Chronic Lymphocytic Leukemia and Associated Disorders

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Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western hemisphere, accounting for 30% of the leukemias in this population. The disease results from a clonal expansion of small B-lymphocytes. CLL always involves the bone marrow and peripheral blood. The disease also can be demonstrated in lymph nodes, liver, and spleen.

Introduction

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western hemisphere, accounting for 30% of the leukemias in this population. The disease results from a clonal expansion of small B-lymphocytes. CLL always involves the bone marrow and peripheral blood. The disease also can be demonstrated in lymph nodes, liver, and spleen. Bone marrow failure may occur as a late event. Staging systems (Rai and Binet) have been developed that correlate with survival, but there is still significant heterogeneity within subgroups. Cytogenetic and molecular analysis may provide information on disease development and prognosis. New therapeutic modalities such as nucleoside analogs and bone marrow transplantation have improved response rates in CLL and create expectations about potential cure of this disease.

Epidemiology

The incidence varies around the world, being more common in the Western hemisphere; in the United States, CLL constitutes 20% of all leukemias, whereas in Asiatic countries, like Japan, it accounts for only 2.5% [1]. The incidence is also age dependent [2], with an increase from 5.2 per 100,000 persons older than 50 years to 30.4 per 100,000 in people older than 80 [3]. The male to female ratio is 2:1. An increase in the incidence of CLL in the last 50 years was suggested by a recent study in a Minnesota population [4], but was considered due to improved diagnostic techniques [5].

Etiology

B-cell CLL (B-CLL) is the only leukemia that has not been associated with radiation exposure, chemicals or drugs [6-8]. On the other hand, an increased risk in relatives of patients with CLL has been found [9-15] that is between twofold to sevenfold higher than in a control population [11,16]. An increase in other lymphoid malignancies has been found as well [12]. Although the leukemic cells of family members sometimes express the same immunoglobulin (Ig) heavy-chain variable region gene [17], the cells of each patient have different Ig heavy-chain variable region genes [13,17,18]. Mitogens or phorbol myristate-acetate (PHA) can induce proliferation of B-CLL cells in vitro; with the
use of G-banding and Q-banding techniques, almost 50% of the leukemic cells of CLL patients have been found to have clonal chromosomal abnormalities [19-22]. The most common cytogenetic abnormalities involve chromosomes 12, 13, and 14. Abnormalities involving chromosomes 6 and 11 are found less often.

**Chromosome 12 Anomalies**
The most common anomaly associated with CLL is trisomy 12 [20,23-25], which was found in 67 (17%) of 391 evaluable CLL patients in a study by Juliusson et al [24]. This trisomy can be found as the only anomaly in B-CLL but is often found with other chromosomal abnormalities. The presence of a complex karyotype may indicate clonal evolution [26,27]. Trisomy 12 may be also detected by using fluorescent in situ hybridization (FISH) [28-31]. Escudier et al found that FISH is more sensitive in detecting this chromosomal abnormality than conventional cytogenetics [30], but others did not demonstrate better sensitivity by FISH [28]. The significance of trisomy 12 as regards prognosis in B-CLL is controversial; some authors report a poor prognosis and advanced disease in patients with the anomaly [27,30] or the presence of lymphocytes with a prolymphocytic-like morphology [29]. Other authors have not found a worse prognosis with this anomaly [26,29].

**Chromosome 13 Anomalies**
Structural anomalies of chromosome 13 were found in 51 (13%) of 391 patients in the study done by Juliusson et al [24], some of them involving the site of the retinoblastoma 1 gene (RB1 gene). This anomaly confers a better outcome than trisomy 12 abnormalities, but is worse than diploid cytogenetics [24].

**Chromosome 14 Anomalies**
Structural anomalies of chromosome 14 were found in 41 (10.5%) of 391 patients by Juliusson and colleagues [24]. Ten of those patients had t(11;14)(q13;q32). This translocation involved rearrangement of a proto-oncogene called **BCL-1** for B-cell leukemia-1 [46,47], and called later **PRADI**, a proto-oncogene involved in the pathogenesis of mantle-cell lymphoma. B-CLL cases with BCL-1 rearrangement may represent the leukemic phase of mantle-cell lymphoma. This translocation involves chromosome 14 at band q32 that includes the Ig heavy chain locus. Other less frequent chromosomal anomalies include t(14;18)(q32;q21), t(14;19)(q32;q13.1) with high expression of the BCL-2 protein in the first case and expression of the proto-oncogene **BCL-3**. Most studies agree on the poor prognosis of either a 14q+ anomaly [24,34] or a complex chromosomal abnormality [24,34].

**Surface Antigen Phenotype**
Freedman et al [35] studied the immunophenotype of 100 B-CLL patients and found that all cases expressed Ia, CD19, and CD20 (pan B-cell antigens). CD5, an antigen present on mature T-cells, was found in 95% of cases. In 90% of cases, CLL cells expressed the Epstein-Barr Virus (EBV) and CD21 (C3d complement) receptors. Surface immunoglobulin expression (sIg) was weakly expressed in 90% of CLL cases. The most common isotype was IgM plus IgD, seen in half of the patients, followed by IgM alone. The light chains were either k or l type. Expression of sIg is important for assessing the clonality of a lymphoid population. In addition, clonality can be proved by the detection of a specific cytogenetic abnormality and rearrangement of Ig heavy and light chains.

**Cell of Origin**
B-CLL cells may derive from a small subclone of normal, activated B-cells that develop clonal expansion by a mechanism that is not well understood. Those cells express the antigens described above. CD5+ B-cells are found in the periphery of the germinal centers of lymph nodes in the adult.

**Clinical Characteristics and Laboratory Findings**
At diagnosis, most patients are older than 60 years old, with more than 90% over 50 years. The diagnosis of CLL is often made incidentally, when an elevated absolute lymphocyte count (ALC) is found at the time of a complete blood count. Other patients may present with autoimmune disorders such as autoimmune hemolytic anemia (AHA) or autoimmune thrombocytopenia (ATP). Presenting symptoms may include infections, fatigue, malaise, or, rarely, B-symptoms. Physical examination may reveal cervical, axillary, or inguinal lymphadenopathy. Splenomegaly and hepatomegaly are also common.

Laboratory findings invariably show lymphocytosis. The ALC can range from 5,000 to 500,000/µL. During smear preparation, abnormal lymphocytes are frequently damaged resulting in “smudge” cells. The degree of infiltration of the bone marrow varies between 30% and 99%, with a diffuse or nodular pattern of infiltration. The number of erythroid, myeloid and megakaryocytic precursors may be normal or decreased. Patients can present with anemia due to bone marrow infiltration or AHA. Pure red-cell aplasia has been described in 1% to 6% of the patients [36]. Other features include thrombocytopenia due to hypersplenism, bone marrow failure, or on an
autoimmune basis. Patients may develop panhypogammaglobulinemia that progresses in frequency and severity with advancing disease [37]. Monoclonal gammopathy is also seen and the frequency varies according to the method used for diagnosis. Other laboratory findings include an increase in beta-microglobulin, and, rarely, serum lactate dehydrogenase (LDH) and hypercalcemia.

Diagnosis

Diagnostic criteria were proposed by the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) in 1989 and are summarized in Table 1 [38]. An ALC of 10,000/µL or higher sustained for at least 4 weeks and involvement of 30% or more of the bone marrow with lymphocytes or evidence of clonality by immunophenotype is required. The National Cancer Institute-sponsored CLL Working Group (NCIWG) only required an ALC of 5,000/µL when the IWCLL bone marrow and clonality criteria are met [39].

<table>
<thead>
<tr>
<th>NCI WORKING GROUP</th>
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<tr>
<td>Lymphocytes &gt; 5,000/µL</td>
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<tr>
<td>&quot;Atypical&quot; cells &lt; 55%</td>
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<tr>
<td>Duration of lymphocytosis ≥ 2 months</td>
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<td>Bone marrow lymphocytes ≥ 30%</td>
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<table>
<thead>
<tr>
<th>IWCLL</th>
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<tr>
<td>Lymphocytes &gt; 10,000/µL and either B phenotype or bone marrow involvement</td>
</tr>
<tr>
<td>Lymphocytes &lt; 10,000/µL and both bone marrow involvement + B phenotype</td>
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<tr>
<td>Bone marrow lymphocytes &gt; 30%</td>
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Morphologically, the lymphocytes are small and mature in appearance. When the number of larger, prolymphocyte-like cells is greater than 10% but less than 55%, this has been considered a variant between CLL and prolymphocytic leukemia (PLL) and classified by the French-American-British group [40] and the NCIWG [39] as CLL/PLL. If more than 55% of the lymphocytes are prolymphocytes, the diagnosis is PLL.

Differential Diagnosis

Clinical, morphologic, immunophenotypic and cytogenetic methods help to make the differential diagnosis between B-CLL and and other diseases such as T-cell CLL (T-CLL), leukemic phase of non-Hodgkin’s lymphoma (mantle cell, follicular and others), other mature B-cell lymphoproliferative disorders such as PLL, hairy-cell leukemia (HCL) and its variants, splenic lymphoma with villous lymphocytes (SLVL) and Waldenström's macroglobulinemia (WM) that can be confused with CLL. Table 2 summarizes the immunophenotypes in the differential diagnosis of these disorders.

<table>
<thead>
<tr>
<th>CD19</th>
<th>CD20</th>
<th>Sig</th>
<th>CD5</th>
<th>CD10</th>
<th>CD11c</th>
<th>FMC7</th>
<th>CD25</th>
<th>TRAP</th>
<th>CD23</th>
</tr>
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<tbody>
<tr>
<td>B-CLL</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-PLL</td>
<td></td>
<td>+</td>
<td>±</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td>Mantle-cell lymphoma</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Follicular lymphoma</td>
<td>+</td>
<td>+</td>
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Staging and Prognosis

The Rai [41] and Binet [42] are the most commonly used staging systems for CLL (Tables 3 and 4). Both systems evaluate the tumor burden by lymphadenopathy, hepatomegaly, splenomegaly, and bone marrow failure by anemia and thrombocytopenia. The original Rai system included five stages from 0 to 4; this has been modified to 3 stages by defining Rai stage 0 as low-risk, joining stages 1 with 2 to form an intermediate-risk group, and stages 3 with 4 to be a high-risk group, with a median survival of > 12.5, 7, and 1.5 years for each risk group respectively [43]. The Binet staging system comprises three categories: A, with two or fewer lymphoid-bearing areas (LBA); B, with three or more LBA; and C, with a hemoglobin (Hb) < 10 g/dL and/or thrombocytopenia (100,000 platelets/µL); with a median survival of > 10, 6, and 2 years respectively. Although both systems have correlated with survival in several prospective studies [44-47], they fail to identify which patients in a given stage will have disease progression.

Table 3. Rai Staging System for Chronic Lymphocytic Leukemia

<table>
<thead>
<tr>
<th>Stage</th>
<th>Modified stage</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>Low risk</td>
<td>Lymphocytosis only</td>
</tr>
<tr>
<td>1</td>
<td>Intermediate risk</td>
<td>Lymphocytosis and lymphadenopathy</td>
</tr>
<tr>
<td>2</td>
<td>Intermediate risk</td>
<td>Lymphocytosis and splenomegaly with and without lymphadenopathy</td>
</tr>
<tr>
<td>3</td>
<td>High risk</td>
<td>Lymphocytosis and anemia (hemoglobin &lt; 11g/dL), with or without lymphadenopathy or hepatosplenomegaly</td>
</tr>
<tr>
<td>4</td>
<td>High risk</td>
<td>Lymphocytosis and thrombocytopenia (platelets&lt; 100,000/µL), with or without anemia, lymphadenopathy or hepatosplenomegaly</td>
</tr>
</tbody>
</table>
Several authors have described alternate prognostic factors. Montserrat et al [48] showed that patients with a lymphocyte doubling time (LDT) of less than 12 months had a median survival, independent of the stage, of 5 years, and, if the LDT was more than 12 months, the survival was more than 12 years. Other authors have confirmed this finding [49]. Expression of the proliferating cell nuclear antigen (PCNA), an estimate of proliferative potential, has been found to correlate with the LDT [50]. Rozman et al [51] and Geisler et al [52] demonstrated that diffuse pattern of bone marrow infiltration was an independent poor prognostic factor in CLL. The term “smoldering CLL” has been proposed for a subgroup of patients who belong to Binet stage A, with a lymphocyte count < 30,000/µL, an LDT > 12 months, Hb level > 13 g/dL, and a nondiffuse pattern (interstitial, nodular, and mixed) of the bone marrow [53]. The risk of progression in 3 years for this group was 5%, compared with 32% in other patients for stage A. Monoallelic \( p53 \) gene deletion has been found to be a strong adverse prognostic factor for survival and response to purine analogs in CLL patients [53a]. Gaidano et al [53b] reported mutations of \( p53 \) in six (15%) of 40 cases of CLL and in three (43%) of seven patients with Richter's transformation. El Rouby and colleagues [53c] found \( p53 \) gene mutations in 15% of 53 patients with CLL. While 27 (93%) of 29 treated patients without \( p53 \) mutations responded to therapy, only 1 (14%) of 7 treated patients with \( p53 \) mutations achieved a partial remission. Other parameters with prognostic relevance in CLL patients are age and sex [44,54,55], lymphocyte count [53,56], serum LDH [44], serum albumin [57], free serum CD23 level [58], presence of prolymphocytes [59], absolute number of prolymphocytes > 15,000/µL [59], presence of myelomonocytic antigens on the lymphocytes [60], karyotype [24,61], \( \beta_2 \)-microglobulin [62], absolute number of white blood cells in S phase in the peripheral blood [63], and serum level of interleukin-2 (IL-2) receptors and CD8 antigen [64,65].

**Treatment**

The time at which treatment should be initiated in a potentially indolent disease is unclear. De Rossi et al [66] reported a retrospective study of 133 patients with a median age of 46 years old and Rai stage 0 disease. They had a 60% likelihood of surviving longer than 10 years. In contrast, older patients had a median survival of 6 to 7 years. Other investigators found a group of patients with survival similar to the general population [44,53,67,68]. Therefore, the treatment of patients in Rai stage 0 to 1 or Binet A is usually limited to those who present with severe hyperlymphocytosis or who have systemic symptoms such as fever, weight loss, night sweats, bulky disease, recurrent infections, immune mediated complications, LDT < 12 months or with diffuse infiltration of the bone marrow. Most investigators treat patients with Rai stage 3 or 4 or Binet stage C disease. The National Cancer Institute [39] and the IWCLL [38] have proposed uniform guidelines for response criteria, which are summarized in Tables 5 and 6.
### Table 5. National Cancer Institute Response Criteria for Chronic Lymphocytic Leukemia

<table>
<thead>
<tr>
<th>Response Level</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>Complete response</td>
<td>Absence of lymphadenopathy, hepatosplenomegaly, and constitutional symptoms; normalization of CBC (neutrophils &gt; 1,500/µL, platelets &gt; 100,000/µL, hemoglobin &gt; 11g/dL, lymphocytes &lt; 4,000/µL); bone marrow biopsy shows normal cellularity; lymphocytes &lt; 30%; nodules and infiltrates in the bone marrow are permitted. Duration of response &gt; 2 months.</td>
</tr>
<tr>
<td>Partial response</td>
<td>At least 50% reduction in absolute blood lymphocyte count and in lymphadenopathy and/or 50% reduction in splenomegaly or hepatomegaly; neutrophils &gt; 1,500/µL or 50% improvement over baseline; platelets &gt; 100,000/µL or 50% improvement over baseline; hemoglobin &gt; 11g/dL (not supported by transfusions) or 50% over baseline. Duration of response: &gt; 2 months.</td>
</tr>
<tr>
<td>Stable disease</td>
<td>No complete or partial response, or no progression.</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>At least one of the following: &gt; 50% increase in size of at least two lymph nodes, or new palpable lymph nodes; &gt; 50% increase in hepatomegaly or splenomegaly or appearance if previously absent; transformation to a more aggressive histology (Richter or PLL); &gt; 50% increase of absolute peripheral blood lymphocyte count.</td>
</tr>
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</table>


### Table 6. International Workshop on Chronic Lymphocytic Leukemia (IWCLL) Response Criteria

<table>
<thead>
<tr>
<th>Response Level</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response</td>
<td>Resolution of lymphadenopathy, hepatosplenomegaly, and constitutional symptoms; normalization of CBC (neutrophils &gt; 1,500/µL, platelets &gt; 100,000/µL, lymphocytes &lt; 4,000/µL); normalization of bone marrow findings (the presence of focal or nodular infiltrates is compatible with complete response).</td>
</tr>
<tr>
<td>Partial response</td>
<td>Change from stage C to A or B, or from stage B to A.</td>
</tr>
<tr>
<td>Stable disease</td>
<td>No change in the stage of the disease.</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>Change from stage A to B or C, or from stage B to C.</td>
</tr>
</tbody>
</table>


### Chlorambucil

A nitrogen mustard derivative, chlorambucil (Leukeran) is the drug most commonly used in CLL. Chlorambucil schedules include 0.1 mg/kg/d, 0.4 to 1 mg/kg every 4 weeks, and 0.4 to 0.6 mg/kg every 2 weeks, alone or with prednisone. The drug is given orally and has excellent gastrointestinal absorption.

Chlorambucil was first used by Galton [69] in 1961, at a dose of 0.03 to 0.3 mg/kg/d for 4 to 8 weeks. He reported that 77% of patients responded to some degree. Other investigators report response rates between 38 and 75% [70]. The variations in response are due to the use of different doses and schedules of administration, the use of corticosteroids in some studies, and different criteria for response.

Two small randomized studies compared daily chlorambucil vs chlorambucil plus prednisone [71,72], with a statistically significant difference in responses favoring the combination group. The 2-year survival was also better in the combination group but did not achieve statistical significance.

Sawitzky et al [71] from the Cancer and Leukemia Group B (CALGB) performed a three-arm study in patients with Rai stage 3 and 4 disease, randomizing between prednisone, prednisone plus daily chlorambucil, or intermittent chlorambucil plus prednisone. He obtained response rates of 11%, 37%, and 47%, respectively.

Jaksic et al treated 181 CLL patients [73], comparing intermittent chlorambucil plus prednisone vs chlorambucil, 15 mg/d, until remission or dose-limiting toxicity. The response rate was 90% (70%)
complete response [CR]) for chlorambucil alone vs 50% (31% CR) in the combination group. The total dose of chlorambucil in the daily-dose group was six times higher than in the intermittent group, showing that dose intensity is very important in alkylating agents.

The French Cooperative Group in CLL (FCGCLL) randomized 612 patients with Binet stage A CLL to daily-dose chlorambucil or observation alone. The results did not show statistically significant difference in the 5-year survival between groups. In fact, the treatment group had a trend toward shorter survival when the disease progressed and an increased incidence of epithelial cancers [67]. Almost all patients will become resistant to treatment with chlorambucil. Sulfhydryl groups, glutathione levels, and glutathione-S-transferase activity may be implicated in alkylator resistance [74-76].

**Cyclophosphamide**

Cyclophosphamide (Cytoxan, Neosar) is as effective as chlorambucil in the treatment of CLL. Patients who do not respond to chlorambucil may respond to cyclophosphamide. The usual dose is 100 mg/d. Other regimens include 500 to 750 mg/m² given intravenously (IV) or orally every 3 to 4 weeks.

**Combination Chemotherapy**

Various combination chemotherapies have been used in patients with CLL (see Table 7). For example, COP (cyclophosphamide, vincristine [Oncovin], and prednisone) has produced a response rate ranging from 44% to 82% [77-80]. In randomized trials, COP was neither better [79,80] nor inferior [81] to chlorambucil plus prednisone. CHOP (COP plus doxorubicin [Adriamycin, Rubex]) obtained better results than COP alone in advanced stages of disease [82,83] and better response rates than chlorambucil plus prednisone [84,85]. No survival differences were observed between patients randomized to CHOP or chlorambucil plus prednisone [86].

<table>
<thead>
<tr>
<th>Table 7. Chemotherapy Regimens for Chronic Lymphocytic Leukemia</th>
<th>Chlorambucil/ prednisone</th>
<th>Chlorambucil/ prednisone</th>
</tr>
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<tbody>
<tr>
<td>COP</td>
<td>Cyclophosphamide, vincristine, prednisone</td>
<td>Cyclophosphamide, vincristine, prednisone</td>
</tr>
<tr>
<td>CHOP</td>
<td>Cyclophosphamide, vincristine, doxorubicin, prednisone</td>
<td>Cyclophosphamide, vincristine, doxorubicin, prednisone</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>25 to 30 mg/m²</td>
<td>25 to 30 mg/m²</td>
</tr>
<tr>
<td>Cladribine</td>
<td>0.1 mg/kg/d</td>
<td>0.1 mg/kg/d</td>
</tr>
<tr>
<td>Pentostatin</td>
<td>4 mg/m² once weekly then every other week then monthly</td>
<td>4 mg/m² once weekly then every other week then monthly</td>
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</table>

Other combination chemotherapy regimens that have been studied in patients with CLL include M-2 (vincristine, carmustine [BiCNU], cyclophosphamide, doxorubicin, melphalan [Alkeran], and prednisone), CMP (cyclophosphamide, methotrexate, and prednisone), CAP (cyclophosphamide, doxorubicin, and cisplatin [Platinol]), and POACH (cyclophosphamide, doxorubicin, cytarabine, vincristine, and prednisone) [70]. Although these regimens induce higher CR rates, they are more toxic and are not clearly better than the chlorambucil plus prednisone combination [70].

**Nucleoside Analogs**

The most important nucleoside analogs in the treatment of CLL are fludarabine (Fludara), cladribine (Leustatin), and pentostatin (Nipent).

*Fludarabine* is the most extensively studied purine analog in CLL, but the agent's exact mechanism of action is unclear. Fludarabine is a fluorinated purine analog, dephosphorylated in the plasma to form 2-fluoro-ara-A [87,88], which enters the cell by a carrier-mediated transport mechanism and is phosphorylated to 2-fluoro-ara-ATP. This is the form of the drug necessary for its cytotoxic effect.
The rate-limiting enzyme in the phosphorylation is deoxycytidine kinase [88]. The accumulation of 2-fluoro-ara-ATP in the cell inhibits DNA synthesis [80] by interference with ribonucleotide reductase and DNA polymerase.

In clinical studies, fludarabine was first used by Grever and colleagues, in 26 previously treated CLL patients, at a dosage of 20 mg/m²/d for 5 days. One patient achieved CR, three had excellent partial responses (PR), and 15 had additional evidence of improvement [90]. Keating et al [91] used fludarabine at 25 to 30 mg/m²/d for 5 days every 3 to 4 weeks in 68 previously treated CLL patients, obtaining 13% CR and 44% PR rates. Using the NCI criteria that allowed persistence of residual nodules in the bone marrow, the CR rate was 29%, and the PR rate was 28%. At 36 months, median survival was the same for patients who achieved CR or nodular CR (nCR, defined as PR with only residual nodular disease of the bone marrow), and at 16 months, median survival of these patients was superior to that of patients achieving PR. The median duration of response was 21 months for the CR patients and 13 months for the PR patients. Ninety two percent of the responders achieved at least a PR after the first three courses. Following this study, 33 previously untreated CLL patients received fludarabine at M.D. Anderson [92]. A CR rate of 33% and PR rate of 45% was obtained. Using the NCI criteria including nCR, the CR rate increased to 72%.

In 1993, Keating et al [93] published the follow-up of 78 previously treated and 35 untreated CLL patients who received fludarabine. Using NCI criteria, the untreated group had a response rate of 80% with a 74% CR rate. The previously treated group was divided into refractory patients, with 28% CR and 10% PR and nonrefractory patients, with an overall response rate of 93% and a CR rate of 57%. The response to the therapy correlated with the number of previous treatments, stage of disease, and whether the patients were refractory to alkylating agents [93].

In another study, prednisone was added to fludarabine in a dosage of 30 mg/m² for 5 days in 256 CLL patients [94]. There was no increase in the response rate compared with fludarabine alone. An increase in the rate of opportunistic infections was noted in the combination therapy group, with 13 patients developing either Listeria monocytogenes or Pneumocystis carinii pneumonia. One patient developed both infections. Four patients died of P carinii pneumonia. Three of the Listeria cases occurred in patients who were in remission and not receiving treatment. In the same study, O'Brien et al noted a persistent decrease in CD4+ lymphocyte levels. In 217 patients for whom CD4 levels were available before the treatment, the median level before the initiation of FAMP and Pdn was 1,015/µL. After 6 months of therapy, the median level was 148/µL in 95 patients analyzed [94]. The main toxicity with the use of fludarabine was myelosuppression and infection. Nausea, vomiting, diarrhea, and neurotoxicity were present in 5% of the cases.

Robertson et al conducted immunophenotypic and molecular studies to assess the completeness of responses in 159 patients treated with six courses of fludarabine. No residual disease was detected by two-color flow cytometry in 89% of the CR, 51% of the nCR and only 19% of the PR patients. For complete responders having no residual disease by flow cytometry, the 2-year progression free survival was 84% vs 39% in patients having residual disease (P < .001) [95].

Different fludarabine schedules of administration have been used. Puccio et al gave fludarabine at a loading dose of 20 mg/m² followed by a 48-hour continuous intravenous infusion of 30 mg/m²/d (a total dose of 80 mg/m², compared with 150 mg/m² used in the daily × 5 schedule), repeated at 4-week intervals. Forty-two patients were evaluated for response. No patient achieved CR; 22 patients achieved PR, and 12% had stable disease [96]. A weekly schedule of 30 mg/m²/wk was used at M.D. Anderson in 47 previously treated patients, with an overall response rate of 24% [97].

A European cooperative group conducted a three-arm randomized study in 247 previously untreated CLL patients, comparing fludarabine at 25 mg/m²/d for 5 days every 4 weeks with CAP (cyclophosphamide, 750 mg/m²; doxorubicin, 50 mg/m²; IV prednisone, 40 mg/m² on days 1 to 4) and miniCHOP (vincristine, 1 mg/m² IV, and doxorubicin, 25 mg/m² IV on day 1; plus cyclophosphamide, 300 mg/m², and prednisone, 40 mg/m², given orally on days 1 to 5). In 174 Binet stage B patients, a higher response rate was noted with fludarabine than with CAP and miniCHOP, with 48% CR and 40% PR in the fludarabine group, compared with 14% CR and 64% PR in the CAP group, and 32% CR and 45% PR in the miniCHOP arm (P = .002). In 73 Binet stage C patients, there was no significant difference in the response rate between the arms. Bone marrow was not assessed as part of the response [98].

Hiddeman et al reported their preliminary results of a randomized trial comparing fludarabine vs CAP in 208 patients, 103 pretreated and 105 untreated, with an overall response of 58% for fludarabine and 42% for CAP. The response to fludarabine was 70% in untreated and 45% in pretreated patients, compared with 58% and 26% in the CAP arm [99].

Deletion of the p53 gene has been associated with poor response to nucleoside analogs and short
survival by Dhner et al [53a]. In their study, 100 patients (90 with B-CLL, 3 with Waldestrm macroglobulinemia [WM], and 7 with PLL) were studied by in situ hybridization. Monoallelic deletion of the p53 gene was found in 17% (11 B-CLL, 1 WM, and 5 PLL). None of the 12 patients with p53 gene deletion, compared with 20 (56%) of 36 without a deletion, responded to therapy with fludarabine or pentostatin.

Finally, fludarabine was used in 15 CLL patients who relapsed after an initial response to the drug. Four patients achieved a second response (27%) [94].

Cladribine: Another purine analog with activity in CLL is cladribine, which has been used in a dosage of 0.1 mg/kg/d by continuous IV infusion for 7 days. A group of investigators from Scripps clinics used cladribine and reported an overall response of 55% in 18 previously treated CLL patients; 4 patients achieved PR (22%), and 6 patients (33%) achieved clinical improvement [100]. In 1991, the same group reported the response to cladribine in 90 previously treated patients, 82 of whom were Binet stage C, eight stage B, and one stage A. The treatment was repeated at 4-week intervals, and the patients received a median of two cycles, with some of the patients receiving bolus schedule. Four patients (4%) obtained CR, and 36 (40%) PR by NCI criteria. The median duration of the response was 4 months (range, 2 to 30 months). The main dose-limiting factor was myelosuppression with persistent thrombocytopenia in 24% of the cladribine-treated patients. Infections were present in 18% of the patients [101].

Other investigators used cladribine 0.12 mg/kg/d over 2 hours for 5 days in 18 previously treated CLL patients and obtained 7 CR (39%) and 5 PR (28%) [102]. Only 10 patients had Binet stage C disease in this study, and the patients received a median of four treatments. Twenty previously untreated patients, eleven with Rai stage 3 or 4 disease, were treated with cladribine by the Scripps group. A median of four courses was given (range, 1 to 9). Using NCI response criteria, 5 patients (2 in Rai stage 2, 2 in Rai stage 3, and 1 in Rai stage 4) obtained CR (25%) and 12 patients (60%) achieved PR, for an overall response rate of 85% [103].

Julliusson and colleagues retreated six CLL patients who previously responded to cladribine (two CR and four PR). The median time to retreatment was 19 months (range, 8 to 28 months). Responses included one CR, two PR, one minimal response, one death, and the last patient had no response. The main problem was hematologic toxicity with persistent cytopenias [104].

Cross-resistance to cladribine in patients previously unresponsive to fludarabine has been studied. Julliusson reported four consecutive patients who responded to cladribine after they failed therapy with fludarabine. One CR and three PR were achieved [105]. Other investigators obtained poorer results with cladribine in this setting [106-108]. At M.D. Anderson, 28 fludarabine-refractory CLL patients were treated with cladribine. Two patients (7%) responded (by NCI criteria), and one had antitumor activity, with decrease of the peripheral blood and bone marrow lymphocytosis, but persistent thrombocytopenia. Overall, 65% of the treatment courses were complicated by febrile episodes, and 10 patients died within 60 days of starting cladribine therapy [107].

Julliusson and colleagues used oral preparations of cladribine at doses of 10 mg/m²/d for 5 days every month for up to 6 months in 17 previously untreated CLL patients. Using NCI response criteria, 7 CR (41%), 5 PR (29%) and 5 treatment failures (29%) were seen [109].

The ratio of deoxycytidine kinase to cytoplasmic 5´-nucleotidase—the enzymes that phosphorylate cladribine and dephosphorylate cladribine 5´-monophosphate, respectively—was found to be predictive for cladribine responsiveness [110].

Pentostatin is another nucleoside analog with significant activity in hairy-cell leukemia that has also been used in CLL. In 25 previously treated CLL patients, Grever found that pentostatin, at doses of 4 mg/m²/wk for 3 weeks, produced a 4% CR and 16% PR [111]. Dillman and colleagues [112] treated 39 patients, 26 previously treated and 13 untreated, obtaining one CR (3%) and 9 PR (23%). Six of the partial responders were previously untreated patients. The most significant toxicity was infection, with frequent stomatitis and rash.

Other Treatment Modalities

In patients refractory to conventional therapies, other investigational treatments have been used, including biologic response modifiers, monoclonal antibodies, and bone marrow transplantation.

Biologic Response Modifiers: Alpha interferon (IFN-alfa), used at doses of 1.5 million to 3 million units three times a week, had less than a 50% response rate in stage A and B CLL patients, and less than 10% [113,114] in stage C patients [115]. It is possible that IFN alfa-2a (Roferon-A) may prolong chemotherapy-induced responses [116,117]. This last issue remains controversial, since in another recent study using IFN-alfa as maintenance in 31 B-CLL patients previously treated with FAMP, there was no difference in the time to progression of the disease compared with that in historical controls [117a].
Recombinant Interleukin-2 (rIL-2) was used to activate natural killer cells, whose activity is decreased in CLL. Minor responses were obtained in two small studies with 12 and 8 patients respectively [118,119].

Monoclonal antibodies (MoAbs) against antigens expressed on the surface of the malignant lymphocytes have been used therapeutically in patients with CLL. Antibodies against CD5 were used with few minimal responses [120,121]. Discouraged by the disappointing clinical results with unconjugated MoAbs, investigators decided to use them as a vehicle to deliver drugs, toxins, or radioisotopes directly to the tumor cell. MoAb-toxin conjugates, also known as immunotoxins, have been examined in preclinical studies and recently incorporated in clinical trials. The antibodies used are directed against CD5, CD19, CD22, and CD25. The most commonly used toxins are the two-chain protein toxins, ricin and diphtheria toxin, and the single-chain toxin, Pseudomonas exotoxin A.

In clinical trials, the monoclonal antibody against CD19 (B4) conjugated to whole ricin was used by Grossbard et al in 25 patients with B-cell malignancies, obtaining one durable CR and two PR in patients with NHL [128]. Based on those studies, this conjugate was used in six CLL patients with only 1 PR [122,123]. LeMaistre et al used a ligand (IL-2) conjugated to modified diphtheria toxin (DAB486) in a patient with refractory CLL, obtaining a PR [124]. Others used IL-2 conjugated with Pseudomonas endotoxin [125]. The highly lytic CAMPATH was used by Janson and colleagues in two patients with refractory CLL, obtaining one CR and one PR [126].

The most significant problems with the use of MoAbs are the lack of the expression of the tumor antigens and the development of antiamouse antibodies.

Other Cytokines: Granulocyte colony-stimulating factor (G-CSF, filgrastim [Neupogen]) and granulocyte-macrophage colony-stimulating factor (GM-CSF, sargramostim [Leukine]) were used in CLL to ameliorate the neutropenia induced by chemotherapy or by the disease. GM-CSF increased the number of neutrophils 1.7- to 29-fold in patients with CLL [127,128], with low doses superior to high doses in some cases [128].

Allogeneic Bone Marrow Transplant: Allogeneic bone marrow transplant (BMT) can be effective in inducing long-term disease-free survival [129-133]. The European Group and the International Bone Marrow registry reported their experience with 47 CLL patients receiving allogeneic BMT from HLA-identical sibling donors. The median age was 42 years (range, 21 to 58); 56% had Rai stage 3 or 4 disease. Total body irradiation (TBI) was given to 96% of the patients as part of the conditioning regimen, and 64% received methotrexate and cyclosporine (Sandimmune) for prevention or treatment of graft-vs-host disease (GVHD). Forty-five patients were evaluated. Engraftment occurred in 43 (95%) and CR was obtained in 33 of 39 assessable patients (70%). Five patients (15%) relapsed between 4 and 54 months after transplant. Acute GVHD > grade 2 was experienced by 38% of the patients. Chronic GVHD was present in 47%, with extensive disease in 17% of the patients. GVHD was the most common cause of death. The projected leukemia-free survival at 5 years was 40%, with the most important prognostic factor being the stage of the CLL at the time of the transplant [131].

Rabinowe and colleagues [132] performed T-cell depleted allogeneic BMT from HLA-identical siblings in eight patients with CLL. The median age was 40 years (range, 31 to 54). Seven patients had Rai stage 2, and one had Rai stage 4 disease. All the patients were treated to reduce the bulk of the disease, six of them receiving fludarabine. At the time of transplant, one patient was in CR, five had minimal disease in the bone marrow and lymph nodes, one had only disease in the bone marrow, and one had residual adenopathy. The conditioning regimen was cyclophosphamide plus TBI. There was one toxic death due to P carinii pneumonia. At a median follow up of 11.7 months (range 6 to 18), seven patients were in CR and one had progressive disease.

At M.D. Anderson, Khouri et al [133] performed allogeneic BMT in 11 CLL patients, nine with identical-HLA siblings, one with a one-antigen mismatch in the HLA-A locus, and one syngeneic transplant. The median age was 42 years (range, 25 to 55). All patients were treated previously with fludarabine, five were primary refractory to fludarabine, and two had refractory relapse. Eight patients had Rai stage 4 and one had Rai stage 3 disease at the time of BMT. Seven patients achieved CR, and one had a nodular CR. One patient died of disseminated Aspergillus infection at day 58. With a median follow-up of 10 months (range, 2 to 36), 10 patients are alive, with 7 patients still in CR, 2 in nodular CR (one was a PR who received a second allogeneic BMT). No acute GVHD > grade 2 occurred in any patient.

The incidence of GVHD in the American series was significantly less than that seen by the European group. Previous treatment with fludarabine in the American group was a common denominator. In contrast, in the European multicenter study, no patients were treated with fludarabine prior to the BMT. An association between pretreatment with fludarabine and lack of development of severe acute
GVHD has been suggested.  

**Autologous BMT:** New techniques, including allogeneic peripheral blood stem-cell transplantation are under investigation. Rabinowe et al [132] treated 12 patients with multiple MoAb purged autologous BMT, using cyclophosphamide plus TBI as a conditioning regimen. The median age was 45 years (range, 27 to 54). At the time of the transplant, two patients were in CR, and the rest had minimal disease. One patient died on day 62 with diffuse alveolar hemorrhage. At a median follow-up of 5 months (range, 2 to 31), five patients were in CR, one had persistent disease, and five were too early to be evaluated.

Khouri et al at M D. Anderson [133] treated 11 patients with autologous BMT. Their median age was 59 years (range, 37 to 66). All patients responded; five patients achieved CR, four achieved nCR, and one achieved PR. At a median follow-up of 10 months (range, 2 to 29), six patients were alive, three in CR, two were relapsed, and one in PR. One patient died at day 100 of cytomegalovirus pneumonia in CR; another died in CR due to a complication of a liver biopsy 2 months after the BMT. Three patients developed Richter's transformation and died at 10, 10, and 11 months, respectively.  

**Splenectomy** has been considered another therapeutic option in patients with CLL. With improved surgical techniques, the operative mortality has been reduced [135-141]. The indications for splenectomy can be summarized as hypersplenism with cytopenias unresponsive to treatment, autoimmune thrombocytopenia, hemolytic anemia, and massive symptomatic splenomegaly. The results have varied between institutions and depending on the year of the study. In the Mayo Clinic, 57 splenectomized CLL patients were reviewed retrospectively, and 50 were analyzed; responses were noted in 77% of patients with anemia, 70% of patients with thrombocytopenia, and 64% of patients with anemia and thrombocytopenia. At 1 year, the response was sustained in more than 80% of responders. The operative morbidity was 20%, the mortality was 4%, and the median survival after splenectomy was 41 months in the responding group vs 14 months in the nonresponders [140].

At M.D. Anderson, the outcomes of 55 splenectomized CLL patients were examined retrospectively. Hemoglobin values were increased by more than 3 g/dL in 25%, and platelets were increased by 50,000/µL or more in 72% of patients. The mortality rate was 9% [141].

Delpero et al [136] studied 44 CLL patients with splenomegaly and hypersplenism. Twenty-six had anemia and 36 had thrombocytopenia. The hemoglobin and platelet counts increased to normal in 85% and 92% of the patients, respectively.  

**Splenectomy** has been used as an alternative to splenectomy, to alleviate cytopenias and symptomatic splenomegaly in patients whose surgical risk is considered unacceptable. SI helps to decrease the number of circulating lymphocytes and relieves the pain, but only improves the cytopenias in 25% of the patients in one series [142] and may aggravate the thrombocytopenia in some patients [143]. In one series, Roncandin et al [144] showed improvement in blood counts in 78% of patients and decrease in the size of the spleen by 50% or more in 63% of the patients.  

**Radiation** has been used to palliate bulky lymphadenopathies when there is no response to the chemotherapy.  

**Intravenous Gammaglobulin (IVIG):** Hypogammaglobulinemia occurs in 10% to 60% of B-CLL patients [145-147], and the severity of this condition has been associated with the stage of the disease, a diffuse pattern of bone marrow infiltration, and an increased incidence of infections [148-152]. An IgG level of < 700 mg/dL was associated with reduction in the survival [148]. In a study done in nine patients, deficiencies in IgG3 and IgG4 were the most significant findings, with only moderate reduction in IgG1 and IgG2 [159]. This selective deficiency of IgG subtypes may explain the pattern of infections seen in CLL patients.  

The etiology of the hypogammaglobulinemia in CLL patients is poorly understood and probably caused by multiple factors. Functional abnormalities in T-cells [153] or dysfunction of the nonclonal CD5-B cells may be implicated [154]. Jaksik et al noted no improvement in the serum Ig in 81% of 282 patients treated with chlorambucil [155]. In contrast, fludarabine improved IgM levels in patients who achieved CR [156].

In 1988, an International Cooperative Group for the study of Immunoglobulins in CLL, initiated a prospective, double-blind, randomized study comparing placebo vs IVIG (400 mg/kg) every 3 weeks for 1 year. The IVIG-treated group had fewer bacterial infections than the placebo group (23 vs 42, P < .001). The infections prevented were of mild to moderate severity without differences found in the frequency of major infections or in survival [157].

Weeks et al showed a gain of 0.8 quality-adjusted days per patient per year of therapy at a cost of $6 million per quality-adjusted 1 year gained [158] and an annual cost of $15,740 (in US$). To reduce the cost, selection of patients with a previous history of infections and...
hypogammaglobulinemia and home administration may be useful.

Autoimmune Disorders

Chronic lymphocytic leukemia has been associated with autoimmune hemolytic anemia (AHA), autoimmune thrombocytopenic purpura (ITP), aplastic anemia, pure red-cell aplasia, and other autoimmune manifestations. Coombs-positive AHA is observed in 1% of CLL at the time of the diagnosis [160], but increases in frequency with the progression of the disease [161], achieving a cumulative incidence of 7% to 35% during the course of the disease [162,163]. The origin of the autoantibodies causing hemolysis is not clear. In most cases, the autoantibodies are produced by the normal B lymphocytes rather than by the clonal B-CLL cells, but the abnormal clone was the origin of the antibodies in two patients [164]. The autoantibodies causing the hemolysis in CLL patients are “warm” anti-red blood cell (RBC) antibodies (IgG) [165] and are usually polyclonal in origin. Since the antibodies often react against a panel of RBC (pan-agglutinins), blood banks may face difficulty in finding compatible blood. In a retrospective study that included 53 patients who had AHA [166], patients who received transfusions did not have severe reactions or increased hemolysis. Therefore, if the anemia is symptomatic, transfusion with the most compatible blood is indicated. Appropriate treatment is prednisone, at doses of 1 to 2 mg/kg/d and then tapered over a few weeks, if possible. Patients who do not respond to prednisone or who require high doses of steroids may benefit from splenectomy [167], splenic irradiation [168], danazol [169], or intravenous Ig [170]. Autoimmune thrombocytopenia is less common and more difficult to demonstrate. The treatment includes steroids, IVIG, and sometimes splenectomy. Other therapeutic options include vincristine, immunosuppression, splenic irradiation, plasmapheresis, and recently, the use of the staphylococcal protein A column.

Pure red-cell aplasia occurs in 1% to 6% [36]. The combination of cyclosporine and prednisone was superior to treatment with prednisone alone [171].

Secondary Malignancies

Patients with CLL have an increased risk of secondary malignancies. The most common solid tumors in CLL patients are melanoma, soft-tissue sarcoma, colorectal carcinoma, and lung cancer. A recent study showed an incidence of 8.9% of second malignancies in 9,456 cases studied. This was 28% higher than expected in a comparable population. The observed/expected ratio for Hodgkin's disease was 7.69; for ocular melanoma, 3.79; for malignant melanoma, 2.79; for brain tumors, 1.98; and for lung cancer, 1.90 [172].

Transformation

Richter's Syndrome

In 1928, Maurice Richter described the association between CLL and “reticulum cell sarcoma” [173]. Since then, many cases of this association have been reported. In general, the development of a higher-grade lymphoma, usually diffuse large-cell (DLCL) or immunoblastic variant, occurs in 1% to 10% of the patients. Robertson et al [174] reported 39 cases of Richter's syndrome (RS) among 1,374 CLL patients seen between 1972 and 1992 (a 3% incidence). The presenting features, in order of frequency in this study, were increase in serum LDH (82%), progressive lymphadenopathy (64%), systemic symptoms (59%), monoclonal gammopathy (44%), and extranodal involvement (40%). Ten patients had no evidence of CLL at the time of transformation. Three of these 10 were also free of disease as assessed by dual-color flow cytometry or restriction analysis for Ig gene rearrangement. The median survival was 5 months, despite treatment. Three of the eight patients who survived more than 1 year had de novo presentation of CLL and RS. Responders to treatment survive longer than nonresponders. Other studies showed similar clinical, laboratory, and survival characteristics [175-177]. The association of Hodgkin's disease and CLL has been reported by some investigators [178-179]. Brecher and Banks called this association a Hodgkin's disease variant of RS [178]. The clinical presentation is similar to RS, and the median survival is 12 months.

CLL/PLL and Prolymphocytic Transformation

Up to 15% of patients with CLL present with a mixture of small lymphocytes and larger cells with prominent nucleolus called prolymphocytes (PL) [180-181]. When PL in the peripheral blood measures between 11% and 55%, this is called CLL/prolymphocytic leukemia (PLL). Patients with
CLL/PLL present with splenomegaly disproportionate to the degree of lymphadenopathy. In a review by Melo et al, an absolute number of PL > than 15,000/µL had an outcome and survival as bad as pure prolymphocytic leukemia (> 55% PL). A scoring system used by these investigators included as adverse prognostic factors: size of the spleen greater than 8 cm, absolute number of PL > 15,000/µL, formation of rosettes 30% or less, and strong surface immunoglobulin stain [181]. Patients with more than two adverse factors had a median survival of 2.5 years [181]. The immunophenotype in CLL transforming to PLL, is the same as that in B-CLL.

Other Transformations
Rare cases of CLL transforming to acute leukemia have been reported [182-185]. Studies of some of these cases suggested that the blasts arise from the same B-cell clone as the CLL cells [183-185]. Isolated cases of CLL transformation into small noncleaved-cell lymphoma, lymphoblastic lymphoma, and hairy-cell leukemia have been reported [186-188].

Prolymphocytic Leukemia
Prolymphocytic leukemia is another lymphoproliferative disorder characterized by massive splenomegaly, a high number of circulating lymphocytes, minimal lymphadenopathy, and median survival less than 3 years. The circulating lymphocytes may be B- or T-cell type, and more than 55% of the circulating white cells should have typical morphologic characteristics. Prolymphocytes are larger and less homogeneous than CLL cells, have a clear and abundant cytoplasm, clumped nuclear chromatin, and a prominent nucleolus. The B-cells in PLL have abundant immunoglobulins on the surface, usually do not express CD5, and are strongly positive for FMC-7. These features enable the differential diagnosis with CLL. PLL is associated with cytogenetic abnormalities such as t(11;14), t(6;12), and abnormalities involving chromosome 14. In 20% of patients with PLL, T-cell markers are expressed. Splenectomy and combination chemotherapy (eg, CHOP) has been used, with short responses. Recently, purine analogs, such as pentostatin and fludarabine, showed activity in this disease.

T-Cell Chronic Lymphocytic Leukemia
Less than 5% of CLL cases involve T-lymphocytes (T-CLL). The cells arise from the postthymic cell population and usually express either CD4 or CD8 on their surface. However, the malignant cells often display aberrations in their phenotype compared with normal T-cells. Many patients with T-CLL present with a prolymphocytic variant of the disease. The physical examination typically reveals minimal lymphadenopathy and prominent splenomegaly. Response to treatment is usually poor, and survival is shorter than in B-CLL patients with similar stage disease.

Large Granular Lymphocyte Proliferation
Large granular lymphocytes (LGL) are a morphologically recognizable lymphoid subset of peripheral blood mononuclear cells. LGL can be divided into two lineages: CD3+ and CD3-. CD3+ are T-cells that express the CD3/T-cell receptor (TCR) complex and rearrange TCR genes. CD3- are natural killer cells and do not express CD3/TCR complex. T-LGL leukemia (T-LGLL) is characterized by clonal proliferation of CD3+ LGL and has also been called Tg lymphocytosis. The median patient age at presentation is 57 years (range, 4 to 88 years) [188a]. Recurrent bacterial infections, occurring as a consequence of neutropenia, and rheumatoid arthritis are the major reasons why these patients seek medical attention. The clinical picture may resemble that of Felty's syndrome. Splenomegaly is present in half of the patients, and anemia (Hct < 36%) are found in almost half of the patients, but thrombocytopenia is less common. Association with pure red cell aplasia has been reported. Bone marrow infiltration is found in 88% of patients [188a]. The typical T-LGL are bigger than normal lymphocytes and have a pale cytoplasm with prominent azurophilic granules. The T-LGL express CD3+ and often CD16+, CD57+, and CD8+; CD56 is usually negative. Most of the patients express TCRab+, whereas few of them express TCRgd+.

The indolent course of this condition in the majority of patients makes observation the most common approach. Treatment is indicated in patients with recurrent infections, severe neutropenia, rapidly progressive disease, or severe autoimmune manifestations. Splenectomy, chlorambucil, cyclophosphamide, combination chemotherapy, low-dose methotrexate, and steroids have been
used without significant success. G-CSF has been used for the treatment of neutropenia. One successful BMT in a patient with aggressive disease was recently reported [188b].
CD3-LGL proliferation usually manifests as a chronic indolent condition with mild cytopenias and a lower incidence of autoimmune phenomena than T-LGL leukemia. Demonstration of clonality in these patients is difficult; in a study of seven women with chronic CD3- LGL leukemia who were heterozygous for certain X-linked loci, X-linked gene analysis did not demonstrate clonality [188c].
A minority of CD3- LGL leukemia patients have a different presentation and prognosis from those of most patients with T-LGLL. The disease is also called natural killer-LGL leukemia (NK-LGLL). The median patient age is 39 years (range, 7 to 70) [188a]. In contrast with T-LGLL, this group of NK-LGLL patients can present with fulminant disease, high fever without evidence of infection, and B-symptoms. Anemia and thrombocytopenia may be severe, but neutropenia is usually mild. Massive hepatomegaly and splenomegaly are common in these patients. Patients with fulminant disease usually die within 2 months due to multiorgan failure and coagulopathy. Some patients have chronic symptoms for long periods of time before they develop more aggressive disease. The phenotype of the LGL is CD3-, CD56+, CD15-, CD57-, CD8-, CD4-. Treatment options including steroids and combination chemotherapy are usually ineffective. Other malignancies such as some acute lymphocytic leukemias, lymphoblastic lymphomas, and other non-Hodgkin's lymphomas can express some LGL surface antigens, such as CD56, CD57, or CD16.

**Hairy-Cell Leukemia**

Hairy-cell leukemia is an uncommon B-cell malignancy usually associated with pancytopenia and splenomegaly and first described by Boroncule et al in 1958 [190]. About 600 cases of HCL are diagnosed every year [189], and the disease represents 2% of all adult leukemias. For many years, the only effective treatment was splenectomy. However, in the last 10 years systemic therapy with interferon, and later with nucleoside analogs, has improved the prognosis of this disease and raised the possibility of cure.

**Etiology and Pathogenesis**
The etiology of HCL is unknown. Radiation exposure or Epstein-Barr virus (EBV) infection have been suggested by some investigators but denied by others. Fifteen cases of familial HCL have been published [191-195]. No specific cytogenetic abnormality has been described.
Based on immunologic and molecular studies, the cell of origin is believed to be of B-lymphocytic lineage [196-199]. Surface phenotype demonstrates expression of pan B-cell surface antigens, CD19, CD20, CD22, and monoclonal surface immunoglobulin. The cells often express heavy-chain isotypes. HCL cells also express CD25, CD11c, B-Ly-7, HC2, and other markers that have been helpful in the differential diagnosis.

**Clinical Features**
HCL is four times more common in males. The median age at presentation is 50 years. The patients may seek medical attention because of abdominal discomfort due to splenomegaly, weight loss, recurrent infections, or symptoms related to anemia. Splenomegaly is present in 90% of patients at the time of diagnosis, sometimes becoming massive. Palpable lymphadenopathy is uncommon, but Mercieca et al, using CT scans, reported enlargement of the mesenteric and/or retroperitoneal lymph nodes in 17% of patients at diagnosis and 56% at relapse [200]. Patients with HCL are more susceptible to infections usually caused by gram-negative bacteria, but infection with atypical mycobacteria are not infrequent. Rarely, patients can present with autoimmune complications like vasculitis and arthritis.

**Laboratory Features**
At the time of diagnosis, 80% of HCL patients have some degree of anemia and/or thrombocytopenia, with platelet count < 100,000/µL. Only 10% have a platelet count < 10,000/µL. Leukopenia with WBC < 3,000/µL is present in 50% of the patients, commonly associated with neutropenia and monocytopenia. Only 10% of patients present with leukocytosis (WBC > 10,000/µL). The malignant cells are larger than normal lymphocytes, with a diameter between 10 and 15 µm. The cytoplasm is pale blue and often has fine projections. The nucleus is round or ovoid with eccentric location, lacy chromat, and often with a visible nucleolus. The hairy cells express high levels of CD19, CD20, CD22, CD25, CD11c, sIg, B-Ly 7 and they are usually CD5-. The cells stain positively for tartrate-resistant acid phosphatase (TRAP). By electron microscopy, pseudopods and microvilli can be seen at the cell surface, and lamellar bodies are noted in 50% of cell samples. The bone marrow is typically difficult to aspirate, and the presentation of a patient with pancytopenia, splenomegaly, and a difficult bone marrow aspiration (dry tap) should raise the
suspicion for HCL; appropriate stains and tests should be done. The bone marrow is usually hypercellular, and an increase in reticulin fibers is shown with silver stains. The spleen can weigh more than 1,000 g in 51% of HCL patients [201]. Microscopic examination reveals infiltration by the malignant cells in the red pulp cords and sinuses. The white pulp is usually atrophic. Postmortem examinations and specimens obtained during splenectomies have revealed lymph-node infiltration by hairy cells [189,201]. Erythropoietin levels are decreased in these patients, and free serum IL-2 receptor levels, serum tumor-necrosis factor (TNF)-alpha, serum IL-1-beta, and free serum CD8 levels correlate with outcome [202-208].

**Differential Diagnosis**
HCL can be confused with malignant lymphomas, HCL variant (HCLv), splenic lymphoma with villous lymphocytes (SLVL), CLL, other non-Hodgkin's lymphoma in leukemic phase, and myelodysplastic syndromes.

**HCLv** is a disorder characterized by prolymphocytic-like cells, with a median WBC count of 90,000/µL. Cells are usually CD25– with CD11c and B-Ly 7 expressed less often. Neutropenia and monocytopenia are not usually present. The bone marrow is easier to aspirate than in typical HCL. SLVL is a very rare B-cell low grade lymphoma recently included within the marginal zone group [209], presenting with significant splenomegaly and minimal or no lymphadenopathy. Anemia and thrombocytopenia are common, but neutropenia and monocytopenia are rare. The cells express CD11c in 47%, CD25 in 25%, and almost never B-Ly-7 or HC2 [210]. A score system has been proposed by Matutes et al [210], giving 1 point for any of the four markers above. Approximately 98% of HCL cases have 3 or 4 points, whereas HCLv and SLVL cases have only 1 or 2. The SLVL cells are usually TRAP negative.

Other low-grade malignant B-cell lymphomas such as follicular and mantle-cell lymphomas in leukemic phase can be differentiated by the morphology of the cells as well as by the expression of CD10+ in follicular lymphomas, of CD5+ in mantle-cell lymphomas, and the absence of typical HCL markers in both disorders. In CLL, the morphology and immunophenotype usually makes the distinction easy.

**Treatment**
The indications for treatment are an absolute neutrophil count (ANC) < 1,000/µL, platelet count < 100,000/µL, or Hb < 10 g/dL; leukemic phase of HCL; symptomatic splenomegaly; recurrent infections; or autoimmune complications.

The criteria for a complete response (CR) require normalization of the complete blood count (CBC), with ANC > 1,500/µL, platelet count > 100,000/µL and Hb > 12 g/dL; regression to normal of organomegaly and bone marrow; and peripheral blood (PB) free of hairy cells. Partial responses require reduction of the hairy cells in the bone marrow to < 50%, < 5% hairy cells in PB, > 50% reduction in the organomegaly, and normalization of the CBC. Minimal response requires the normalization of at least one of the peripheral blood cell elements and decrease of the PB circulating cells by at least 50%.

**Observation With No Treatment:** A small group of patients having an indolent course can be observed without any treatment [211]. In general, in patients with one or two cytopenias without any other symptoms and not requiring transfusion, observation is a reasonable approach. At M.D. Anderson, only 2% of the HCL patients belong to that category. Few cases of spontaneous remission of HCL have been reported [212,213].

**Splenectomy:** Splenectomy was the first-line treatment until systemic chemotherapy was started in 1984. The interpretation of the studies is difficult because no prospective studies have been done. Golomb and Verdiman [214] performed a retrospective study in 65 HCL patients who underwent a splenectomy. In their study, they note the adverse effect of bone marrow infiltration by the leukemic cells on response to splenectomy. Ratain et al showed that patients with bone marrow cellularity of < 85% and platelet count > 60,000/µL before splenectomy required further antileukemic treatment at a median of 56.5 months, as compared with less than 1 year in the other subset [215]. Splenectomy is no longer necessarily front-line treatment in patients with HCL and, instead, is reserved for special cases such as splenic rupture, infarcts, massively enlarged spleens, severe hypersplenism, or failure to systemic chemotherapy.

**IFN-alfa:** The initial observations on natural IFN-alfa were published by Quesada et al in 1984 [216]. In that report, three of seven patients obtained a CR and four achieved a partial response. Since then, multiple studies were conducted using natural and recombinant IFN-alfa at daily doses of 3 million U/d by intramuscular or subcutaneous injections for 6 months, followed by 3 million U/d three times a week for 12 and 24 months [217-222].
The overall results in 10 different studies and a total of 417 HCL patients were evaluated by Jaiyesimi and colleagues. The CR rate is 8%, PR rate 74%, minor responses 7%, and no responses 8% [221]. The median time to response was 6 months for patients achieving PR and 14 months to achieve CR. Patients frequently relapse between 12 and 24 months after discontinuation of therapy. Reinduction with IFN-alfa is successful in most previous responders [219]. The most common side effects of IFN-alfa are flu-like symptoms, fatigue, depression, neurologic symptoms, cytopenias, and elevation of hepatic enzymes. The presence of neutralizing antibodies against recombinant IFN-alfa has been associated with refractoriness to treatment [223].

**Purine Analogs**, such as pentostatin and cladribine, have been shown to be potent and effective drugs in the treatment of HCL. Pentostatin is a purine analog synthesized by *Streptomyces antibioticus*. This drug was developed based on the observation that patients with adenosine deaminase deficiency had a combined immunodeficiency with severely depressed levels of both T and B lymphocytes. Pentostatin binds to adenosine deaminase (ADA). The recommended dose is 4 mg/m² IV bolus every other week until CR is obtained. Usually, patients require a median of 8 courses (range, 4 to 15). The CR rate varies between 59% and 89% in different studies, and the PR between 4% and 37%. Responses can last for many years, and patients who relapsed often responded to retreatment with pentostatin [227-229]. Cladribine is an active drug for the treatment of HCL, achieving similar activity to pentostatin. Due to this and the advantage of one cycle of a 7-day infusion, this drug is sometimes the first choice for treatment of this disorder. Cladribine inhibits ADA and results in selective accumulation of deoxypurine nucleotides, 2-deoxy ATP. Lymphocytes may accumulate deoxypurine nucleotides more than other cells, because the rate of deoxyadenosine phosphorylation exceeds the rate of nucleotide phosphorylation. Intracellular accumulation of 2’-chloro-deoxy ATP inhibits ribonucleotide diphosphate reductase, which inhibits DNA synthesis. In addition, inhibition of DNA polymerase and DNA ligase prevent DNA repair, resulting in increased DNA strand breaks, which, in turn, may accelerate the process of apoptosis.

The first (and later, the largest) clinical trial was performed by Piro and colleagues, who treated 144 HCL patients with cladribine, 0.1 mg/kg/d by continuous IV infusion for 7 days. A total response rate of 97% was obtained, with 85% CR and 12% PR. The response was independent of previous treatment with IFN or splenectomy, and three patients refractory to pentostatin were responsive to cladribine. With a median follow-up of 14.2 months (range, 8.1 to 68.3 months), only four patients relapsed. Fever was common, occurring in 43% of patients, usually on the sixth day of treatment and thought to be due to release of cytokines from dying cells. Recovery of the blood counts occurred by day 61 (range, 11 to 268 days) [224].

At M.D. Anderson, Estey et al treated 46 HCL patients and noted 78% CR, 11% PR, and one minimal response. Febrile episodes were present in 46% of the patients. In this study, the median CD4+ lymphocyte count before treatment was 588 cells/µL, and posttreatment decreased to 126 cells/µL [225].

Four HCL patients who relapsed after responding to cladribine were retreated again with the same drug, resulting in two CR and one PR [226]. There is very limited clinical data on the use of fludarabine in HCL [230,231]. Kantarjian et al treated three patients (two with HCL and one with HCL variant), observing two PR.

**Other Treatments**: Some patients occasionally responded to chlorambucil, cyclophosphamide, and combination chemotherapy. A successful syngeneic bone marrow transplantation has been reported [232].

**References**


182. Zarrabi M, Grunwald HW, Rosner F: Chronic lymphocytic leukemia terminating in acute


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