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# 2 Chronic Lymphocytic Leukemia

## *Diagnosis and Management*

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### **CONTENTS**

|   |
|---|
| INTRODUCTION  |
| EPIDEMIOLOGY  |
| ETIOLOGY  |
| CLINICAL AND LABORATORY FEATURES                                      |
| THE NORMAL CELLULAR COUNTERPART OF B-CLL                              |
| CYTOGENETIC ABNORMALITIES IN CLL                                      |
| MOLECULAR ABNORMALITIES IN CLL  |
| THE DIAGNOSIS OF CLL  |
| THE DIFFERENTIAL DIAGNOSIS OF CLL                                     |
| STAGING OF CLL  |
| PROGNOSTIC FACTORS IN CLL   |
| THE TREATMENT OF CLL  |
| AUTOLOGOUS STEM-CELL TRANSPLANTATION IN CLL                           |
| THE ROLE OF ALLOGENEIC STEM-CELL TRANSPLANTATION (ALLO<br>SCT) IN CLL |
| THE TREATMENT OF YOUNGER PATIENTS WITH CLL                            |
| AUTOIMMUNE DISEASE AND ITS TREATMENT IN CLL                           |
| REFERENCES  |

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### **INTRODUCTION**

CLL is characterized by the gradual accumulation of malignant lymphocytes in the bone marrow, blood, lymph nodes, liver, and spleen. Thus, the major clinical features are the sequelae of bone marrow failure and the compressive syndromes that result from gross enlargement of lymphoid organs. In addition, CLL is associated with humoral and cell-mediated immunodeficiencies as well

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as a greatly enhanced risk of autoimmune cytopenias—particularly autoimmune hemolytic anemia (AIHA).

The pathogenesis of CLL is dominated by the gradual, yet seemingly inexorable, accumulation of leukemic cells in the bone marrow and lymph nodes. This process results largely from an intrinsic failure of physiological programmed cell death or apoptosis rather than from an increased proliferative rate (1,2). The fact that CLL cells cannot die also explains their inherent resistance to conventional chemotherapy. Indeed, at the present time, CLL is best considered an incurable disease, and therapeutic interventions are largely aimed at improving bone-marrow function and resolving those complications resulting from enlargement of lymphoid organs rather than eradicating or curing the disease.

The course of CLL exhibits a striking degree of clinical heterogeneity. Unfortunately, for many patients, CLL is a clinically progressive disease that results in death within 1–2 yr from initial diagnosis. For others, in contrast, the disease is extraordinarily indolent and results in no reduction in life expectancy. Since contemporary management options range from simple observation to high-dose therapy with allogeneic bone-marrow transplantation (Allo BMT), a major challenge in the care of patients with CLL is to select appropriate interventions for each affected individual.

## EPIDEMIOLOGY

CLL is the most common type of leukemia in adults, comprising 25% of all cases diagnosed in North America and Europe. Its incidence increases with age with a median age of 55 yr at diagnosis, and is more common in males (male:female ratio 2:1). The incidence of CLL varies geographically, and is much less common in the Far East (3).

## ETIOLOGY

The etiology of CLL is uncertain. Interestingly—unlike virtually all other hemopoietic malignancies—exposure to ionizing radiation does not appear to be causative (4). There is an increased risk of CLL in first-degree relatives of patients with CLL and other B-cell malignancies, and the phenomenon of anticipation is observed within affected kindreds (5,6).

## CLINICAL AND LABORATORY FEATURES

The cardinal clinical and laboratory features of CLL are summarized in Table 1.

Anemia can be multifactorial in CLL, and potential mechanisms include bone-marrow failure caused by leukemic infiltration, AIHA, hypersplenism, pure red-cell aplasia (PRCA), the effects of chemotherapy, and coincident hematinic deficiency. All of these possibilities should be considered when assessing the CLL patient with anemia, since important alternative causes of anemia caused by

Table 1  
The Clinical and Laboratory Features of CLL

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|   |
|---|
| Bone-marrow failure (anemia, thrombocytopenia, and neutropenia)     |
| Lymphadenopathy   |
| Hepatosplenomegaly  |
| Hypersplenism   |
| Autoimmune disorders (AIHA, ITP, PRCA)                              |
| Positive Direct Antiglobulin Test (15–35% of cases)                 |
| Systemic symptoms (sweats, weight loss)                             |
| Hypogammaglobulinemia (recurrent bacterial infections)              |
| T-cell immunodeficiency (shingles, <i>Pneumocystis carinii</i> )    |
| Transformation to an aggressive lymphoma (Richter's Transformation) |
| Paraprotein (rare)  |

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iron deficiency, such as colon cancer, occur in a similar age group. Autoimmune cytopenias, most commonly AIHA, are particularly common in CLL. Only a minority of patients with a positive direct antiglobulin test (DAGT) will develop hemolysis, although approximately 4–5% of CLL patients will develop clinically significant AIHA at some stage of their disease (7,8). The pathogenic high-affinity IgG autoantibodies responsible for AIHA are polyclonal, and therefore are not the product of the CLL clone itself (9). Rather, the autoimmune disorders associated with CLL result from a dysregulated immune system, which is itself a fundamental feature of CLL.

In approx 3% of patients with CLL, an aggressive diffuse large-cell lymphoma develops (Richter's syndrome) (10). Usually, this presents with asymmetric or discordant lymph-node enlargement, often with progressive systemic symptoms. The prognosis for this group of patients is poor, with a median survival of less than 6 mo. The clonal relationship of such lymphomas to the underlying CLL has been investigated by analyzing the sequence of the rearranged immunoglobulin genes in both lymphoma and CLL. The majority of cases of Richter's syndrome appear to result from clonal evolution of the CLL clone itself (11). Presumably, the accumulation of further genetic abnormalities results in a dramatic alteration in the biological and clinical phenotype of the disease. Similar events are well-documented in CML during the transition to blast crisis. However, 30–40% of cases of Richter's syndrome are clonally distinct from the preceding CLL (11). It is possible that these cases are analogous to the lymphomas that arise in other states of immunodeficiency, such as the post-transplant lymphoproliferative disorders (10). Certainly, in the rarer Hodgkin's disease variant of Richter's syndrome, the malignant Reed-Sternberg cells contain EBV, which is known to be central to the development of many lymphomas that arise in the setting of chronic immunosuppression (12).

## THE NORMAL CELLULAR COUNTERPART OF B-CLL

Although CD5+ B-cells are relatively rare in the adult, they are found in high numbers in fetal and neonatal blood. Essentially, the antibodies produced by these naïve cells are the expression of the germline immunoglobulin repertoire, since no antigen exposure with consequent somatic hypermutation has occurred before birth. These naturally occurring antibodies are polyspecific, bind with low affinity, and generally react with autoantigens (e.g., IgG) as well as with microbial epitopes (13,14). The autoreactive germline repertoire is highly conserved phylogenetically, and persists into adult life so that all normal individuals possess low levels of auto-antibodies. It is probable that in early life, these act as a first line of defense against invading microbes and function as templates upon which the antigen-driven somatic hypermutation machinery can operate in order to produce high-affinity antibodies characteristic of secondary immune responses (15).

In adults, CD5+ B-cells with many of these features are found, in low numbers, in blood, primary follicles, and the mantle zone surrounding secondary follicles. CD5+ mantle-zone B-cells share many features with B-CLL cells such as the absence of extensive somatic hypermutation and the production of polyreactive autoantibodies. However, unlike mantle-zone B-cells, CLL cells express very low-level SmIg and high levels of bcl-2. Therefore, the precise normal counterpart of the CLL cell remains uncertain (13).

Very weak expression of surface-membrane immunoglobulin, as well of other important signaling molecules such as CD22, are characteristic features of CLL that are shared by anergic B-cells. It has been hypothesized that in CLL, the malignant cell can be operationally defined as an anergic self-reactive CD5+ B-cell committed to the production of autoantibodies (1,13).

The most important information about the cellular derivation of CLL derives from the analysis of the Ig V<sub>H</sub> gene mutation status. Hamblin et al. found that approx 50% of CLL cases had unmutated V<sub>H</sub> genes indicative of a naïve B-cell of origin (16). The remainder had somatically mutated V<sub>H</sub> genes characteristic of B-cells that had been exposed to the follicular microenvironment. Importantly, Ig gene mutation status correlated with certain clinical features. Unmutated cases tended to have atypical cytology, advanced stage with progressive disease, and inferior survival. Indeed, patients with stage A disease had very different median survivals in the two groups: unmutated 95 mo, mutated 293 mo. There have also been reports that trisomy 12 is associated with unmutated, and 13q<sup>-</sup> with mutated status. Very similar findings have been reported by Damle et al., who also correlated unmutated V<sub>H</sub> gene status with CD38 positivity (17).

It is now clear that there are two subsets of B-CLL. The first is a malignancy derived from naïve B-cells, although the precise normal counterpart is still uncertain. The second is a malignancy of somatically mutated memory B-cells, and is associated with a significantly more favorable outcome. It seems highly

**Table 2**  
**Chromosomal Abnormalities in CLL as Detected**  
**by Conventional Metaphase Cytogenetics**

| <i>Abnormality</i> | <i>Frequency</i> | <i>Comments</i>   |
|--------------------|------------------|---|
| Trisomy 12         | 33%              | Atypical cytology<br>Poor outcome   |
| Del 13q            | 15%              | Similar prognosis to cases with<br>normal karyotype   |
| Del 11q23          | 11%              | Extensive lymphadenopathy<br>Poor prognosis<br>Deletions may occur at sites of<br>CCG trinucleotide repeats |
| Del 6q             | 5%               |   |
| t(11;14)           | 2%               | Probably cases of MCL   |

likely that these striking differences in prognosis will in turn relate to the presence of other established genetic abnormalities such as *p53* or ataxia telangiectasia mutated gene (ATM) inactivation. Current research is directed at determining whether particular cytogenetic abnormalities are correlated with unmutated or mutated phenotypes.

## CYTOGENETIC ABNORMALITIES IN CLL

Early cytogenetic analyses in CLL were restricted by the fact that the malignant cells intrinsically have a low proliferative rate. Even with the use of potent B-cell mitogens, chromosomal abnormalities were only found in approx 50% of cases with admixed normal T-cells accounting for most normal metaphases (18,19) (Table 2). It was found that the presence of a cytogenetic abnormality by itself, as well as the percentage of abnormal metaphases in a particular case, correlated with poor survival. In addition, complex clonal abnormalities were associated with particularly poor outcomes. Interestingly, unlike the situation in CML or follicular lymphoma (FL), clonal cytogenetic evolution was not a feature of disease progression, although *p53* inactivation may well prove to be associated with transformation.

Importantly, the presence of clonal abnormalities was strongly associated with advanced disease and with cases with a higher percentage of prolymphocytes (20,21). Therefore, it can be argued that the association of abnormal metaphase cytogenetics with poor prognosis results simply from an increased likelihood of detecting any clonal abnormality in those cases which are more clinically advanced, with a higher proliferative rate. Whatever the case, the abnormalities detected by conventional cytogenetics successfully identified regions of interest

Table 3  
Chromosomal Abnormalities in CLL Detected by Interphase FISH

| <i>Abnormality</i>   | <i>Frequency</i> | <i>Implicated Gene</i>                               |
|----------------------|------------------|--|
| Del 13q              | 55%              | ?  |
| Del 11q              | 18%              | ATM  |
| Trisomy 12           | 16%              | ?  |
| Del 17p              | 7%               | <i>p53</i>   |
| Del 6q               | 6%               | ?  |
| 14q32 rearrangements | 4%               | IgH translocations with multiple potential oncogenes |
| t(11;14)             | 0%               | bcl-1  |

that could be analyzed in more detail using more sensitive tests such as interphase FISH. This technique does not rely on the presence of metaphases, and is thus independent of proliferative rate.

Using interphase cytogenetics, Dohner et al. analyzed 325 patients with CLL (22). In all, 82% of the cases were abnormal. Of these, approx 65% had one, 25% had two, and 10% had more than two abnormalities (Table 3). Several striking observations are made in this study. First, cytogenetic abnormalities occur in the vast majority of cases of CLL. Second, 13q- is by far the most common abnormality in CLL. Third, the proportion of cases with trisomy 12 is significantly lower than would be expected by extrapolation of data derived from conventional cytogenetics. Also, trisomy 12 was not associated with a poor prognosis in the analysis, although this remains a contentious issue. Fourth, 14q32 translocations appear to be rather unusual events in CLL in contradistinction to myeloma—for example, in which such IgH rearrangements are nearly universal. Fifth, no cases with a t(11;14) were found. Earlier series often reported a 2–5% incidence of t(11;14), and the most likely explanation for this discrepancy is that these were misdiagnosed cases of mantle-cell lymphoma (MCL).

The correlation with clinical data showed that 17p- and 11q- were both associated with more advanced and 13q- with less advanced disease. The data was analyzed in such a way as to generate a hierarchical model of genetic subgroups: 17p-, 11q- but not 17p-, trisomy 12 but not 17p- or 11q-, normal karyotype and 13q- alone). These are associated with different clinical outcomes (Table 4). However, using multivariate analysis, only 17p- and 11q- retained prognostic importance along with age, Binet stage, LDH, and white-blood-cell count (WBC).

## MOLECULAR ABNORMALITIES IN CLL

Although there is increasing knowledge about the cytogenetic abnormalities that occur in CLL, their precise pathogenetic molecular consequences are largely

Table 4  
Cytogenetic Abnormalities and Prognosis

| <i>Karyotype</i> | <i>Median survival (mo)</i> |
|------------------|-----------------------------|
| 17p–             | 32 mo                       |
| 11q–             | 79 mo                       |
| Trisomy 12       | 114 mo                      |
| Normal           | 111 mo                      |
| 13q–             | 133 mo                      |

unknown. Indeed, *bcl-2* overexpression, which occurs in 85% of cases, is not a consequence of any known chromosomal translocations, as is the case with the t(14;18) characteristic of FL (2). In CLL, possible mechanisms include hypomethylation of the *bcl-2*-promoter region (23) or production of basic fibroblast growth factor (bFGF) by the CLL cells themselves (24), which in turn induces *bcl-2* expression. Whatever the case, *bcl-2* is a negative regulator of apoptosis although it is likely that the *bcl-2:bax* ratio is more critical in setting the precise threshold for programmed cell death. Indeed, in CLL, a high *bcl-2:bax* ratio correlates with progressive disease and resistance to treatment (25,26).

Usually *p53* mutations accompany 17p deletions, are found in 12–25% of cases of CLL, and are strongly associated with advanced disease and Richter's transformation (27). *ATM* is another gene that may function as a classical tumor-suppressor gene in CLL. *ATM* is located at 11q22–11q23, which is the region deleted in CLL. Inherited homozygous *ATM* mutations result in ataxia telangiectasia, which is itself associated with a greatly increased risk of lymphoid neoplasia and acquired homozygous *ATM* mutations are also known to occur in T-PLL. Schaffner et al. analyzed 22 cases of B-CLL with monoallelic deletions of 11q22–23, and found that 5 of 22 also possessed point mutations in the remaining allele (28). Furthermore, germline mutations in *ATM* have been found at a much higher frequency in patients with CLL than in the general population (29). Taken together, these observations suggest that *ATM* may function as a classical tumor-suppressor gene in a subset of patients with CLL, although it is possible that another tumor-suppressor gene at 11q23 is operative in CLL.

Since 13q– is a frequent abnormality in CLL, it is another likely site for a tumor-suppressor gene. The entire minimal region of deletion (MDR) has now been sequenced and analyzed. The MDR contains two pseudogenes and three transcribed genes (*CAR*, *Leu1*, and *Leu2*). However, no mutations have been found in these genes in 20 cases of CLL with 13q– (30). These findings suggest that the 13q– MDR does not actually harbor a classical tumor-suppressor gene, although it is possible that haploinsufficiency of one of these genes may be involved in the pathogenesis of CLL.

Table 5  
Minimal Criteria for a Diagnosis of CLL (NCI Working Group)

1. Peripheral-blood lymphocytosis  $>5 \times 10^9/L$
2. Lymphocytes must be morphologically mature-appearing with less than 55% atypical cells (prolymphocytes or lymphoplasmacytoid cells)
3. Greater than 30% lymphocytes in a normocellular or hypercellular bone-marrow aspirate (bone-marrow not absolutely required for diagnosis)
4. Monoclonal light-chain expression
5. Expression of B-lineage markers (at least one of CD19, CD20, and CD23)
6. CD5 expression

The advent of gene-expression profiling will undoubtedly have a major impact on efforts to unravel the molecular phenotypes of CLL. Currently available data demonstrate that the expression profile of CLL is analogous to that of resting B-cells, although there are additional genes expressed by CLL, the so-called CLL signature. Notably, there is a complete lack of gene expression typical of germinal center B-cells (31). A more detailed analysis of gene expression is currently underway, and will undoubtedly further highlight differences between mutated and unmutated cases, and also shed light on the molecular basis of drug resistance.

## THE DIAGNOSIS OF CLL

CLL can be defined as a neoplasm of small, round B-lymphocytes found in the peripheral blood, bone marrow, and lymph nodes. Guidelines for diagnosis and treatment of CLL have been published and subsequently revised by an NCI-sponsored Working Group (32,33). The major purpose of these guidelines was to facilitate comparisons between clinical trials. The minimal criteria for a diagnosis of CLL are listed in Table 5. The International Working Group on CLL and The French-American-British Cooperative Group have published very similar diagnostic criteria (34,35).

In the great majority of cases, a diagnosis of CLL can easily be made after examination of a peripheral blood smear and cell-marker analysis. Occasionally, bone-marrow biopsy, lymph-node histology, cytogenetics, or molecular studies are required in order to distinguish CLL from rarer lymphoproliferative disorders. On a peripheral-blood smear, CLL cells appear small and uniform with scanty cytoplasm and clumped nuclear chromatin, giving rise to a mosaic or 'cracked mud' appearance. Characteristically, numerous smear cells are also present. The bone marrow is infiltrated in an interstitial, nodular, or diffuse pattern, and lymph nodes are effaced by monomorphic small lymphocytes with scattered, paler-appearing proliferation centers containing larger nucleolated



Table 6  
The Characteristic Immunophenotype of CLL

|   |
|---|
| κ or λ light-chain restriction                                      |
| Weak SmIg   |
| IgM or IgM/IgD SmIg   |
| CD5   |
| CD23  |
| Pan-B markers (with normal levels of intensity): CD19, CD24, CD79a  |
| Pan-B markers (with reduced levels of intensity): CD20, CD22, CD79b |
| Lack of expression: FMC7, CD11c, CD25                               |

cells known as prolymphocytes or paraimmunoblasts. Occasionally, patients will present with lymph-node involvement without significant blood or marrow involvement. Nevertheless, CLL and small-cell lymphocytic lymphoma (SLL) are biologically the same entity, and the WHO classification highlights the fact that CLL and SLL are one disease at different stages (36).

The immunophenotype of CLL is highly characteristic. Surface-membrane immunoglobulin (SmIg) is usually of IgM or IgM/IgD isotype, is very weakly expressed, and can sometimes be undetectable. By definition, there is κ or λ light-chain restriction. Almost universally, there is co-expression of CD5 and CD23. CD5 is conventionally considered as a T-lineage antigen, but is also expressed by approx 10–15% of normal blood B-cells, which may be the normal cellular counterpart of CLL. CD23 is the low-affinity FcR that is expressed by activated B-cells and eosinophils, by cases of CLL but rarely by other B-cell malignancies. Pan B-lineage markers such as CD19, CD24, and CD79a are expressed at normal levels, whereas others such as CD20, CD22, and CD79b are characteristically expressed at very low levels. Antigens that are expressed by other B-cell lymphoproliferative disorders such as FMC7 (most B-cell malignancies) or CD11c and CD25 (hairy cell leukemia) (HCL) are typically absent in CLL (Table 6). The characteristic spectrum of antigen expression in CLL has encouraged the development of a highly predictive scoring system for its positive identification and discrimination from other leukemias (37,38) (Table 7).

The FAB group recognized two types of atypical CLL, which together are termed CLL of mixed cell type (35). In CLL/PL, prolymphocytes account for 10–55% of circulating cells, whereas if there are more than 55% prolymphocytes, a diagnosis of B-PLL is made. The other variety of atypical CLL is characterized by a spectrum of small to large lymphocytes, but with less than 10% prolymphocytes. Two groups have reported that atypical CLL tends to be a more aggressive disease with an earlier requirement for therapy and a shorter survival (39,40). In addition, trisomy 12 and abnormalities of *p53* may be more common in atypical CLL (39,41). These findings must be reanalyzed along with immunoglobulin gene

Table 7  
A Scoring System for the  
Immunophenotypic Diagnosis of CLL

| <i>Marker</i>   | <i>Score</i> |
|-----------------|--------------|
| Weak SmIg       | +1           |
| CD5+            | +1           |
| CD23+           | +1           |
| FMC7—           | +1           |
| CD22 weak or —* | +1           |

A score of 4/5 or 5/5 confirms CLL.

\*As an alternative to CD22, negativity for CD79b can score +1. This refinement results in greater discrimination between CLL and other B-cell malignancies.

mutation status, since it seems probable that atypical cytology, the presence of trisomy 12 by conventional metaphase cytogenetics, and *p53* abnormalities may be surrogate markers for an unmutated genotype or an increased proliferative rate.

## THE DIFFERENTIAL DIAGNOSIS OF CLL

In the great majority of cases, the characteristic cytology and immunophenotype of CLL result in the rapid and unequivocal recognition of this entity. In addition, in those cases with atypical morphology, the immunophenotype usually makes a diagnosis of CLL relatively straightforward (Table 7). A variety of reactive conditions can produce a peripheral-blood lymphocytosis but the clinical scenario is usually suggestive (e.g., infectious mononucleosis). Yet if any doubt remains after appropriate investigations (such as testing for the presence of heterophile or EBV-specific antibodies), immunophenotyping will rapidly exclude a clonal B-cell disorder.

A relatively large number of B- and T-cell neoplasms can result in peripheral-blood lymphocytosis. The acute lymphoblastic leukemias (ALLs) are readily distinguished by their blastic morphology, but if any doubt persists, immunophenotyping will readily distinguish these disorders from mature, post-thymic B-cell malignancies. Similarly, Burkitt's lymphoma in the leukemic phase has a characteristic morphology, phenotype, and cytogenetics.

Although collectively rare, the most frequently encountered mature T-cell leukemias are T-prolymphocytic leukemia, Sézary syndrome, adult T-cell leukemia-lymphoma, and T-cell large granular lymphocyte (T-LGL) leukemia. These leukemias have characteristic morphologies and display a mature T-cell phenotype (CD3+, CD4/8+), and are thus readily distinguishable from B-cell neoplasms.

Table 8  
Chronic B-Cell Leukemias in the Differential Diagnosis of CLL

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|  |
|--|
| B-CLL/SLL  |
| B-cell prolymphocytic leukemia   |
| Lymphoplasmacytic lymphoma   |
| Splenic marginal zone B-cell lymphoma with circulating villous lymphocytes |
| Hairy cell leukemia  |
| Follicular lymphoma  |
| Mantle cell lymphoma   |

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Therefore, in routine clinical practice, the major entities that can be confused with CLL include other types of peripheral B-cell lymphoid malignancies with a propensity to involve the blood (Table 8). A unifying feature that helps to distinguish B-CLL from other entities is the highly characteristic immunophenotype of CLL. The bulk of the other peripheral B-cell neoplasms express a “consensus” phenotype with typically strong expression of the pan-B antigens CD19, CD20, CD22, CD24, and CD79a/b, as well as strong FMC7 and SmIg. Weak expression of CD20, CD22 and CD79b is highly characteristic of B-CLL, as is the co-expression of CD23 and CD5.

B-PLL typically presents in elderly men with a high WBC and splenomegaly. The leukemic cells have a characteristic cytological appearance with a prominent, central nucleolus and express the consensus B-cell leukemia immunophenotype with negativity for CD23 and CD5. Lymphoplasmacytic lymphoma occasionally presents in leukemic phase. The diagnosis is suggested by typical lymphoplasmacytic cytology, and the consensus immunophenotype, and often by the presence of a serum paraprotein.

HCL seldom presents diagnostic difficulties in view of its characteristic clinical and laboratory features. Typical HCL presents with pancytopenia and massive splenomegaly along with typical hairy cells in the blood and monocytopenia. A highly predictive HCL scoring system has been developed in which a score of one is given for positivity with CD25, CD11c, HC2, and CD103 (42). Virtually all cases of HCL will have a score of 3–4/5. Two other disorders are associated with the presence of ‘hairy cells’ in the blood, namely variant hairy cell leukemia (HCL-V) and splenic marginal B-cell lymphoma with circulating villous lymphocytes (SLVL). HCL-V typically presents with a higher count than HCL and has typical cytology with a hairy cytoplasm, but a prominently nucleolated nucleus and preserved monocyte numbers. The HCL score is typically 0–2/4, with CD11c most often expressed. SLVL presents with splenomegaly and lymphocytosis, with the malignant cells demonstrating ‘polar’ villi rather than the circumferential ‘hairs’ of HCL and HCL-V.

The two entities that most often must be differentiated from CLL are FL in leukemic phase and mantle cell lymphoma (MCL). FL typically presents with prominent lymphadenopathy and bone-marrow infiltration, but can occasionally be leukemic. The circulating cells have typical centrocytic cytology and are small—often the size of normal red cells—with inconspicuous but deep nuclear clefting and minimal amounts of cytoplasm. They possess the consensus phenotype, and the majority are also CD10+. The bone marrow is infiltrated in a characteristic paratrabecular pattern, but if any doubt remains, the lymph-node histology will distinguish FL, as will the presence of a t(14;18). MCL usually presents with lymphadenopathy, but frequently involves the gastrointestinal tract and bone marrow, with circulating malignant cells. The leukemic cells are often heterogeneous in appearance, and, this lack of homogeneity is often the first clue to a diagnosis of MCL. The immunophenotype of MCL conforms largely to the consensus phenotype, with the important exception of CD5 positivity. This feature accounts for the occasional difficulty in distinguishing MCL from CLL, and in the past, cases of MCL were probably misdiagnosed as CLL. Again, if doubt persists, lymph-node histology and detection of the characteristic t(11;14) with resulting cyclin D1 expression can confirm a diagnosis of MCL. Cyclin D1 expression can be identified by flow cytometry or immunocytochemistry. It is of significant clinical importance to distinguish MCL from CLL, because the former has a particularly poor clinical outcome, for which intensive and experimental therapies are appropriate.

In summary, CLL can usually be easily distinguished from other leukemic B-cell malignancies. In those rare cases in which the clinical features, cytology, pattern of bone-marrow infiltration, and immunophenotype cannot provide a definitive diagnosis, then lymph-node biopsy and cytogenetic analysis are likely to increase diagnostic certainty.

## STAGING OF CLL

The major staging systems for CLL (Rai and Binet) were originally devised 20–25 years ago, and the fact that these systems are still widely used is an indication of their overall clinical utility and robust nature. The Rai clinical staging system was first published in 1975, with formal modifications made in 1987 (43,44) (Table 9). The modified system was introduced to assist in the design of prospective trials, and its use is recommended by the NCI Guidelines for the diagnosis and treatment of CLL. It is important to remember that when anemia or thrombocytopenia is immune in etiology, the patient should not be assigned to stage III/IV, because these stages refer to cytopenias that result from bone-marrow failure rather than autoimmune destruction. The Binet staging system consists of three stages (45). Stage C disease is defined by anemia or thrombocytopenia resulting from bone-marrow failure, whereas stages A and B are defined according to the

Table 9  
Rai Clinical Staging Systems

| <i>Risk Group<sup>b</sup></i> | <i>Stage<sup>a</sup></i> | <i>Description</i>   |
|-------------------------------|--------------------------|--|
| Low                           | 0                        | Lymphocytosis only   |
| Intermediate                  | I                        | Lymphocytosis plus enlarged nodes  |
|                               | II                       | Lymphocytosis plus enlarged liver or spleen with or without enlarged nodes   |
| High                          | III                      | Lymphocytosis plus anemia (Hb <11 g/dL) with or without enlarged nodes, liver, or spleen                                       |
|                               | IV                       | Lymphocytosis plus thrombocytopenia (Plats <100 × 10 <sup>9</sup> /L) with or without anemia, enlarged nodes, liver, or spleen |

<sup>a</sup>Original Rai system (1975).  
<sup>b</sup>Modified Rai system (1987).

Table 10  
Binet Clinical Staging System

| <i>Stage</i> | <i>Clinical features</i>  |
|--------------|---|
| A            | <3 palpable lymphoid areas  |
| B            | 3 or more palpable lymphoid areas                                 |
| C            | Anemia (<10 g/dL) or thrombocytopenia (<100 × 10 <sup>9</sup> /L) |

number of palpably enlarged lymphoid areas. For these purposes, five lymphoid areas are described: cervical, axillary, and inguinal nodes, and spleen and liver (Table 10). There have been various attempts to devise new staging systems, including the IWCLL’s proposal to integrate the Rai and Binet systems (46), but none of these have achieved widespread acceptance.

PROGNOSTIC FACTORS IN CLL

By far the most important prognostic factor in CLL is clinical stage, whether defined by the Rai or Binet systems (43–45) (Table 11). The prognostic significance of clinical stage is of major importance, and many putative prognostic factors have failed to maintain statistical significance after multivariate analysis. Nevertheless, it remains impossible to predict outcomes for individual patients regardless of their clinical stage, because the natural history of CLL is so highly variable.

After multivariate analysis, only a few variables retain prognostic power above that provided by clinical stage, and these include age, sex, and lymphocyte doubling time (LDT). A number of studies have identified increasing age as indica-

Table 11  
Survival in CLL According to Clinical Stage

| <b>Rai Clinical Staging System</b>          |                             |
|---|-----------------------------|
| <i>Stage</i>                                | <i>Median survival (yr)</i> |
| 0   | >12                         |
| I   | 8.5                         |
| II  | 6                           |
| III   | 1.5                         |
| IV  | 1.5                         |
| <b>Modified Rai Clinical Staging System</b> |                             |
| <i>Risk Group (Rai stage)</i>               | <i>Median survival (yr)</i> |
| Low (0)                                     | >10                         |
| Intermediate (I/II)                         | 7                           |
| High (III/IV)                               | 1.5                         |
| <b>Binet Clinical Staging System</b>        |                             |
| <i>Stage</i>                                | <i>Median survival (yr)</i> |
| A   | >10                         |
| B   | 5                           |
| C   | 2                           |

tive of inferior outcome, although it is difficult to allow for the influence of co-existing morbidities. For instance, one study demonstrated a clear survival disadvantage associated with advancing age (age <50 yr, median survival 7.1 yr; age >50 yr, median survival 4.1 yr) (47). However, there was no difference in survival after adjustment for non-CLL mortality. Intriguingly, female patients survive longer than male patients of equivalent stage, for unknown reasons. Galton was the first to suggest that LDT was a poor prognostic indicator (48), and Montserrat subsequently reported that patients with a LDT of less than 12 mo had an overall survival of less than 5 yr whereas at the time of publication, the survival of those with a LDT of over 12 mo had not yet been reached (49). Importantly, although there was a weak correlation of LDT with stage, the prognostic power of LDT was independent of stage. Utilizing the prognostic importance of the LDT, Montserrat was able to identify a subgroup of patients with stage A disease who had a particularly favorable outcome. Such patients with smoldering CLL must fulfill the following criteria: stage A disease, non-diffuse bone-marrow infiltration, hemoglobin >13 g/dL, lymphocytes <30 × 10<sup>9</sup>/L, and LDT >12 mo (50). These patients are most unlikely to progress (approx 15% risk at 5 yr) and have life expectancies equivalent to age-matched controls without CLL.

Various groups have suggested that the pattern of bone-marrow infiltration in CLL has prognostic importance. In a retrospective analysis, Rozman et al. found that patients with a non-diffuse pattern of infiltration had a better survival rate than patients with a diffuse pattern (51). However, the difference was only significant in those patients with stage B disease. Similarly, in a prospective analysis of untreated patients, Desablens et al. found no difference in survival in patients with stage A or B disease with diffuse and non-diffuse infiltration (52). Therefore, although there are somewhat contradictory findings, it would appear that the prognostic significance of the pattern of bone-marrow infiltration adds little to that supplied by clinical stage alone. Indeed, it seems probable that diffuse marrow infiltration is strongly associated with advanced stage itself, and this in turn defines prognosis. Other variables, such as the levels of soluble intercellular adhesion molecule-1 (ICAM-1) or soluble CD23, have been reported to have prognostic importance, but their contribution, beyond that supplied by clinical stage, remains uncertain (53,54). Of these biological variables, serum  $\beta$ 2-microglobulin ( $\beta$ 2M) may well be the most important, since high levels have been associated with shortened survival within each of the three modified Rai clinical stages (55). Further large prospective studies are required to formally assess the significance of these biological variables, and are not presently used to routinely guide therapeutic strategies.

The importance of cytogenetic abnormalities is addressed in the preceding section 'Cytogenetic Abnormalities in CLL.' Perhaps the most significant development in our current understanding of the biology of CLL is that there appear to be two distinct types of CLL, one with unmutated and the other with mutated Ig V<sub>H</sub> genes (16,17). These findings have been discussed previously, but are likely to have major importance in defining prognosis for individual patients. Among patients with stage A disease, those with unmutated, pre-germinal-center CLL have a survival of 95 mo whereas those with mutated, post-germinal-center have a survival of 293 mo (16). The finding that there are two variants of CLL, as defined by Ig V<sub>H</sub> gene mutation status, has potentially major implications. It is imperative to determine the importance of mutation status for prognosis within each clinical stage and to dissect out its relationship with other cytogenetic and molecular abnormalities as well as with response to therapy. Mutation status is likely to become increasingly important as a prognostic indicator and to direct new therapeutic strategies—particularly for younger patients with early-stage disease.

In summary, at the present time it is fair to say that clinical stage is overwhelmingly the most important prognostic indicator in CLL. No other disease-related variables have found a universally applicable role in predicting outcome. However, the fundamental observation that Ig V<sub>H</sub> gene-mutation status divides CLL into two distinct disease groups is likely to have far-reaching implications for the understanding and management of CLL.



## THE TREATMENT OF CLL

In the 1950's, chlorambucil was first shown to have activity in CLL by reducing the lymphocyte count, improving bone-marrow function, and reducing the size of the lymph nodes, liver, and spleen (56). This drug, with or without prednisolone, remains the most commonly used agent in CLL, despite a large number of trials of alternative and combination regimes. Response rates, which have been variably defined, range from 38–75% (57) and although small studies have suggested a higher response rate with the combination of chlorambucil and prednisolone (58), there is no clear evidence to suggest that the addition of a steroid is beneficial. Indeed, although chlorambucil can relieve symptoms related to progressive CLL, there is no definitive proof that it actually prolongs survival. Chlorambucil has been used in a variety of ways, including continuous daily therapy, and as pulsed or intermittent high-dose schedules. Again, no advantage to any particular regime is evident (57).

### *Treatment of Early CLL*

One important question is whether therapy should be instituted in the early stages of the disease, before clear symptomatic indications for treatment have appeared. Although initial randomized studies had already demonstrated no benefit from early treatment (59), the definitive trials investigating the utility of early treatment were performed by the French Cooperative Group on CLL (60,61). Two consecutive trials investigated daily chlorambucil and pulsed chlorambucil with prednisolone vs no treatment. In all, 1535 patients were treated, with follow-up periods of 11 and 6 yr, respectively. Although treatment with chlorambucil delayed the time to disease progression, there was no difference in overall survival between the treated and untreated groups. Importantly, 49% of the untreated group did not progress or require therapy on prolonged follow-up of up to 11 yr or more.

These results clearly demonstrate that early treatment does not prolong survival of stage A patients. Furthermore, exposure to alkylating agents may result in unwanted complications, such as myelodysplasia or acute myelogenous leukemia (AML). In the first French trial, an increased rate of secondary epithelial cancers was noted, although the second and successive studies have not confirmed this finding. It is also possible that early exposure to chlorambucil may result in acquired drug resistance, which would result in reduced efficacy of therapy at a future date when it is required for symptomatic reasons.

### *Recommendations for the Initiation of Therapy in CLL*

It is generally accepted that patients with stage A disease should be monitored without intervention until there is evidence of disease progression. Most patients with stage C disease will benefit from therapy at diagnosis, although a minor-



Table 12  
NCIWG Criteria for Initiating Treatment on a Protocol

- 
1. A minimum of any one of the following disease-related symptoms must be present:
    - a. Weight loss >10% within the previous 6 mo
    - b. Extreme fatigue (cannot work or unable to perform usual activities)
    - c. Fevers >100.5° F for > 2 wk without evidence of infection
    - d. Night sweats without evidence of infection
  2. Progressive marrow failure: developing or worsening anemia or thrombocytopenia that is not autoimmune in nature
  3. Autoimmune anemia or thrombocytopenia that is poorly responsive to steroid therapy
  4. Progressive splenomegaly (>6 cm below the costal margin)
  5. Massive nodes or clusters (>10 cm in longest diameter) or progressive lymphadenopathy
  6. Progressive lymphocytosis with an increase of >50% over a 2 mo period or an anticipated LDT of less than 6 mo
- 

ity—as with many patients with stage B disease—can simply be monitored without treatment until progressive or symptomatic disease develops. If there is doubt about the need for intervention, it is advisable to delay therapy and to review the situation after 1–2 mo of observation.

The NCI-sponsored Working Group has published clear guidelines for the definition of progressive or active disease, and these should be followed in clinical trials so that comparative analyses between studies are possible (32,33) (Table 12). They can also be applied to general clinical practice, although each case must be considered based on its own merits.

### ***Treatment of CLL with Alkylator-Based Multi-Agent Therapies***

A number of clinical trials have attempted to determine whether combination regimes are more effective than single-agent chlorambucil in the treatment of advanced-stage or progressive CLL. In 1985, Montserrat et al. published the results of a randomized study comparing chlorambucil and prednisolone with cyclophosphamide, vincristine, and prednisone (COP) (62). Although the response rate was higher for the chlorambucil arm in this study (59% vs 31%,  $p < 0.01$ ), there was no difference in overall survival. Similar results were observed by the French Cooperative Group, in which patients with stage B disease were randomized to treatment with chlorambucil or COP (63). In this study, there was no difference in the overall median survival of approx 5 yr. Again, an ECOG randomized trial of chlorambucil and prednisolone vs (COP) showed equivalent response rates (72% vs 82%) and survival (64).

The addition of an anthracycline has also been investigated. An early report suggested that mini-CHOP was superior to COP in stage C patients (65). Overall 3-yr survival was significantly higher in the CHOP arm (71% vs 28%), which resulted in early closure of the trial. However, this was a small study, and the outcome of patients in the COP arm was unusually poor. Subsequently, two further studies comparing CHOP with chlorambucil and prednisolone failed to show any advantage with CHOP, although in both the response rate was higher in the anthracycline-containing arm (66,67).

Thus, a recurring theme is that although these more intensive, multi-agent regimes result in higher response rates, they fail to improve survival. This conclusion is confirmed by a meta-analysis of 10 randomized trials comparing chlorambucil with a variety of combination chemotherapies (68). Indeed, there is no compelling evidence to suggest that any particular combination of chlorambucil and prednisolone, COP, or CHOP, offers any major advantage as the initial treatment for CLL.

### *Treatment with Purine Analogs*

Of the three purine analogs currently available, fludarabine has been used most extensively in the treatment of CLL. The pivotal phase II studies were performed at the MDACC, and reported response rates of 38–57% in previously treated patients and 78% in treatment naïve patients (69–71). These single-center results suggest that fludarabine is the most active single agent currently available for the treatment of CLL. Many of the practical difficulties associated with the conventional intravenous (iv) administration of fludarabine can be overcome with the recently developed oral form of the drug (72). However, it remains to be seen whether it is equally effective when administered in this way. There is less clinical experience with 2-CDA and deoxycytosine in CLL, and no comparative trials with fludarabine have been performed.

Fludarabine is metabolized to fluoro-ara-A that is resistant to the enzyme adenine deaminase, which is found at high levels within T- and B-cells. The phosphorylated metabolite accumulates within cells, inhibits DNA and RNA synthesis, inhibits ribonucleotide reductase, and results in apoptosis (73). The drug causes significant T-cell depletion, with the greatest effect on CD4+ T-cells. Thus, the drug leads to significant immunosuppression, and indeed, this effect has been successfully used in non-myeloablative conditioning regimes for allogeneic stem-cell transplantation (74). This effect has led to concerns about the potential for a high incidence of infective complications following fludarabine treatment, particularly with pathogens associated with cellular immunodeficiency, such as *Pneumocystis carinii* and varicella zoster. However, it is likely that only the combination of fludarabine with steroids has a significant effect in this regard (69).

Table 13  
Results of the European Trial of Fludarabine vs CAP Chemotherapy

|                                      | <i>Fludarabine</i> | <i>CAP</i> |              |
|--------------------------------------|--------------------|------------|--------------|
| <i>Previously untreated patients</i> |                    |            |              |
| CR                                   | 23%                | 17%        | NS           |
| Overall RR                           | 71%                | 60%        | NS           |
| Median response duration             | NYR                | 6.9 mo     | $p < 0.0001$ |
| Median OS                            | NYR                | 52.5 mo    | $p = 0.087$  |
|                                      | <i>Fludarabine</i> | <i>CAP</i> |              |
| <i>Previously treated patients</i>   |                    |            |              |
| CR                                   | 13%                | 6%         | NS           |
| Overall RR                           | 48%                | 27%        | $p = 0.036$  |
| Median response duration             | 10.8 mo            | 6 mo       | NS           |
| Median OS                            | 24.3 mo            | 24.4 mo    | NS           |

Three major randomized, prospective phase III trials have compared fludarabine with conventional treatments. The European Trial randomized a total of 196 patients with either untreated stage B or C disease or relapsed disease to treatment with 6–10 cycles of fludarabine or CAP chemotherapy (75). Fludarabine was better tolerated, with significantly less nausea, vomiting, and alopecia than in the CAP arm. Furthermore, there was no difference in the infection rate between the two arms, although fludarabine had to be stopped in 5% of patients because of the development of autoimmune cytopenias. Overall, the response rate was higher for fludarabine-treated patients (60% vs 44%,  $p = 0.023$ ). Results for the two groups of patients are shown in Table 13. Fludarabine resulted in a higher response rate in previously treated patients and prolonged remission duration in previously untreated patients, with a trend toward prolonged overall survival in this group.

The second ‘French’ study compared six cycles of treatment with fludarabine vs ChOP (mini-CHOP) or CAP in 938 previously untreated patients with stage B or C disease (76). The CAP arm was closed early because of a significantly reduced response rate, and the interim results as published in abstract form are summarized in Table 14. Treatment with fludarabine resulted in higher initial response rates, but this was not translated into longer progression-free or overall survival. Notably, there was no increase in the rate of autoimmune cytopenia in the fludarabine arm.

The third study of this type, conducted by the US Intergroup, compared fludarabine with chlorambucil in previously untreated patients (77). A third arm with combined fludarabine and chlorambucil was stopped early because of

Table 14  
Preliminary Results of the French Trial of Fludarabine vs CHOP vs CAP

| <i>Fludarabine</i> | <i>CHOP</i> | <i>CAP</i> |       |                    |
|--------------------|-------------|------------|-------|--------------------|
| RR                 | 41%         | 30%        | 15%   | $p = 0.006/0.0001$ |
| PFS                | 30 mo       | 28 mo      | 27 mo | NS                 |
| Median OS          | 74 mo       | 68 mo      | 70 mo | NS                 |

Table 15  
Results of the US Intergroup Study of Fludarabine vs Chlorambucil

|           | <i>Fludarabine</i> | <i>Chlorambucil</i> |             |
|-----------|--------------------|---------------------|-------------|
| CR        | 20%                | 4%                  | $p < 0.001$ |
| RR        | 63%                | 37%                 | $p < 0.001$ |
| PFS       | 20 mo              | 14 mo               | $p < 0.001$ |
| Median OS | 66 mo              | 56 mo               | NS          |

excess toxicity. A total of 509 patients with Rai stage III/IV or I/II with active disease were treated for up to 12 monthly cycles of therapy. The chlorambucil was given monthly at a dose of 40 mg/m<sup>2</sup>. The results of this study are summarized in Table 15. Patients with primary treatment failure were allowed to cross over to the other arm. For chlorambucil treatment failures, there was a subsequent 46% response rate to fludarabine, whereas for patients who failed fludarabine, only 7% responded to chlorambucil. The authors conclude that fludarabine resulted in higher complete and overall response rates and in prolonged response duration. However, this did not result in a prolongation of overall survival, perhaps because of the relative success of fludarabine as salvage therapy in patients who failed chlorambucil.

What are the general conclusions that can be drawn from these three studies? First, fludarabine results in a higher response rate and longer response duration than alkylator-based regimes in previously untreated patients with CLL. However, this superiority has not resulted in prolonged survival, and this is a result—at least in part—to the clear efficacy of fludarabine as a salvage therapy. Yet, the achievement of higher response rates with fludaribine is highly encouraging, and is stimulating further clinical investigation designed to improve on this success. Such strategies include the use of fludarabine in combination with other agents, and the use of high-dose therapy with stem-cell support to consolidate remission.

### ***Combination Therapy with Fludarabine and Cyclophosphamide***

In vitro studies have indicated that the combination of cyclophosphamide and fludarabine is likely to be synergistic. Normally, the DNA interstrand crosslinks induced by alkylating agents are rapidly repaired. However, the addition of

Table 16  
New Therapeutic Targets in CLL

| <i>Target</i>                      | <i>Example of drug</i>    |
|------------------------------------|---------------------------|
| Histone deacetylase inhibition     | Depsipeptide              |
| Protein kinase C inhibition        | Staurosporine derivatives |
| Protein kinase C activation        | Bryostatins               |
| Cyclin-dependent kinase inhibition | Flavopiridol              |
| Phosphodiesterase inhibition       | Theophylline              |
| Anti-angiogenesis                  | Thalidomide               |
| Proteasome inhibition              | PS-341                    |

fludarabine results in significant inhibition of crosslink repair, presumably because of its ability to directly inhibit DNA repair enzymes (78,79). Two single-center studies have investigated the clinical efficacy of this combination in vivo.

Flinn et al. studied the combination of cyclophosphamide (600 mg/m<sup>2</sup> on d 1) with fludarabine (20 mg/m<sup>2</sup> on d 1–5) supported by granulocyte-colony-stimulation factor (G-CSF) in a variety of previously untreated indolent lymphoid malignancies. For the 17 patients with CLL, 51% and 41% achieved a complete remission (CR) and partial remission (PR) respectively (80). A larger number of patients with CLL at various stages have been treated at the MDACC with a slightly different regime comprised of cyclophosphamide at doses ranging from 300–500 mg/m<sup>2</sup> for 3 d and fludarabine at 20 mg/m<sup>2</sup> for 3 d (81). Excess toxicity was observed at the higher cyclophosphamide doses, and the majority of patients were therefore treated at the lowest dose level. For the previously untreated patients, the total response rate (RR) was 88%, with 35% achieving CR. Although the CR rate was not significantly higher than the 27% rate observed with fludarabine as a single agent in the Intergroup Study (77), a potentially important observation was that only 8% of the patients with CR had detectable CD5+ B-cells in the marrow by flow cytometry. The suggestion is that the combination regime results in a better quality of remission in responding patients.

The potential value of these findings has not yet been established in prospective randomized studies. Certainly, the ability to induce remissions associated with the elimination of disease by the most sensitive techniques is encouraging. Indeed, such responses are likely to be the most favorable platforms from which to launch high-dose therapies with the intent of cure, since such approaches are known to be most effective in the setting of minimal disease.

### ***Potential New Therapies for CLL***

A variety of biological therapies are currently under development for the treatment of CLL. The underlying concept is that these agents will interfere with molecular or biological targets that are operative in CLL. It is beyond the scope

of this chapter to review these promising agents in any detail, but further information can be found in a recent comprehensive review (82). Examples of such potential targets and drugs are provided in Table 16.

Two monoclonal antibodies (MAbs) have been examined in CLL. Anti-CD20 has shown only marginal activity when administered in a conventional fashion (375 mg/m<sup>2</sup> weekly for 4 wk) with a response rate of only 12% (83). Intuitively, this is not surprising, since CD20 is expressed only at low levels by CLL cells. Attempts to overcome this problem have included using the drug at significantly higher concentrations or with a 3x weekly dosage regime (84,85). Although high response rates have been observed, the considerable cost associated with such strategies means that this approach is unlikely to be widely applicable.

Anti-CD52 (CAMPATH-1H) has shown promise in CLL, with response rates of 42% in previously treated patients (86). Notably, responses were more marked in blood and bone marrow than lymph nodes. In a large pivotal study (CAM211), patients with fludarabine-refractory CLL showed a 33% response rate (87). CD52 is expressed by T- and B-lymphocytes as well as by monocytes. A major concern is that its use will result in clinically significant immunosuppression. Indeed, in a series of 56 treated patients, 7% experienced CMV reactivation, and one death resulted (88).

How these new treatments will impact on the treatment of CLL remains uncertain, and there is a clear need for well-planned trials investigating their use both as single agents and in combination with established therapies. Nevertheless, it is highly encouraging that these new agents under development offer distinct modes of action and considerable potential for the future.

## AUTOLOGOUS STEM-CELL TRANSPLANTATION IN CLL

Autologous stem-cell transplantation can be curative in a proportion of patients with relapsed Hodgkin's disease and aggressive non-Hodgkin's lymphomas. These results have encouraged the investigation of this treatment strategy in CLL, with conflicting results. The MDACC experience was that, in a group of heavily pretreated patients, transient remissions at best were achieved following high-dose therapy with stem-cell rescue (89). However, investigators at the DFCI, have reported superior results in a group of 12 patients with poor-prognosis CLL (90). Ten of 12 patients achieved a CR after autografting with cyclophosphamide/total body irradiation (TBI) conditioning. These patients were generally treated earlier in the natural history of their disease, and were treated with conventional chemotherapy to a state of complete remission or minimal disease prior to high-dose therapy. The bone-marrow harvests were also purged with a cocktail of anti-B-cell MAbs. Of the first five patients who achieved a CR, all remained in CR for a median of 25 mo (range 6–31 mo). Perhaps most importantly, all three patients with

prolonged follow-up showed the absence of a clonally rearranged immunoglobulin gene by Southern blot analysis.

A retrospective analysis of 321 patients with CLL who have undergone autologous transplantation has been performed by the EBMT (91). Overall, there was a transplant-related mortality rate of 6%. Improved survival was correlated with a shorter interval from diagnosis to transplant, CR status at the time of transplant, and the use of TBI in the conditioning regime. No benefit was observed from *in vitro* purging.

In summary, there are indications that high-dose therapy with autologous stem-cell transplantation results in molecular remissions in CLL, and it is possible that these favorable responses will result in long-term survival. It is also evident that this approach is most likely to be of benefit early in the course of the disease, and in the presence of minimal residual disease at the time of transplant. Important questions remain concerning the role of purging, the most appropriate conditioning regime, patient selection, and, most significantly, the impact on overall survival. These questions can only be answered definitively in prospective randomized trials.

## **THE ROLE OF ALLOGENEIC STEM-CELL TRANSPLANTATION (ALLO SCT) IN CLL**

In the light of the success of Allo SCT in a variety of hematological malignancies, this treatment modality has been investigated in patients with CLL. Retrospective analyses of conventional ablative transplants have been reported, the largest from the EBMT/IBMTR (89,90,92,93). The majority of patients in these series had advanced, often chemorefractory, disease and transplant-related mortality ranged from 35–46%. Nevertheless, overall survival ranged from 46% to 62% at 3–5 yr. Furthermore, in those cases that were analyzed, molecular remissions were often achieved at intervals greater than 6 mo following transplantation, suggesting that a graft vs leukemia effect (GVL) may be operative in CLL (89). The most convincing evidence that a GVL effect is operative in hematological malignancies derives from observing responses to the cessation of immunosuppressive therapy or the administration of donor lymphocyte infusions in the setting of persistent or relapsing disease following Allo SCT (94). There are only anecdotal reports of such responses for CLL, and in all cases, GVL has been associated with GVHD (95–97). Therefore, it remains to be seen whether there is a specific GVL effect in CLL that can be separated from GVHD.

In an update of the MDACC series, which included ablative and non-ablative transplants, the 3 yr overall and disease-free survivals were 47% and 34%, respectively (98). The outcome was significantly better for patients with chemosensitive disease and for those who had received fewer lines of chemotherapy. Of particular interest is the observation that survival following non-



ablative transplants was equivalent to that following conventional SCT. In view of the reduced toxicity of such transplants, this treatment should be investigated in both younger and older patients with CLL.

At present, it can be concluded that the precise role of Allo SCT in CLL remains uncertain. There is only limited evidence to support the existence of a GVL effect in CLL, and further information is required concerning the efficacy of donor lymphocyte infusions to clarify this issue. As is the case with many hematological malignancies, it is likely that Allo SCT will be most effective in early-stage chemosensitive disease, but these issues must be formally addressed in prospective studies.

### THE TREATMENT OF YOUNGER PATIENTS WITH CLL

The management of younger patients presents special difficulties, because life expectancy is likely to be reduced by up to 20 yr overall in this subgroup of patients with CLL (99). Estimates of the proportion of younger patients with CLL have varied from 6–20% with youth usually defined as less than 55 yr of age (100,101). A representative series of 204 younger patients showed that, in general, the presenting features and response to therapy were similar to those in older patients. Of this group, 34% had smoldering CLL and an identical median overall survival of 10 yr (101). However, an important point is that, for younger patients, death was much more likely to result from CLL itself rather than any other extraneous comorbidity. In this series, younger patients with CLL had a higher male-to-female ratio than older patients (2.85 vs 1.29) and a greater risk of developing Richter's syndrome (5.9% vs 1.2%), although other reports have not confirmed these differences (102). In addition, only dynamic parameters such as active disease (as defined by the NCI criteria) and a short LDT were prognostically significant. In contrast to older patients, on multivariate analysis, the stage of disease had no prognostic importance. Two subgroups were easily identifiable—60% of younger patients had progressive disease with a median survival from the time of initial therapy of only 5 yr. The remaining 40% had long-lasting stable disease, which did not require therapy and was associated with 94% survival at 12 yr from diagnosis.

This study and others clearly indicate that young patients with CLL who do not have conventional indications for treatment should be managed expectantly. However, for the group with progressive disease, innovative therapies with or without stem-cell transplantation are appropriate within the context of clinical trials.

### AUTOIMMUNE DISEASE AND ITS TREATMENT IN CLL

A characteristic feature of CLL is the frequent development of both autoimmune disease and immunodeficiency. The autoimmune phenomena that occur in CLL are largely restricted to hemopoietic tissues with autoimmune hemolytic



anemia (AIHA), immune thrombocytopenia (ITP) and pure red-cell aplasia (PRCA) observed with decreasing frequency (103). The intriguing co-existence of immunodeficiency with autoimmunity is also observed in a number of other primary and acquired immunodeficiency states, such as common variable immunodeficiency, X-linked immunodeficiency with hyper IgM, and HIV infection. The hyper IgM syndrome is caused by inherited defects of the gene for CD40-ligand (CD154) and is characterized by profound immunodeficiency and a greatly increased risk of immune hematological abnormalities (104). The interaction of CD154 with CD40 present on antigen-presenting cells (APC) is required for the generation of normal secondary immune responses that are characterized by isotype-class switching and somatic hypermutation. Intriguingly, CD4+ T-cells from patients with CLL appear to have an acquired CD154 deficiency and fail to express this immunoregulatory molecule after CD3 ligation. Furthermore, the addition of CLL cells results in decreased CD154 expression in vitro by normal activated CD4+ T-cells (105). These similarities between the hyper IgM syndrome and CLL suggest that the acquired CD154 deficiency characteristic of CLL is likely to be central to the immunodeficiency and autoimmunity observed in CLL. Furthermore, transduction of CLL cells with CD154 may be a novel strategy for circumventing the defective immune responses seen in CLL, and may allow for restoration of effective anti-tumor activity (106).

Whatever the case, AIHA is the most common immune disorder found in CLL, and its occurrence poses particular challenges to management. Highly variable incidence rates for AIHA have been observed, ranging from 3–37% (103). It has been reported that AIHA may be related to advanced-stage disease, and indeed, the NCI guidelines suggest that AIHA or ITP that is poorly responsive to steroid therapy is indicative of active disease, and is thus an indication for the initiation of chemotherapy (32,33). However, it is important to emphasize that in the vast majority of cases AIHA is caused by polyclonal autoantibodies that are not the product of the CLL clone itself (107). Rather, they are the result of the disturbed immunoregulatory pathways that are characteristic of CLL, particularly the imbalance and deficiency of CD4+ T-cell subsets. In this regard, it has long been noted that AIHA can be triggered by the initiation of chemotherapy, whether this is in the form of alkylating agents (108) or purine analogs (109,110). A number of reports have suggested that fludarabine is especially likely to result in AIHA and in the European Cooperative Study autoimmune cytopenias developed in 5% of patients of patients treated with fludarabine, but none treated with CAP (75).

Mauro and colleagues retrospectively analyzed their large cohort of patients with CLL for the occurrence of AIHA (111). AIHA developed in 4.3% (52 of 1203) of their patients, and in 90% of cases was associated with active CLL, although 75% of instances occurred in previously untreated cases of CLL. Using

multivariate analysis, AIHA was associated with increasing age, male sex, and a high lymphocyte count. By itself, AIHA had no detrimental effect on survival. It is clear from this large study that AIHA is associated with CLL itself, and most cases occur prior to therapy. Furthermore, there was no difference in frequency between patients treated with chlorambucil and fludarabine, although both groups received concurrent prednisolone in this study. Therefore, it seems likely that the triggering effect of drugs such as fludarabine, which are themselves potent immunosuppressive agents, results from a further disturbance of immunoregulatory mechanisms in addition to those caused by the disease itself.

The relative rarity of autoimmune blood disorders associated with CLL means that most therapeutic strategies are informed by largely anecdotal reports, and there is a distinct lack of evidence-based guidelines to assist the physician. Nevertheless, the generally accepted practice is to treat coexistent AIHA, ITP, and PRCA in a similar manner to that adopted for idiopathic cases (103). Thus, the mainstay of treatment remains the use of steroids in conventional doses with the use of immunosuppressive agents as second-line agents. Similarly, there is not enough evidence to provide firm guidelines about the efficacy of splenectomy in such cases. Our practice is to treat autoimmune disorders in their own right and to reserve specific CLL-directed therapies for patients who have alternative conventional indications for therapy or who fail standard immunomodulatory treatments. The increased risk of opportunistic infections is a particular concern in patients treated with steroids and other immunosuppressive agents, and careful clinical evaluation of this group of patients is warranted. The role of prophylactic antimicrobials remains unproven, and indeed, in the series reported by Mauro et al., prophylactic cotrimoxazole, did not improve survival (111). In summary, despite the introduction of new therapies for CLL, the disease remains incurable. There is an urgent need to assess treatment strategies with the aim of curing patients with CLL.

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