Combination Chemotherapy of Advanced Chronic Lymphocytic Leukemia: The M-2 Protocol (Vincristine, BCNU, Cyclophosphamide, Melphalan, and Prednisone)

By Sanford Kempin, Burton J. Lee III, H. Tzi Thaler, Benjamin Koziner, Suzanne Hecht, Timothy Gee, Zalmen Arlin, Claudia Little, David Straus, Lillian Reich, Elizabeth Phillips, Hamid Al-Mondhiry, Monroe Dowling, Klaus Mayer, and Bayard Clarkson

The M-2 protocol (vincristine, cyclophosphamide, BCNU, melphalan, and prednisone) was administered monthly to 63 evaluable patients with advanced chronic lymphocytic leukemia. Complete remission (absence of all clinical and bone marrow evidence of leukemia) and partial response (>50% decrease in organ enlargement and reduction of WBC count to below 15,000 x 10^3/liter) were achieved in 17% and 44%, respectively, for a total response rate of 61%. The median survivals from therapy of patients achieving a CR, PR, or no response were 73, 40, and 14 mo respectively. The median survival time from onset of treatment for stages II, III, and IV disease were 47, 20 and 19 mo, respectively, which was not statistically different from historical controls. However, when untreated patients are compared to this latter group, a significant survival advantage from diagnosis was found (p = 0.01), stressing the importance of prior therapy as the only unfavorable prognostic factor. Although complete remissions in CLL, as reflected in apparently normal bone marrow B-lymphocyte markers, can be induced with acceptable morbidity, the majority of patients relapse after cessation of therapy. An alternative approach to the M-2 protocol will be needed to eradicate the disease.

The clinical variability of the disease, the fear of worsening an already impaired immune system, and the advanced age of many patients with chronic lymphocytic leukemia (CLL) have represented obstacles to a systematic attempt at disease eradication. Therapy has most often been utilized during periods of symptomatic or advancing illness when infections, anemia, thrombocytopenia, enlarging lymphoid tissue, and splenomegaly complicate what was previously a long asymptomatic period of lymphocytosis and minimally enlarged lymph nodes.

Initially, CLL is a slowly progressive disorder with a median survival of 4–5 yr. A number of studies have attempted to correlate survival of CLL patients with their clinical state or with the biologic properties of CLL cells. Retrospective analysis of the Memorial Hospital experience as well as that of Santoro et al. have confirmed the utility of the Rai staging classification as a useful clinical staging system with respect to survival. Boggs et al. using a different staging system, concluded that survival was dependent primarily on the length of the preclinical phase of the disease. Although these studies appear to demonstrate that clinical variability is most probably a reflection of disease progression, rather than an intrinsic heterogeneity of CLL de novo, Knope et al. concluded from their investigations that in vitro lymphocyte response studies were more reliable guides to survival than clinical staging.

In spite of disagreement as to the most applicable staging system, most authors feel that progressive disease when manifested by anemia and thrombocytopenia has a poor prognosis. The M-2 protocol was employed to test a more aggressive approach in advanced disease using transfusional and antibiotic support with the hope of inducing higher numbers of complete remissions, improving survival and ultimately, curing the disease. The regimen (Fig. 1), based on one designed by Harley et al., has been successfully used for the treatment of patients with multiple myeloma. Both multiple myeloma and CLL represent low growth fraction lymphoproliferative disorders, and a trial of the M-2 protocol for CLL was begun in 1973. Complete remissions in CLL have been obtained with this program and the present report represents an update as well as conclusions derived from our 8-yr experience.

MATERIALS AND METHODS

For purposes of definition, CLL is a monoclonal lymphoproliferative disorder, consisting of blood lymphocytosis (>15,000 B lymphocytes x 10^3/liter), a bone marrow containing at least 40% lymphocytes, enlarged lymph nodes, and hepatosplenomegaly. Patients in whom the enlarged lymph nodes antedated the appearance of peripheral blood and bone marrow lymphocytosis were considered to have diffuse well differentiated lymphoma (DWDL) and were not included in the analysis of the protocol. All patients were staged according to the Rai classification. Those patients who were stage II (<60 yr of age) and stages III and IV initially or who progressed to...
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Only rarely was palpable abdominal disease (exclusive of an enlarged liver or spleen) noted (a problem encountered in only 2 patients) in the M-2 group. Abdominal lymph node enlargement was considered a nonresponse. Prophylactic cholecystectomy was not performed. The pathologic description of normal splenic tissue was documented by bone marrow biopsy, separation studies of the spleen, and cell marker analyses were conducted on fractions so obtained.  

In all patients a complete blood count, bone marrow aspirate and biopsy, Coombs' test, and SMA-12 were obtained prior to therapy. Bone marrow by differential velocity sedimentation were performed to be administered to 14 responding patients. Studies of surface immunoglobulin, mouse erythrocyte rosetting (SRBC-rosette), Fc receptor (MRFC), sheep erythrocyte rosetting (SRBC-rosette), Fc receptor (MRFC), and cell marker analyses were conducted on fractions so obtained.  

A complete remission (CR) is defined as a normal white blood cell count with satisfactory control of her disease for 8 mo, after which anemia recurred, necessitating alternative therapy. One patient in complete remission underwent an incidental splenectomy while undergoing a protocol failures. One patient underwent splenectomy for hemolytic anemia resistant to the M-2 protocol. She then resumed the protocol and was considered a nonresponder. Patients were considered nonevaluable if they received at least two cycles of therapy and died before receiving another cycle, or the decision of the attending physician (5). Therapy was discontinued in 7/11 CR patients, either because of disease progression or hypersplenism resistant to the M-2 protocol. Two of these patients were begun on the M-2 protocol. Two others had progressive disease after follow-up of 1 mo. Of the 63 evaluable patients, there were 36 males and 27 females, for a male:female ratio of 1.3:1. The age of patients entering the protocol was 58.4 yr (range 36-80). Sixty-nine patients (2 stage I (enlarged lymph nodes), 25 stage II (lymph node enlargement), 18 stage III (lymph node and bulkiness of abdominal lymph nodes could not be determined), and 27 stage IV (lymph node enlargement) were considered stages III and IV disease. Patients with stage I1 disease did not undergo lymphangiography, sonography, or computerized axial tomographic analysis as minimally pretreated and were analyzed with the untreated group. Patients with advanced disease were admitted to the program consisting of vincristine, prednisone, cyclophosphamide, and melphalan, and BCNU (Fig. 1) for immunoglobulin G, and complement (C3) receptors were performed.  

Immunoelectrophoresis and quantitative immunoglobulins (Igs) were measured by an automated immunoprecipitation or immunodiffusion procedure. An increase in Igs is defined as a 25% elevation of the IgG and a 50% elevation of the IgA and IgM, respectively. When a complete remission was documented by bone marrow biopsy, separation studies of the spleen, and cell marker analyses were conducted on fractions so obtained. Patients were considered to be in complete response if no lymphocytic cells were present in the peripheral blood or bone marrow, and if the serum albumin or total protein was normal and there was no evidence of immune thrombocytopenia. 

Fig. 1
organomegaly and palpably enlarged lymph nodes and a normal bone marrow biopsy must be documented. Lymphangiography was not performed in these patients to confirm completeness of nodal response. A complete remission confirmed by lymphocyte markers on separated bone marrow (CR-BMM) is defined as the presence of a polyclonal B-cell population, reflecting a normal ratio of kappa to lambda light-chain-bearing cells (absence of a clonal excess) and normal levels of MRFCs. Antiidiotype antisera against the surface immunoglobulin of each patient's CLL cells was not raised, and normal levels of immunoglobulins, when decreased, was not considered in the definition of response.

Statistical Analysis

The survival and remission duration curves were obtained utilizing the Kaplan-Meier product limit method. The survival times were measured from diagnosis as well as from the beginning of the M-2 protocol. The log-rank procedure was used for comparison of survival and remission duration in two or more groups. All p values reported refer to two-sided t tests.

RESULTS

Complete remission (CR) was achieved in 11/63 (17%) evaluable patients (Table 1). A median of 14 cycles (range 5–21) of the M-2 and a median duration of 18.6 mo (range 6–35) of therapy was required for a CR. The age, sex, stage at the beginning of therapy, pretreatment immunoglobulin levels, and white blood cell counts of CR patients did not differ significantly from those patients who attained a partial or no response. The median duration of complete remission is 42 mo. Of the seven CR patients who have relapsed, five have died (one of cardiovascular disease, one of progressive CLL, one from peritonitis, one from a subdural hematoma and subsequent Listeria monocytogenes meningitis, and three of acute myelogenous leukemia). The median survival of the 11 CR patients from the onset of treatment is 76 mo. This survival is statistically better than those patients attaining a PR or NR (p = 0.001). Of the two patients remaining alive after relapsing, one is being maintained with prednisone alone because of immunohemolytic anemia and one is receiving no therapy.

Of the 11 CR patients, 10 underwent separation of their bone marrow lymphocyte subpopulations to determine the presence of residual disease by cell marker analysis. Results of this analysis, previously reported in 8 of these patients, and clinical follow-up are shown in Table 2. An increased population of mouse rosette-forming cells was found in three patients, one of whom had a clonal excess of surface immunoglobulin (SmIg). One patient had persistence of a paraprotein in spite of normal numbers of mouse rosette-forming cells and a normal bone marrow biopsy. Seven patients had normal bone marrow studies and were felt to be CR-BMM. However, five have relapsed at 6, 8, 15, 19, and 30 mo (median 15 mo) after the protocol was stopped. Three patients remain in remission; two being treated with daily cyclophosphamide and one receiving the regimen at bimonthly intervals. The 11 CR patients were maintained on the protocol for a median of 22 mo (range 0–46) after remission was achieved. Relapses occurred in these CR patients 6, 7, 8, 8, 15, 19, and 30 mo (median 8 mo) after the M-2 protocol was stopped. One patient relapsed while being treated with the protocol, having had prolonged treatment-free intervals because of herpes zoster infections.

Twenty-eight patients have achieved a PR (44%). The median number of cycles to reach a PR for the

<table>
<thead>
<tr>
<th>Table 1. Response, Response Duration, and Survival on the M-2 Protocol</th>
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<tbody>
<tr>
<td>Response No. (%)/Total</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>CR</td>
</tr>
<tr>
<td>PR</td>
</tr>
<tr>
<td>CR + RR</td>
</tr>
<tr>
<td>No response</td>
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</table>

<p>| Table 2. Surface Marker Analysis of Separated Bone Marrow Lymphocyte Fractions |
|---------------------------------|-----------------|-----------------|-------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Patient</th>
<th>SmIg (%)</th>
<th>MRFC (%)</th>
<th>SRFC (%)</th>
<th>Interpretation</th>
<th>Clinical Status</th>
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<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>2</td>
<td>76</td>
<td>CR-BMM</td>
<td>Relapse</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>54</td>
<td>CR-BMM</td>
<td>Relapse</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0</td>
<td>78</td>
<td>CR-BMM</td>
<td>Remission</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>3</td>
<td>67</td>
<td>CR-BMM</td>
<td>Relapse</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>2</td>
<td>34</td>
<td>CR-BMM</td>
<td>Remission</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>2</td>
<td>56</td>
<td>CR-BMM</td>
<td>Relapse</td>
</tr>
<tr>
<td>7</td>
<td>36 K – 30 L – 0</td>
<td>36</td>
<td>49</td>
<td>Persistent leukemia</td>
<td>Relapse</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>0</td>
<td>64</td>
<td>CR-BMM</td>
<td>Relapse</td>
</tr>
<tr>
<td></td>
<td>(lgG serum paraprotein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>70</td>
<td>20</td>
<td>25</td>
<td>Persistent leukemia</td>
<td>Relapse</td>
</tr>
<tr>
<td>10</td>
<td>5.5</td>
<td>14</td>
<td>70</td>
<td>Persistent leukemia</td>
<td>Remission</td>
</tr>
</tbody>
</table>
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of patients was 3, with 92% achieving this status after 7 cycles. Of 11 patients considered in CR, 10 (91%) had attained a PR within 5 cycles, whereas only 26/45 or 58% of patients who did not achieve a CR reached a PR within 5 cycles. The median duration of PR is 24 mo (Table 1). Of these patients, 14/28 are still alive with a median survival of 40 mo from the start of therapy. Although a worse survival than CR patients (p = 0.002), it is still significantly better than those who did not respond (median of 14 mo), suggesting a survival advantage of even a partial response. Of 28 PR patients, 11 continue to be treated with the protocol. The median survival of responding patients (CR + PR) was 45 mo.

Prior therapy was found to be the only significant variable, albeit negative, with respect to response (p = 0.001) and survival (p = 0.001). The CR rate of those patients who received no prior therapy was 30% (11/37) as compared to 17% of the entire group of patients. No patient achieving a CR had received prior therapy, whereas 31% (9/28) of the PRs and 71% (17/24) of NRs had been previously treated. The median duration of survival for patients having received no prior therapy was 47 mo versus 15 mo for prior treated individuals (p = 0.001). The median duration of response (CR and PR) was 34 mo for untreated versus 11 mo for prior treated cases. The difference in response duration patterns, however, was not statistically significant (p = 0.10).

Of seven untreated patients who did not achieve a partial response, only one patient appeared to be completely resistant in terms of an inability of the regimen to decrease the white blood cell count. The six other patients achieved varying degrees of lymphocyte count depression and decrease in lymphadenopathy. One of these six elected to discontinue therapy because of intolerable nausea, vomiting, and malaise after each cycle of the protocol in spite of a satisfactory clinical response. Two patients are continuing to receive therapy and may achieve a PR, and four have died. All seven patients were men. They did not differ significantly from the rest of the CLL patients with respect to age, stage, or initial WBC count.

The median survival from the beginning of the M-2 protocol of stages II, III, and IV patients was 47, 20, and 19 mo, respectively (Fig. 2). There is no significant difference in survival among these stages. When the entire group of M-2 patients are compared to the survival of CLL patients treated at Memorial Hospital prior to the use of the M-2 protocol, there is no improvement in survival from diagnosis (Fig. 3). There was, however, a significantly longer survival from diagnosis for the M-2 untreated patients when compared to the historical control (p = 0.012).

Immunoglobulin levels of 49 patients were studied prior to therapy and 32 (66%) were found to have at least one immunoglobulin class decreased (18 IgG, 22 IgA, 16 IgM). There was no significant difference in pretreatment immunoglobulin levels between patients who achieved a CR, PR, or NR. Of 34 patients who had serial studies before and after therapy, 15 had an increase in at least one immunoglobulin class. A significant improvement in immunoglobulins was observed in 4/10, 2/16, 5/12 patients with an initially low IgG, IgA, and IgM, respectively. Significant rises in individual immunoglobulin levels were not dependent on the response to therapy. One CR patient has had a significant rise in IgG and IgM after stopping chemotherapy. The immunoelectrophoresis pattern was studied in 54 patients and 6 (11%) were found to have a paraprotein (2 kappa light chain in urine, 1 lambda light chain in urine, 2 IgM kappa in serum, 1 IgG kappa in serum). Chemotherapy decreased the paraprotein level in one patient (IgG kappa: 9500 mg/dl to 2700 mg/dl). We were unable to document the complete disappearance of the paraprotein in any of these six patients. A Coombs’ antiglobulin test was ordered prior to therapy in 54 patients and 17% (9/54) had a positive direct test. These were all classified as panagglutinins of the warm reacting type. Five of these patients were anemic (stage III) and three were thrombocytopenic as well (stage IV). All patients with immune hemolytic anemia had extensive marrow involvement as well as adenopathy. In three of these patients the direct Coombs’ test became negative while on therapy and their hemoglobin level rose significantly. Two patients had a decrease in titer of their panagglutinin. In four patients there was no improvement in either anemia or the positive direct Coombs’ test. One stage II patient with a positive Coombs’ test developed a severe immunohemolytic anemia shortly after beginning chlorambucil and prednisone. She was then placed on the M-2 with resolution of the hemolytic process. She remained Coombs’ positive. This worsening of the autoimmune process after beginning therapy, first reported by Lewis et al., represented the only incidence of this phenomenon seen. There was no significant difference in response or survival between those stage III patients with and without a positive direct Coombs’ test.

Side Effects and Complications

The protocol was well tolerated by most patients and, with the exception of one patient who suffered intolerable nausea and vomiting after the administration of BCNU and cyclophosphamide, no patient refused therapy. Two elderly patients were unable to follow instructions concerning their oral medications,
prednisone and melphalan. Vincristine neurotoxicity was seen in most patients, and paresthesias and hyposthesias were occasionally troublesome and long-lasting but not disabling. Hypercalcemia and aseptic necrosis of the femoral head, both previously reported in the lymphoproliferative disorders, were each seen in one patient respectively. Continuous corticosteroid administration was responsible for the cushingoid habitus seen in four patients.

Infections were the cause of 32 of 40 deaths (80%). With the exception of one patient whose cellulitis disseminated while he was neutropenic after receiving the M-2 protocol and one patient who died of inanition after a severe herpes simplex infection of the oral cavity, all other infection-related deaths occurred after the M-2 protocol was stopped and in the setting of progressive disease. The initial infectious sites in decreasing order of incidence were lung (14), unknown (8), cellulitis (3), kidney (2), oropharynx (1), orbit (1), meninges (1), and peritoneal cavity (1). Three patients developed interstitial pneumonia; two possibly related to chemotherapy and one due to documented Pneumocystis carinii. In two patients, no obvious cause of death could be determined. An autopsy in one of these revealed leukemic deposits in the myocardium.

One patient with stage III CLL became thrombocytopenic and developed a liver abnormality resulting in a vitamin-K-resistant decrease in the prothrombin complex factors. He succumbed to pulmonary hemorrhage. Apart from this patient with fatal pulmonary hemorrhage, no other patient on the protocol developed significant clinical bleeding, including those patients who were initially thrombocytopenic. A decrease in platelet count while on therapy was a frequent finding and occasionally resulted in an inability to give the protocol at 28–35-day intervals as planned. Of the 63 evaluable patients, 38 were followed closely enough after their initial cycle of the M-2 to evaluate myelosuppressive toxicity. Thrombocytopenia, in those patients who were not stage IV initially,
Fig. 3. Survival of M-2 patients from diagnosis compared to a historical control of patients treated at Memorial Hospital using conventional CLL therapy.

was seen in seven. This was considered mild (75–100,000 × 10⁶/liter) in 3, moderate (50–75,000 × 10⁶/liter) in 2, and severe (less than 50,000 × 10⁶/liter) in 2. The nadir occurred at a mean of 24 days after the M-2 was given. Of 8 patients with stage IV disease, 3 had a significant rise in platelet count shortly after initiation of therapy and 6/8 by the beginning of the second cycle. A platelet count greater than 100,000 × 10⁶/liter was never achieved in two stage IV patients.

Fourteen patients developed a second malignancy after the diagnosis of CLL was made: five prior to the M-2 and nine after the M-2 was begun. The diagnosis of cancer antedated the diagnosis of CLL in two patients (rhabdomyosarcoma, carcinoma of the colon). In six of these cases, the second malignancy was the direct cause of the patient’s demise: rhabdomyosarcoma, acute myelogenous leukemia (2), erythroleukemia evolving into acute myelogenous leukemia, epidermoid carcinoma of the lung, and adenocarcinoma of the rectum. These acute leukemias developed after 30, 40, and 44 mo of therapy, respectively. Two patients died shortly after remission induction therapy with daunomycin, cytosine arabinoside, and thioguanine. The third patient died before therapy could be instituted.

DISCUSSION

With the exception of BCNU, melphalan, and vincristine, there is an abundant literature on the response of patients with CLL utilizing ACTH, corticosteroids, chlorambucil, and cyclophosphamide (Table 3). The combination of chlorambucil and corticosteroids was found to be superior to that of chlorambucil alone with respect to response and survival. This combination has become the standard therapy of CLL, although Kuang et al., in a randomized trial, found that chlorambucil and cyclophosphamide had an identical response rate.

Vincristine is incorporated in several chemotherapy trials of CLL but has not been found to be useful as a single agent. Whether it is effective after initial tumor regression and subsequent increase in the growth fraction, as has been suggested in multiple myeloma, has not been demonstrated in CLL. BCNU is an active agent in multiple myeloma and has been reported to give a significant improvement in the frequency of objective responses when added to alkylating agent regimens either as primary therapy or in cases found to be resistant to melphalan. Alexanian et al., however, were unable to confirm the added benefit of this nitrosourea. Too few patients with CLL

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have been treated with BCNU as a single agent to determine its activity in this disease.\textsuperscript{43} BCNU in combination with prednisone induced one complete and one partial response in diffuse well differentiated lymphoma, a disease closely related to CLL.\textsuperscript{44} Therefore, it is not possible to determine whether the addition of BCNU and vincristine to a multiple alkylating-agent–steroid combination such as the M-2 has played a significant role in our response to a drug that is not known. Scouros et al.\textsuperscript{55} recently reported a 44% complete remission rate for advanced CLL utilizing cyclophosphamide, vincristine, and prednisone (COP). The patient population consisted of both treated and untreated patients. Oken et al.\textsuperscript{54} had previously reported an 11% CR rate in patients refractory to daily alkylating agent therapy utilizing a similar COP program. This decrease in complete response in previously treated patients was observed in the present study. Of 37 untreated patients, 11 (30%) achieved a CR as compared to 13% if all patients are included. This is a statistically significant difference ($p = 0.002$). Whether this difference in response rate is due to the expansion of a population of resistant cells after initial therapy, a progressive malignant transformation of cells with time, or a decrease in immune surveillance due to progressive disease or chemotherapy is not known. Scouros et al.\textsuperscript{55} recently reported a 45% CR rate by adding an anthracycline, Adriamycin, to a conventional cyclophosphamide and prednisone program. Both the Liepman and Scouros studies appear to show a significant improvement in response rate, but longer follow-up will be needed to determine if this can be translated into improved survival.

Whereas nearly one-half of previously untreated patients can, with moderately aggressive therapy, achieve a complete remission, it is not certain, as noted above, that long-term survival is improved. Patients who achieve a CR or PR and those patients having received no prior therapy are living longer than either

<table>
<thead>
<tr>
<th>Author/Group</th>
<th>No. of Patients</th>
<th>Treatment</th>
<th>CR (%)</th>
<th>PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rundles et al. (1959)</td>
<td>23</td>
<td>CLB 12 mg p.o. daily</td>
<td>3 (13)</td>
<td>12 (52)</td>
</tr>
<tr>
<td>Galton et al. (1961)</td>
<td>47</td>
<td>CLB 0.03–0.3 mg/kg/p.o. daily</td>
<td>24 (50)</td>
<td></td>
</tr>
<tr>
<td>Huguley (1957–1962)</td>
<td>104</td>
<td>CLB 12 mg p.o. daily</td>
<td>12 (11)</td>
<td></td>
</tr>
<tr>
<td>Ezhiki et al. (1965)</td>
<td>51</td>
<td>CLB 6–12 mg p.o. daily</td>
<td>37 (73)</td>
<td></td>
</tr>
<tr>
<td>Han et al. (1967)</td>
<td>144</td>
<td>CLB 6–12 mg p.o. daily PREM 20–60 mg p.o. daily</td>
<td>5 (3)</td>
<td></td>
</tr>
<tr>
<td>Han et al. (1973)</td>
<td>15</td>
<td>CLB 6 mg p.o. daily PREM 30 mg p.o. daily</td>
<td>3 (20)</td>
<td>10 (66)</td>
</tr>
<tr>
<td>Hansen (1973)</td>
<td>26</td>
<td>CLB 5–10 mg p.o. daily</td>
<td>15 (57)</td>
<td></td>
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<tr>
<td>Knospe et al. (1974)</td>
<td>62</td>
<td>CLB 0.4 mg/kg/p.o. biweekly</td>
<td>5 (8)</td>
<td>22 (35)</td>
</tr>
<tr>
<td>Sawitsky et al. (1977)</td>
<td>38</td>
<td>CLB 0.4 mg/kg/p.o. monthly PREM 0.8–0.5 mg/kg/p.o. monthly</td>
<td>5 (13)</td>
<td>10 (26)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PRED 0.8–0.5 mg/kg/p.o. daily</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CLB 30 mg/sq m p.o. biweekly PREM 80 mg/p.o. × 5 bi-weekly</td>
<td>21 (21)</td>
<td>56 (58)</td>
</tr>
<tr>
<td>Liepman et al. (1978)</td>
<td>36</td>
<td>CYT 400 mg/sq m p.o. × 4 monthly VCR 1.4 mg/m i.v. monthly PREM 100 mg/sq m/p.o. × 5 monthly</td>
<td>16 (44)</td>
<td>10 (28)</td>
</tr>
<tr>
<td>Scouros et al. (1980)</td>
<td>42</td>
<td>CYT 500 mg/sq m i.v. monthly AD 50 mg/sq m i.v. monthly PREM 100 mg/p.o. × 5 monthly</td>
<td>19 (45)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CLB 6–12 mg p.o. daily PREM 0.25 mg/kg/p.o. × 4 monthly BCNU 0.5 mg/kg i.v. monthly VCR 0.03 mg/kg i.v. monthly PREM 1.0 mg/kg/p.o. × 7 monthly</td>
<td>11 (17)</td>
<td>28 (44)</td>
</tr>
</tbody>
</table>

CLB, chlorambucil; CYT, cyclophosphamide; PRED, prednisone; AD, Adriamycin; BCNU, 1,3-bis(B-chloroethyl)-1-nitrosourea; MEL, melphalan; VCR, vincristine.
nonresponders or prior treated patients. The survival of stage II patients at 36 mo appears to be better than stages III and IV. However, with longer follow-up, this difference does not approach statistical significance (Fig. 2). Although the survival from diagnosis of the entire group of M-2 patient does not differ from those treated at Memorial Hospital prior to the M-2 protocol (Fig. 3), untreated stage IV patients show a slight survival advantage (p = 0.03).

Sawitsky et al.51 reported 96 patients with advanced disease treated with intermittent chlorambucil and prednisone, daily chlorambucil and prednisone and daily prednisone alone. The median duration of survivals were 37 mo, 21 mo, and 32 mo, respectively, compared to a median duration of survival of 38 mo for the entire group of M-2 patients. CR and PR patients in this earlier series had a combined median survival time of 50 mo compared to 45 mo for the same response groups in the M-2 series. Binet et al.,5 employing a different staging system, reported a median survival of 23 mo for stage IV patients (anemia or thrombocytopenia) treated with several different regimens, including chlorambucil (72 cases) and MOPP combination therapy (23 cases). When stages III and IV (Rai classification) are combined in the present series, a median survival of 20 mo is achieved.

Lieberman et al.,53 using CVP, demonstrated a median survival of approximately 32 mo for stages I and II disease, greater than 36 mo for stages III and IV, and a combined median survival of 35 mo for the entire group. Whether CVP was given as initial or subsequent therapy made no apparent difference in the response or survival. Although cyclophosphamide, vincristine, and prednisone are included as part of the M-2 regimen, conceivably the higher dose of cyclophosphamide and its more frequent administration in the Lieberman study may have been responsible for the improved results. Keller et al.,52 utilizing intermittent chlorambucil and prednisone, reported a median survival for stages 0–II of >40 mo and a median survival for stages III–IV of 19.7 mo, with an overall median survival of greater than 40 mo for their entire group of patients. In spite of the improvement in the rate of remission induction, the long-term survival of patients with CLL appears to be similar in most reported series, with the exception of the report by Lieberman et al.53

The median duration of complete remission was 42 mo with the M-2 protocol. Once therapy was stopped (unmaintained remission), the median time to relapse was 11.5 mo. After high-dose chlorambucil and prednisone, the duration of unmaintained remission was 19 mo.52 Scouros et al.55 reported 4/19 CR patients off therapy for approximately 1 yr. Although both high-dose chlorambucil with prednisone, and adriamycin, cyclophosphamide, and prednisone appeared to maintain patients in complete remission longer than the M-2, once therapy was stopped, a longer follow-up time and more accurate documentation of complete response will be needed to determine if CLL has been eradicated in these patients.

The use of cell marker analysis of bone marrow samples to distinguish lymphocyte subpopulations represents a sensitive technique for determining the completeness of remission. Gordon et al.56 found an abnormal distribution of lymphocytes in 3/5 complete remission patients based on Fc receptor analysis. Although no B-cell monoclonality and/or increased MRFCs were found in 7/11 patients who achieved a complete remission, as documented by bone marrow biopsy, the relapse of 4/7 of these patients after discontinuing intensive chemotherapy would suggest the presence of residual malignant cells at levels insensitive to these techniques but capable of proliferating and repopulating bone marrow and lymph nodes. A recently described cytofluorometric technique57 using labeled antisera to light chains may represent a more sensitive indicator of persistent leukemic cells.

The immune defect in patients with CLL is a complex one. Abnormalities of B-cell function,58 T-cell number59–61 and function,62–63 and immune modulation65–67 have been described. Hypogammaglobulinemia is a common finding, occurring in 44%–87% of patients.67–70 In the present series, 64% of patients were found to have at least one immunoglobulin class decreased prior to treatment. Neither stage of disease nor response to therapy were related to the pretreatment immunoglobulin levels. Miller et al.69 also observed no correlation between progression of disease and immunoglobulin levels. Improvement in B-cell function, as reflected by improved immunoglobulin levels, has been shown by Knope et al.10 using biweekly chlorambucil and prednisone as well as by Johnson71 applying total body irradiation. Improvement in immunoglobulins were seen in a number of patients on the M-2 protocol, but with no relationship to response. Miller et al.69 and Gordon et al.56 were unable to demonstrate a rise in immunoglobulins with treatment. One CR patient has had a significant rise in IgG and IgM after chemotherapy was stopped, emphasizing the impairment of immunocompetence induced by treatment.

Infections are a common cause of morbidity and mortality in chronic lymphocytic leukemia. Aroesty et al.72 reviewed 61 cases of CLL and found that 61% of patients had had significant infections, many of which were life-threatening. Of 50 patients with CLL in Bogg’s series,7 46% of deaths were attributable to infections. Nearly 2/3 of the deaths in Hansen’s series
of 161 CLL patients were infection related. In the present study, 80% (32/40) of deaths were due to infections, almost all occurring in the setting of progressive CLL. Of these 32 deaths, only one was directly related to chemotherapy-induced myelosuppression. The inability to demonstrate a consistent rise in immunoglobulins, even in patients achieving a complete remission, may be a manifestation of chemotherapy-induced immunosuppression. Limiting the amount of chemotherapy once CR is attained and stimulating immunocompetence at that point may improve infection fighting capability and are reasonable future goals.

Pulmonary toxicity related to chemotherapy is being reported with increasing frequency. Although the incidence of this complication was low in the present series (2/63), it was the probable cause of one patient's demise. Cyclophosphamide, BCNU, and melphalan have each been implicated as causative agents. Physicians using the M-2 protocol, containing the above three agents, should be aware of this potential complication and consider altering therapy if unexplained pulmonary symptoms arise.

Second malignancies are reported to be more frequent in patients with CLL than in the general population, with an incidence, depending on the series, of 2.5%–34.4%. Phillips et al. studied the occurrence of second malignancies in patients with CLL at Memorial Hospital and recorded an 11% incidence of nondermatological cancers and a 6.6% and 3.6% incidence of basal cell and squamous cell carcinomas of the skin, respectively. In the present series, 13/63 patients (20%) developed a nondermatological second malignancy and 3 patients (4.7%) developed a cutaneous cancer. Of these 16 malignancies, 7 (44%) occurred prior to the M-2 protocol. Willshaw analyzed the incidence of carcinomas in relation to disease and chemotherapy in 106 patients. Eight solid tumors were diagnosed before, 5 simultaneous, and 7 after CLL was diagnosed. Cyclophosphamide has been implicated as a cause of bladder cancer. One patient developed this tumor prior to therapy of CLL. It is not possible to conclude that chemotherapy increased the incidence of solid tumors. More likely, this apparently increased incidence of second malignancies in patients with CLL is a manifestation either of impaired immune function secondary to disease, decreased immune surveillance that accompanies aging, or both.

Although the role of chemotherapy in solid tumor production is unclear, the leukemogenic potentials of alkylating agents, nitrosoureas, and radiation therapy are well known and may be partly responsible for the occurrence of acute nonlymphocytic leukemia in patients treated for CLL. Zarrabi et al. in reviewing the world's literature, recorded 31 cases of acute leukemia in patients with CLL. The overwhelming majority of these patients were treated with alkylating agents and/or radiation therapy. Twelve of the cases were seen in the untreated state and may have represented malignant progressions of CLL rather than a second leukemia. Kyle et al. reported myelomonocytic leukemia in four patients with plasma cell dyscrasias treated with melphalan. Leukemia appeared 30, 33, 44, and 57 mo after melphalan was started. Three patients in the present series (4.7%) have developed acute myelogenous leukemia. In view of the long latency period before the development of "chemotherapy-induced" leukemia, and given the fact that one of these cases was the first patient admitted to the protocol, it is conceivable that a higher incidence of leukemia may be seen.

The M-2 chemotherapy program is an effective treatment for chronic lymphocytic leukemia. The complete and partial remission rate and survival of patients appears to be similar to most reported series of comparably staged CLL patients. The application of the Rai staging system is still valid; however, there appear to be some patients with aggressive disease in whom clinical staging is not sufficient to define their prognosis. Advanced stage patients are as likely to respond to the M-2 as those with early stage disease, although prior therapy remains a poor prognostic factor with respect to response and survival. Chemotherapy is capable of decreasing tumor burden significantly; however, the reaccumulation of malignant B lymphocytes in marrow and nodal sites once therapy is stopped would suggest that eradication of the disease with conventional chemotherapy may not be possible without causing significant toxicity to normal marrow stem cells. Recent advances in the understanding of the immune deficiency of aging, the phenotypic properties of CLL cells, the ability to differentiate these cells in vitro, and the potential usefulness of idiotypic antibodies should help to improve our basic understanding of the malignant change in this disease and result in more specific therapy aimed at preventing the reaccumulation of persistent malignant cells and curing CLL.

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Combination chemotherapy of advanced chronic lymphocytic leukemia: the M-2 protocol (vincristine, BCNU, cyclophosphamide, melphalan, and prednisone)

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