Potential negative effect of treatment with chlorambucil before indications
- 3/4 of stage A (50% CLL) patients may never require treatment

systems. One large, prospective randomized trial comparing two standard treatment approaches has been published (52). The results are summarized in table 14. There were no significant differences in response or survival for the two arms. A smaller uncontrolled study by the SEG utilized a possibly more intense intermittent chlorambucil plus prednisone regimen in patients with intermediate risk disease (50). The response rates were higher and the survival longer than either of the arms in the French study. It should be recalled that Rai intermediate risk patients may be a better prognostic group than Binet stage B, however. Limited data suggest that response may be higher to regimens containing doxorubicin (54-56,61). The UK MRC trial observed Rai stage I and II to determine static or progressive disease. If static at one year, they were randomized to no

Table 14 Results of French Cooperative Trial in Stage B CLL

DESIGN

- **Binet stage B**
- **No prior therapy**
- **Daily chlorambucil versus intermittent cyclophosphamide, vincristine prednisone (COP)**
  - **Post progression treatment standardized**
  - **151 patients chlorambucil - 140 COP**

RESULTS

- **No difference in overall, 3 year or 5 year survival (p = 0.48)**
- **No difference in time to progression to stage C (p = 0.4)**
- **Response rates not different (p = 0.4) (not evaluated with bone marrow) combined results:**
  - **Clinical remission 16% (Would be maximum for CR + nodular PR)**
  - **Partial response 43%**
- **Causes of death similar**

CONCLUSIONS

- **Response and survival in stage B CLL is same with chlorambucil or COP**
- **Response to standard chemotherapy lower in stage B versus stage A**

treatment or standard treatment. Patients with progressive disease were randomized to various standard regimens. There was no difference in overall survival in any of these subgroups including analysis as Binet stage B patients (32,43). Therefore, many recommend withholding therapy except for indications listed in table 15. When treatment is initiated, no standard regimen has demonstrated clear superiority.

Stage C or High Risk Patients. This also is an heterogeneous group of patients. Table 12 shows that **survivals** in several prospective therapy trials are quite different, but, perhaps more importantly, are generally much better than the 18 month to 2 year median survivals originally proposed for this cohort. In contrast, **response rates**, especially complete remissions, are low but generally uniform (table 11). Thus, the explanation for this apparently better survival over historical series is unclear. The uniformity of response to several "standard" regimens is of interest, however, when one contemplates the introduction of new therapeutic interventions. One prospective randomized trial which added doxorubicin to standard COP purported to demonstrate a substantial improvement in survival over COP (59). This study has been criticized for its small size and early termination. An ECOG randomized trial of COP versus chlorambucil and prednisone demonstrated survival durations similar to the CHOP arm in the

Table 15 Indications for Treatment of CLL

- Disease related symptoms
- Bone marrow failure
- Autoimmune cytopenias
- Massive splenomegaly
- "Bulky"or progressive mass disease
- LDT < 12 months ?
- Recurrent bacterial infections ?
- Diffuse BM pattern ?

French study (60). The investigators suggested that their results, compared to the French COP arm were attributable to a more protracted duration of therapy (up to 18 months). Other small trials also suggest a higher response to doxorubicin containing regimens, but don't clarify the question of impact on survival (54,55,61). Finally, preliminary results of another cooperative group trial are said to demonstrate a significant improvement in both response and survival when comparing high dose chlorambucil to standard dosage (62). Although the optimal regimen and duration of treatment for these advanced stage patients is not settled, it is generally accepted that therapy should be initiated for this subset of CLL patients because of the potential for a very short survival and the suggestion that survival has improved with the systematic early employment of therapy (1). Because of the diversity of survival outcomes between studies, it will be imperative that future trials be conducted in a randomized setting where the multiplicity of prognostic factors can be controlled.

New Therapeutic Approaches. Recent observations have suggested that more effective therapeutic options may soon exist for the treatment of selected patients with CLL. These include both new chemotherapeutic agents as well as novel biologic modalities.

Chemotherapy drugs. Three new agents have been recently demonstrated to have activity in CLL. The most promising of these is a synthetic purine analog, 9-ß-D-arabinofuranosyl-2-fluoroadenine-5'-monophosphate or **fludarabine** phosphate.

With the major activity of cytosine arabinoside, a pyrimidine analog, in acute leukemia, purine analogues with similar activity were sought. Adenine arabinoside was too rapidly inactivated in vivo by adenosine deaminase. The 2-fluoro derivative was not rapidly inactivated but was poorly soluble. The 5'-monophosphate was both soluble and resistant to ADA (63). Structural relationships of these

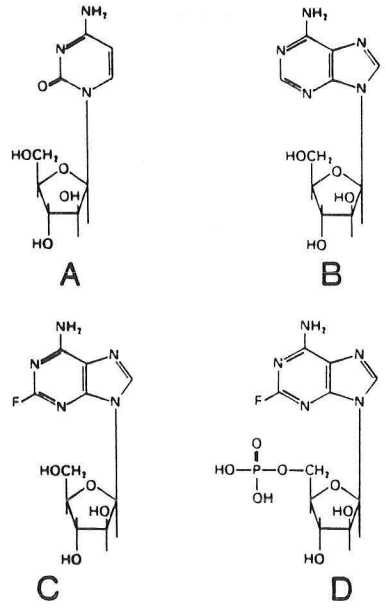

Fig 1. Chemical structures of pyrimidine and purine nucleosides and fludarabine phosphate. (A) Cytarabine, (B) vidarabine (ara-A), (C) 2-fluoro-ara-A, and (D) fludarabine phosphate (2-fluoro-adenine arabinoside-5'-phosphate, NSC-312887).

Figure 6

Table 16 Characteristics of Fludarabine Phosphate

- 9-ß-D-arabinofuranosyl-2-fluoroadenine-5'-monophosphate
- Mechanism of Action:
  - Dephosphorylated in serum to F-ara-A
  - Enters cells by carrier mediated transport
  - Intracellular phosphorylation to F-ara-ATP by deoxycytidine kinase
  - Accumulates in cells
  - Suppresses DNA synthesis by inhibition of ribonucleotide reductase and DNA polymerase α
  - Incorporated into cellular RNA and DNA
- Pharmacokinetics and Metabolism:
  - Plasma clearance triexponential
  - Terminal T1/2: plasma-8 hrs, intracellular-15 hrs; not dose dependent; AUC x time is dose dependent
  - Primarily renal elimination
- Toxicity:
  - Myelosuppression, especially neutropenia - dose limiting at levels used in CLL
  - Nausea, vomiting, diarrhea - mild
  - Somnolence, fatigue - infrequent
  - ↑ liver enzymes and creatinine - occasional
  - Fatal encephalopathy - rare at CLL doses
  - Acute pulmonary toxicity - idiosyncratic
  - Tumor lysis syndrome reported

compounds are shown in figure 6. Characteristics of the drug are shown in table 16.

Clinical trials in patients with CLL, initially refractory to standard therapy and subsequently in untreated patients have demonstrated remarkable results (64-67) (table 17). The overall response rates in previously treated patients were at least equivalent to those in untreated patients utilizing standard therapy (table 11). The criteria for response were those of the NCI shown in the appendix. To put it in the context of studies shown in table 11, the CR + nodular PR would be a CR in all of the French Cooperative Group studies. A nodular PR appears to have the same survival potential as a CR. There may be a dose-response effect of this drug in CLL. Another study that utilized lower dose intensity, when applying the above criteria for response, found a lower response rate than in the MDA series (67a). The impact of this drug on survival outcome in CLL is still unknown. There are not good data on survival after treatment failure and the median survival in the untreated group has not been reached.

2-deoxycoformycin (Pentostatin) is an adenosine deaminase inhibitor. It has exceptional activity in hairy cell leukemia. Limited studies in patients with high risk or stage C previously treated CLL indicate a 10-30% response rate (68-70). Data on

activity in previously un-treated patients is even more limited, but it appears to be inferior to fludarabine (69).

Table 17 Response to Fludarabine. M.D. Anderson Experience

Rx	Prior Rx	Stage	# PTS	%CR* (90%CI)	%CR+PR (90%CI)	Ref
F ± P	Yes	Low - Int	75	-	77[1] (68-84)	64, 67
F ± P	Yes	High	104	-	44 (36-52)	64, 67
F	No	Int	19	74 (55-87)	84 (66-93)	66
F	No	High	14	71 (49-86)	71 (49-86)	66

F = fludarabine; P = prednisone; *includes residual lymphoid nodules in marrow (nodular PR); [1]In the combined F ± P studies there were 13% CR, 16% nodular PR, 30% PR

2-chlorodeoxyadenosine (2-CDA) is a synthetic purine analog closely related to fludarabine. Initial limited studies showed a partial response rate of 17% in previously treated stage C patients (71). With more total cycles, the response rate is stated to reach 50% with some CR's (72). Another interesting observation is a high response of associated auto-immune cytopenias. This activity approaches that of fludarabine and its role deserves further study.

A number of phase II and III trials are now on-going to evaluate combinations of these newer agents with standard drugs as well as to compare the relative impact of these on response and survival (1,73).

Biologic therapy of lymphoid malignancies has attracted attention of investigators. These lesions are well characterized phenotypically, tend to be widely disseminated and are susceptible to manipulation by a variety of cytokines. Some approaches that have been employed or proposed are outlined in table 18. **Monoclonal antibodies** with a variety of specificities for surface antigens have been administered. Although effective in vitro, pharmacodynamic problems as well as development of human anti-murine antibodies (HAMA), neutralization by soluble antigen and development of phenotypic change have made these studies ineffective (74). The use of **conjugates** to monoclonal antibodies have shown more promise. These include radionuclides **(radioimmunotherapy or RAIT)** and toxins **(immunotoxins)**.

Table 18 Biotherapy in CLL

ANTIBODIES (Specificity)
CD5
CD19
CD20
CD22
CDw52 (CAMPATH-1 Ab)
cCLLa*
Anti-idiotypic (personalized)
Anti-idiotypic (shared)*
Unknown (LYM-1 Ab)
CONJUGATES
Radionuclides (^{131}I, ^{90}Y)
Toxins
Ricin A chain
Blocked Ricin
Saporin*
CYTOKINES
IL-2
Interferon α
rIL-2 - Diphtheria Toxin

* Proposed

Significant responses have been reported for RAIT in CLL (75). The primary limitation is the total body irradiation dose which results in significant bone marrow toxicity. Other, shorter range radionuclides are being examined. Immunotoxin therapy has had mixed results in CLL and a number of modifications to improve this approach are under investigation (76-79). The most tumor specific antibodies possible are the ultimate carrier. Since the idiotype of the sIg on the CLL cell is tumor specific for that patient, anti-idiotypic antibodies are a prime candidate for immuno-conjugate therapy. This requires, however, production of individualized or "personalized" antibody for each patient. The discovery of the high frequency of shared idiotypes or CRI among CLL patients makes these attractive targets (8,19,20,80,81). One group of investigators has described a monoclonal antibody that is alleged to react with a surface antigen that is expressed by cells from all patients with B CLL and its variants and not found on any normal cells or other neoplasms, the common CLL antigen (cCLLa). This would be the ideal type of target for immunotherapy and in-vitro studies have been encouraging (82,83). However, the presence of this antigen has not been confirmed by other investigators. Another novel approach to biotherapy is presently being investigated. This utilizes a recombinant toxin assembled from human IL2 cDNA fused to a truncated diphtheria toxin gene and expressed in E. Coli. This has demonstrated activity in T cell malignancies (84) and some B cell tumors (F Lemaistre, personal communication). The IL2 receptor consists of an intermediate affinity (p75 subunit), low affinity (p55 subunit, CD25) and the high affinity hybrid (p55/p75 subunits). This immunotoxin requires the high affinity receptor for IL2 binding. Only 25-50% of CLL patients have cells that express the CD25 antigen although the intermediate subunit is ubiquitous on leukemia/lymphoma cells. It has been found that phytohemagglutinin stimulates the expression of CD25 on CLL cells that did not express the antigen before exposure and makes them sensitive to the IL2-toxin (85). This could make this agent effective in a larger group of patients with CLL since PHA can be

administered in vivo to humans. **Interferon-α** has demonstrated activity in CLL, but only in low stage disease (86-88). It is possible that it could be utilized as maintenance therapy after achievement of maximum response with cytoreductive agents in a fashion similar to that in multiple myeloma. The use of other cytokines, alone, or with additional agents is presently being explored and no meaningful results are available.

Other therapeutic modalities. Irradiation has been utilized in various approaches. **Splenic radiation** has been utilized as a primary therapeutic modality and, in randomized trials, was associated with survival equivalent to standard chemotherapy (43). **Whole body irradiation** has not provided any survival benefit over standard chemotherapy and is more myelotoxic (89). In general, radiotherapy is reserved for local palliation of symptomatic mass disease. **Splenectomy** has been utilized successfully for the management of refractory autoimmune cytopenias or hypersplenism (90-92). **Allogeneic bone marrow transplantation** has been utilized in a small series of advanced CLL patients with the achievement of a high CR rate. It is too early to determine whether a curative outcome might occur (93). This modality is limited to only the younger patients with CLL. **Autologous marrow re-infusion** following high dose ablative therapy is being evaluated in patients who have achieved a bone marrow CR as determined by sensitive polymerase chain reaction assay (Nadler L, unpublished data). This provides a broader age range for this high intensity therapy approach.

One indisputable point emerges from all studies in chronic lymphocytic leukemia. Almost all patients will eventually progress or relapse after the initiation of presently employed therapeutic regimens and will succumb to the disease or its complications unless unrelated causes intervene. The only exception appears to be a proportion of the subset with "smoldering" CLL. The primary goal at present, therefore, is to determine those strategies that will produce the longest potential survival. Questions that are being addressed to achieve this end include timing of initial therapy, comparison of regimens in terms of response rates, the role of biotherapy, techniques for detecting minimal residual disease and the signifiance of such detection, the duration of therapy, and alternate approaches for the ablation of residual diease. It would be hoped that answers to these questions might ultimately lead to the development of strategies that would be aimed at permanent eradication of the disease.

IMMUNOLOGIC ABNORMALITIES

A number of immunologic defects have been described in CLL, some of which may lead to the significant increase in propensity to bacterial as well as opportunistic infections. A number of these (cited in reference 1) are not convincingly reproducible but span humoral and cellular immune processes (table 19). A clear definition of the status of the immune system in CLL may provide clues to the role of the CD5+ B cell in normal immune-modulation.

Table 19 Immunologic Abnormalities in CLL

Hypogammaglobulinemia
↓ Humoral Ab response 1° and 2°
↓ CD4+ cells
↑ CD8+ cells
↑ CD4+/CD8+ cells
↓ T cell proliferation
↓ T helper function
↑ T suppressor function?
↓ Delayed hypersensitivity 1° and recall
↓ Ab dependent cytotoxicity
↑ Natural killer cells
↓ NK function
Autoimmune cytopenias

The most reproducible defect in CLL is the development of hypogammaglobulinemia. The mechanism is unknown but it has been proposed that this is a major etiologic factor in the development of bacterial infections. The presence of hypogammaglobulinemia correlates with both the duration and stage of disease (94,95). The majority of patients with CLL become hypogammaglobulinemic with time (96). The rate of infections and severe infections correlates with hypogammaglobulinemia, especially a decrease in IgG, and to a lesser extent with IgA and M (94). These patients also have impairment of the humoral immune response both to recall and primary antigens (97). The most common cause of death in patients with advanced stages of CLL is bacterial infection (32). Hypogammaglobulinemia does not tend to improve with therapy of the CLL, but treatment does not appear to significantly increase the propensity to infection except as it relates to treatment induced neutropenia (94).

Intramuscular administration of gamma globulin does not reduce the incidence of infection. With the availability of IV **gamma globulin (IVGG)**, a double blind, randomized prospective trial of IVGG in patients with CLL who were hypogammaglobulinemic and/or had a history of infection was performed (98). The overall incidence of bacterial infections was reduced significantly in the IVGG group, but a statistically significant reduction in serious bacterial infections (requiring hospitalization) was not seen, perhaps due to sample size or lack of efficacy. There was a significant delay in the time to development of serious bacterial infections in the treated group, however. In a second, much smaller study with a cross-over design, there was a reduction in serious bacterial infections during the time that IVGG was

received (99). Due to the high cost of this material, it is not clear at the present time what patients are candidates for chronic prophylaxis with IVGG (100). It is reasonable to consider patients with more than one episode of moderate to severe bacterial infection for this therapy.

A number of abnormalities in cell mediated immunity have been described (96,97). These do not correlate with the incidence of infection and, in part, may be related to cytotoxic therapy. It is also possible that they reflect changes induced secondarily by the expansion of the CD5+ B CLL population of cells and, in turn, provide clues to the interaction of the normal CD5+ B cell and cell mediated immune functions.

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FEATURES OF LEUKOCYTE ANTIGENS REFERENCED IN THIS PROTOCOL

CD#	Other Name	Characteristics and Cellular Distribution
CD5	T1,Leu1	T cells, subpopulation B cells; possible cytokine receptor
CD10	CALLA	Pre-B;common ALL; follicular B lymphomas; granulocytes; kidney; neutral endopeptidase
CD11		Member Integrin supergene family of heterodimeric receptors/adhesion molecules; share B subunit (CD18)
CD11b	Mac-1	Granulocytes; monocytes; B subset; C3bi receptor
CD11c	LeuM5	Granulocytes; monocytes; hairy cell leukemia
CD14	My4	Monocytes; Kupfer cells; (granulocytes); B subset
CD19	Leu12,B4	Pan B; regulates proliferation and differentiation
CD20	Leu16,B1	Mature B; transmembrane ion flux
CD21	CR2,B2	B; dendritic reticulum cells; C3d/EBV receptor
CD22	Leu14	B
CD23	FcϵRII	B subset; actB; monocytes; eosinophils; dendritic reticulum cells; low affinity receptor for IgE
CD24	BA-1	Some B; mature granulocytes
CD25	Tac	Activated cells; macrophages; component IL-2 receptor
CD38	T10	T; germinal center B; plasma cells
CD71	T9	Transferrin receptor

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RESPONSE CRITERIA IN CLL AS DEFINED BY THE INTERNATIONAL WORKING PARTY AND THE NATIONAL CANCER INSTITUTE

	CR		PR	
	IWCLL	NCI	IWCLL	NCI
Physical Exam			Shift to a lower Binet stage	
Nodes	None	None		≥50% decrease
Liver/spleen	Not palpable	Not palpable		≥50% decrease
Symptoms	None	None		N/A
Blood				
Neutrophils	≥1500/μL	≥1500/μL		>1500/μL or ≥50% ↑
Platelets	>100,000/μL	>100,000/μL		>100,000 or ≥50% ↑
Hemoglobin	Not specified	>11 gm/dL		>11 gm/dL or >50% ↑
Lymphocytes	<4000/μL	<4000/μL		≥50% ↓
Bone Marrow	Normal aspirate and biopsy*	<30% lymphs normal biopsy*		N/A

* Nodules or focal aggregates of lymphocytes compatible