Early Results of a Chemoimmunotherapy Regimen of Fludarabine, Cyclophosphamide, and Rituximab As Initial Therapy for Chronic Lymphocytic Leukemia

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ABSTRACT

Purpose
Fludarabine and cyclophosphamide (FC), which are active in treatment of chronic lymphocytic leukemia (CLL), are synergistic with the monoclonal antibody rituximab in vitro in lymphoma cell lines. A chemoimmunotherapy program consisting of fludarabine, cyclophosphamide, and rituximab (FCR) was developed with the goal of increasing the complete remission (CR) rate in previously untreated CLL patients to ≥ 50%.

Patients and Methods
We conducted a single-arm study of FCR as initial therapy in 224 patients with progressive or advanced CLL. Flow cytometry was used to measure residual disease. Results and safety were compared with a previous regimen using FC.

Results
The median age was 58 years; 75 patients (33%) had Rai stage III to IV disease. The CR rate was 70% (95% CI, 63% to 76%), the nodular partial remission rate was 10%, and the partial remission rate was 15%, for an overall response rate of 95% (95% CI, 92% to 98%). Two thirds of patients evaluated with flow cytometry had less than 1% CD5- and CD19-coexpressing cells in bone marrow after therapy. Grade 3 to 4 neutropenia occurred during 52% of courses; major and minor infections were seen in 2.6% and 10% of courses, respectively. One third of the 224 patients had one episode of infection, and 10% had a fever of unknown origin.

Conclusion
FCR produced a high CR rate in previously untreated CLL. Most patients had no detectable disease on flow cytometry at the end of therapy. Time to treatment failure analysis showed that 69% of patients were projected to be failure free at 4 years (95% CI, 57% to 81%).

INTRODUCTION

The introduction of purine analogs, such as fludarabine, into the treatment of chronic lymphocytic leukemia (CLL) has improved the complete remission (CR) and overall response (OR) rates beyond those seen with alkylating agents such as chlorambucil and cyclophosphamide. Fludarabine was associated with a CR rate of 20% to 30% as initial single-agent therapy for CLL.1-3 On the basis of in vitro evidence of synergism between fludarabine and cyclophosphamide (FC), a combination regimen was developed and was associated with increased CR and OR rates compared with the rates seen with treatment with fludarabine alone.5-7

Rituximab is a chimeric monoclonal antibody that targets the CD20 antigen; cytotoxicity may occur through complement-mediated lysis, antibody-dependent cellular cytotoxicity, and direct induction of
apoptosis.8 In a pivotal trial using rituximab in the treat-
ment of relapsed low-grade lymphoma, disappointing re-
sults were noted in patients with small lymphocytic
lymphoma (SLL), the lymphomatous counterpart of CLL.9
This finding was attributed to the lower expression of CD20
on the surface of SLL cells and the shorter half-life of the
antibody in patients with SLL compared with patients with
follicular lymphoma.9 The shorter half-life was postulated
to be a result of an antigen sink. Subsequent studies showed
that a soluble form of CD20 is present in the plasma of
patients with CLL and that immune complex formation
with the antigen sink may explain the shorter half-life of the
antibody.10 Rituximab had limited activity in previously
treated patients with CLL, with a partial response rate of
10% to 15% at conventional doses.11,12 Subsequent studies
have shown that more intensive dose regimens can in-
crease the OR rate in previously treated CLL patients to
40% to 75%,12,13 and higher response rates are noted
when rituximab is used as initial therapy at the conven-
tional dose and schedule.14,15
Studies in lymphoma cell lines have shown that ritu-
ximab enhances the cytotoxicity of both fludarabine and
cyclophosphamide.16,17 In some cell lines, fludarabine ex-
posure results in downregulation of the complement-
defense proteins CD55 and CD59.18,19 Clinical trials in
low-grade and intermediate-grade lymphoma strongly sug-
gest higher response rates and even longer survival when
rituximab is added to chemotherapy regimens. These ob-
servations provided the rationale for the combination of
fludarabine, cyclophosphamide, and rituximab (FCR) as
therapy for CLL. Subsequently, later studies confirmed
the activity of chemotherapy combined with rituximab in
lymphoma.20,21
The goal of this study was to improve the CR rate using
the National Cancer Institute Working Group (NCIWG)
criteria for response to ≥ 50% for patients receiving initial
therapy for CLL.22 There is a clear correlation between
the response to treatment of CLL and the time to progression
(TTP) and survival.2,24 The NCIWG criteria for CR23 do not
incorporate an evaluation of residual CD5- and CD19-
coexpressing cells by flow cytometry of the bone marrow at
the time remission is documented. In many cases of CR by
NCIWG criteria, cells that coexpress CD5 and CD19 are still
detectable.25 We report that the FCR combination as initial
therapy for CLL achieves a high frequency of CRs with no
detectable disease by flow cytometry.

PATIENTS AND METHODS
Between July 1999 and April 2001, 224 patients with CLL requiring
therapy as indicated by the NCIWG guidelines23 were treated after
informed consent was obtained according to The University of
Texas M.D. Anderson Cancer Center (MDACC) institutional
guidelines. All patients had a pretreatment evaluation including
history, physical examination, CBC with differential, platelet
count, liver and renal function tests, bone marrow aspiration and
biopsy, and bone marrow sample for immunophenotyping. All
patients had a monotypic expansion of CD5-, CD19-, and CD23-
coexpressing lymphoid cells in the peripheral blood (> 10 ×
10^9/L), which were morphologically consistent with CLL, more
than 30% lymphocytes in the bone marrow, and adequate renal
(creatinine < 2 mg/dL) and hepatic (total bilirubin < 2 mg/dL)
function. Fluorescent in situ hybridization studies and analysis of
the mutation status of the immunoglobulin (Ig) gene or ZAP70
expression were not performed.
Flow cytometry testing was performed in the MDACC labora-
tory using established techniques.26 The serum level of beta-
2 microglobulin (β2M) was obtained in 220 patients. One hundred
fifty-five patients (69%) were men. The median age was 58 years
(range, 24 to 86 years). Thirty patients (13%) were older than 70
years. Seventy-five patients (33%) had advanced Rai stage disease
(stage III to IV), and the rest had evidence of progressive stage 0 to
II disease. Nine patients with Rai stage 0 were treated because of
repeated infections, B symptoms, or a lymphocyte doubling time of
less than 6 months. The Zubrod performance status was 0 in 77
patients (34%), 1 in 138 patients (62%), and 2 in nine patients
(4%). The median time from diagnosis to treatment was 26
months (range, 0 to 157 months).

Therapy
On day 1 of the first cycle of FCR, patients received 375
mg/m^2 of rituximab with 25 mg of intravenous (IV) diphenhydra-
mine and 650 mg of oral acetylsalicylic acid as premedication. No
corticosteroids were administered as premedication. The infusion
was interrupted if patients showed signs of grade 3 or 4 toxicity.
and 25 to 50 mg of meperidine was administered IV for chills. If
patients had an elevation of temperature to more than 39°C, 100
mg of hydrocortisone was administered IV. After symptoms sub-
side, the infusion was reinitiated at a slower rate and was com-
pleted in all patients on the first day of therapy. On days 2, 3,
and 4 of the first cycle of therapy, the first nine patients received 30
mg/m^2 of fludarabine IV over 30 minutes and 300 mg/m^2 of
cyclophosphamide IV over 1 hour. Because of significant tumor
lysis noted in three of the first nine patients, all subsequent patients
received fludarabine 25 mg/m^2/day and cyclophosphamide 250 mg/
m^2/day for 3 days; in addition, allopurinol was administered to all
patients in the study at a dose of 300 mg daily for 7 days on the first
cycle. In cycles 2 to 6, the rituximab dose was increased to 500
mg/m^2 on day 1. Fludarabine and cyclophosphamide were admin-
istered on days 1, 2, and 3. Courses were repeated every 4 weeks,
depending on recovery of blood counts, with courses delayed until
the platelet count was greater than 80 × 10^9/L and the absolute
neutrophil count was more than 1 × 10^9/L. For patients begin-
ing therapy with thrombocytopenia, courses were delayed only if
the platelet count had not returned to the baseline level by 4 weeks.
Doses of fludarabine and cyclophosphamide were reduced if
blood counts had not recovered to the levels described earlier 5
weeks after the last course of therapy or if major infections oc-
curred. Both fludarabine and cyclophosphamide were reduced
by one dose level (20 and 200 mg/m^2, respectively) or two dose levels
(15 and 150 mg/m^2, respectively). The rituximab dose was not
reduced. One hundred nine patients received oral trimethoprim-
sulfamethoxazole (TMP-SMX) twice weekly as Pneumocystis cari-
nii pneumonia prophylaxis, and 154 patients received 500 mg
daily of valacyclovir for herpes simplex and herpes zoster infection
prophylaxis. The use of prophylactic antimicrobials, therapeutic
Response Criteria

Response criteria were those previously defined by the NCIWG.23 CR required disappearance of all palpable disease and normalization of blood counts (neutrophils > 1.5 x 10^9/L, platelets > 100 x 10^9/L, and hemoglobin > 11 g/dL), less than 30% lymphocytes on bone marrow aspirate, and no evidence of disease on bone marrow biopsy. A nodular partial remission (NPR) required the same criteria as CR except that one or more lymphoid nodules or aggregates were present on bone marrow biopsy. The criteria for partial remission (PR) included at least 50% reduction in measurable disease and one or more of the following features: neutrophils ≥ 1.5 x 10^9/L or a 50% improvement over baseline, platelets more than 100 x 10^9/L or a 50% improvement over baseline, and hemoglobin more than 11.0 g/dL or a 50% improvement over baseline without transfusions. Computed tomography scans were not required to evaluate response. Remission (partial or complete) occurred only if it persisted for more than 2 months. Bone marrow evaluation was usually performed at the end of therapy, although it was not required for determination of PR. After completion of therapy, patients were re-evaluated at 3-month intervals with history, physical examination, and blood counts. If possible, bone marrow examination was performed 6 and 12 months after treatment.

Flow Cytometry

Bone marrow samples were evaluated by multicolor immunostaining and flow cytometry analysis before treatment and after the third and sixth cycles of therapy.26 Flow cytometry CR was defined as CD5- and CD19-coexpressing cells of less than 1%, with normalization of the kappa:lambda ratio (< 3:1 in patients with monotypic kappa and > 1:3 in patients with monotypic lambda). Flow cytometry relapse was defined as a return to ≥ 5% CD5- and CD19-coexpressing cells, together with an abnormal kappa:lambda ratio.

Statistical Section

The CR rate in previously untreated patients receiving fludarabine as a single agent was 29%,3 and the CR rate with FC in a similar patient population was 35%;3 the major infection rates were 19% and 18% of patients, respectively. The goal of this study was to achieve a CR rate of more than 50%, with less than 40% of patients experiencing major infection. The design by Bryant and Day27 was used to test this outcome. If there was a CR rate of more than 22% or there were less than 38 major infections in the first 57 patients, the study was to continue until the 95% CI did not include a CR rate of 50%. This was accomplished, and after internal discussion, additional patients were recruited onto the study to broaden experience with this effective regimen. Type I and II errors were less than 10%. Because no systematic evaluation of remissions confirmed by flow cytometry had been performed in earlier trials to allow comparison,25,26 this analysis was descriptive.

Survival was measured from the time of the initiation of therapy in all patients. The last follow-up data available for this analysis was from July 31, 2003. Time to relapse was measured from the time of final response evaluation to the development of new lymphadenopathy, splenomegaly, or Richter’s syndrome; to a 50% increase in the size of nodes or the spleen in patients who achieved a PR; to an increase in the peripheral lymphocyte count to more than 10 x 10^9/L on two occasions; or to an increase in bone marrow lymphocytes to more than 50%. This definition of time to relapse is similar to other MDACC studies.25,26 The time to treatment failure was measured from the time of the initiation of therapy until patients failed to respond to FCR and were removed from the study, died, or suffered progressive disease or Richter’s syndrome. Response was evaluated with bone marrow examination on completion of the therapy and again 6 months later. Some patients who had residual disease in the bone marrow or cytopenia on completion of therapy had normal values when re-evaluated 6 months later. The final response, using NCIWG criteria, was identified at the time of the better response. A general linear mixed model was used to create three models for the repeated measurements, IgG, IgA, and IgM, as a function of time since baseline measurement. Because of skewed distributions, each outcome variable was log transformed before analysis. Ig measurements were taken before treatment, after 6 months of treatment, and 12 months after treatment began. However, patients did not necessarily have all the measurements. Partial data was included in the model. Significance was assessed at the 5% level. Separate models were built for each of the three Ig measures studied.

RESULTS

One hundred fifty-six patients (70%; 95% CI, 63% to 76%) achieved a CR, 23 (10%) achieved a NPR, and 34 (15%) achieved a PR (Table 1). Eleven patients did not respond to FCR; six of these patients had an inadequate antitumor response with persistent cytopenia, one developed Richter’s transformation after the first course of treatment, two died of pneumonia after one or two courses of treatment, and two were lost to follow-up after one and two courses and were considered as having experienced treatment failure. The responses in 34 patients who had PRs were classified as such for various reasons. Five patients had residual lymphadenopathy. Four patients had a clinical CR but did not have a confirming marrow. One of these four patients later had a Richter’s transformation after five courses of therapy. Twenty-five patients were classified as PRs because of persistent anemia (five patients) or thrombocytopenia (20 patients). These 25 patients often had no measurable residual disease in nodes (16 of 18 assessable patients), spleen (10 of 11 assessable patients), liver (five of five assessable patients), and peripheral blood (25 of 25 assessable patients). Thirty-four patients who achieved a PR had a follow-up marrow; 16 of these patients had a marrow CR, eight had persistent nodules, two had 50% reduction in marrow infiltrate but more than 30% marrow lymphocytes, and four failed to respond. No marrow evaluation was performed in four patients. Applying the NCIWG criteria for response to various sites of disease, CR was seen in 214 (96%) of 224 assessable patients in the blood, 177 (91%) of 195 patients...
in the lymph nodes, 108 (92%) of 117 patients in the spleen, and 48 (91%) of 53 patients in the liver. One hundred seventy-four patients (78%) had a CR in the marrow, and 48 (91%) of 53 patients in the liver. One hundred eighty-six (92%) of 207 patients had CR in the lymph nodes, 108 (92%) of 117 patients in the spleen, and 30 (65%) of 46 patients in the liver. One hundred sixty-two (97%) of 162 patients were still alive at 5 and 14 months.

Fifty-eight patients (26%) did not complete the prescribed six courses of therapy. The major cause of premature discontinuation of therapy was persistent cytopenia, which was noted in 28 patients. Neutropenia led to discontinuation of therapy in 21 patients. Other reasons for discontinuation were patient preference (n = 6), infection (n = 3), Richter’s syndrome (n = 3), resistant disease (n = 3), cardiac symptoms (n = 3), myelodysplastic syndrome (n = 1), gall bladder cancer (n = 1), rash (n = 1), elevated creatinine (n = 1), and early death (n = 2). Early discontinuation of therapy was significantly associated (P < .05) with advanced Rai and Binet stages, older age, higher WBC counts, lower platelet counts, higher serum β2M level, bone marrow biopsy cellularity more than 50%, and splenomegaly ≥ 5 cm below the left costal margin.

Patients were usually evaluated for response after three courses of therapy and at the end of their therapy (Table 2). Fifty-eight of the 60 patients in CR after three courses were still in CR at the end of therapy. One patient was found to have residual nodules, and one patient developed thrombocytopenia and was classified as a PR. The corresponding values for obtaining CR at the end of therapy for patients with NPR, PR, and stable disease after three courses of therapy were 58 (87%) of 67 patients, 39 (59%) of 66 patients, and three (43%) of seven patients, respectively.

**Survival and Time to Treatment Failure**

The overall survival and time to treatment failure are shown in Figure 1. One hundred fifty-four (99%) of the 156 patients who achieved CR are alive (Fig 2). One patient died of infection after developing delayed pancytopenia, and one patient died in CR from gall bladder cancer. Nine patients have relapsed (Fig 3), and all were still alive as of 2003. One patient relapsed with a Richter’s transformation. Eight of 23 patients who had a NPR have relapsed, and two have died. Nine of 34 patients who achieved a PR have had disease progression (one with Richter’s syndrome, seven with recurrent CLL, and one with worsening cytopenia), and six have died. Three additional patients died of infection while still in PR. Five of the nine patients whose CLL did not respond to therapy have died; one of these patients died after a Richter’s transformation, two died from pneumonia while on therapy, and two died from infection after being taken off the study because of resistance. Two patients experienced early deaths from infection (4 and 7 weeks). Two additional patients who were classified as having experienced treatment failure because of lack of follow-up information were still alive at 5 and 14 months.

**Response As Measured by Flow Cytometry**

Two hundred seven patients had flow cytometric studies on bone marrow aspirates to evaluate residual disease

| Table 1. NCI Working Group Response to FCR by Pretreatment Characteristics |
|---------------------------|-----------------|-----|
| Characteristic            | No. of Patients | CR (%) |
| Total                     | 224             | 70   |
| Rai stage                 |                 |      |
| 0 to II                   | 149             | 76   |
| III to IV                 | 75              | 64   |
| Binet stage               |                 |      |
| A                         | 61              | 82   |
| B                         | 109             | 70   |
| C                         | 54              | 56   |
| Age                       |                 |      |
| < 55 years                | 81              | 80   |
| 55-69 years               | 113             | 68   |
| ≥ 70 years                | 30              | 47   |
| WBC                       |                 |      |
| < 100 × 10⁹/L             | 130             | 75   |
| 100-199 × 10⁹/L           | 77              | 66   |
| ≥ 200 × 10⁹/L             | 17              | 41   |
| Spleen                    |                 |      |
| 0-4 cm below LCM          | 166             | 74   |
| ≥ 5 cm below LCM          | 58              | 57   |
| Beta₂-microglobulin       |                 |      |
| < 3 mg/L                  | 68              | 88   |
| 3.1-4 mg/L                | 59              | 78   |
| > 4 mg/L                  | 93              | 53   |
| Marrow cellularity, biopsy|                 |      |
| < 50%                     | 53              | 79   |
| 50%-80%                   | 98              | 65   |
| ≥ 90%                     | 32              | 62   |

Abbreviations: NCI, National Cancer Institute; FCR, fludarabine, cyclophosphamide, and rituximab; CR, complete remission; OR, overall response; LCM, left costal margin.

P < .05

<p>| Table 2. Comparison of End of Therapy Response With Response at the End of Three Courses |
|-----------------------------------------------|-------|-----|-----|-----|</p>
<table>
<thead>
<tr>
<th>Course 3 Response</th>
<th>Total No. of Patients</th>
<th>CR</th>
<th>NPR</th>
<th>PR</th>
<th>Fail</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>63</td>
<td>58</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>NPR</td>
<td>68</td>
<td>58</td>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>72</td>
<td>39</td>
<td>11</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>NR</td>
<td>13</td>
<td>3</td>
<td></td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete remission; NPR, nodular partial remission; PR, partial remission; NR, no response.
after six courses of therapy (or after their last course of therapy, if only three to five courses were administered). One hundred thirty-eight patients (67%) had less than 1% CD5- and CD19-coexpressing cells (flow cytometry CR), 41 patients (20%) had 1% to 5% coexpressing cells, and 28 patients (14%) had more than 5% coexpressing cells. The proportion of patients in CR with CD5- and CD19-coexpressing cells less than 1% was 78% (120 of 153 patients), which was significantly higher than the five (24%) of 21 patients in NPR (P < .001) and 13 (50%) of 26 patients in PR (P < .001). Flow cytometry CR was significantly less common in patients with marked splenomegaly, β2M levels more than 3 mg/L, and marrow cellularity ≥ 90% (Table 3).

The relapse rate was analyzed according to the NCIWG criteria and the percentage of CD5- and CD19-coexpressing cells in the marrow aspiration at the end of FCR treatment. There was strong correlation between probability of relapse and NCIWG responses, as well as the CD5 and CD19 flow cytometry response (Fig 4 and Table 4). Too few deaths have occurred to analyze risk of dying according to flow cytometry response.

**Toxicity**

The first dose of rituximab was associated with infusional symptoms, which were usually responsive to meperidine or hydrocortisone; grade 3 to 4 toxicities were rare (Table 5). Adverse reactions to rituximab in courses 2 through 6 were noted in only three patients. In courses 2 through 6, rituximab was usually infused over 2 to 4
hours. Toxicities associated with FC were similar to the toxicities seen in previous studies, with nausea occurring during 23% of the courses and vomiting occurring during 7%. Nausea and vomiting tended to occur late and often began on day 3 of the chemotherapy or after it had been discontinued. Prophylactic ondansetron was administered to all patients. One patient complained of hair loss, and one patient developed hemorrhagic cystitis.

Cytopenia and Infection

The adverse effects observed with FCR were myelosuppression and infections. Grades 3 and 4 neutropenia occurred in 24% and 28% of 927 assessable courses, respectively. Grades 3 and 4 thrombocytopenia occurred in 4% and less than 1% of courses, respectively. Despite the significant incidence of neutropenia, only 2.6% of the courses were associated with major infections, including pneumonia (20 episodes) or septicemia (11 episodes; Table 6). Minor infections, such as fever of unknown origin, cellulitis, urinary tract infections, upper respiratory infections, sinusitis, or bronchitis, were reported in 10% of the courses. The organisms identified in the documented infections were *P. carinii* (*n* = 3), *Aspergillus* (*n* = 2), torulopsis glabrata (*n* = 1), *Pseudomonas* (*n* = 2), *Escherichia coli* (*n* = 1), *Enterococcus* (*n* = 2), and cytomegalovirus (*n* = 1). Eleven patients experienced a reactivation of herpes simplex (*n* = 8) or herpes zoster (*n* = 3).

TMP-SMX (one 800 mg/160 mg tablet twice a day) was administered to 109 patients on a Saturday and Sunday or every Monday, Wednesday, and Friday regimen for *P. carinii* pneumonia prophylaxis. There was no difference in the incidence of grade 3 to 4 neutropenia or major or minor infections in patients administered TMP-SMX compared with patients who did not receive prophylaxis. Prophylactic valacyclovir (500 mg) was administered to 154 patients; none of these patients developed herpes zoster reactivation.

Table 5. Toxicities Associated With the First Infusion of Rituximab

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 1 to 2</th>
<th>Grade 3 to 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever and chills</td>
<td>94 (42)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>22 (10)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>28 (13)</td>
<td>— (—)</td>
</tr>
<tr>
<td>Nausea</td>
<td>25 (11)</td>
<td>— (—)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>10 (5)</td>
<td>— (—)</td>
</tr>
<tr>
<td>Back pain</td>
<td>7 (3)</td>
<td>— (—)</td>
</tr>
<tr>
<td>Urticaria</td>
<td>7 (3)</td>
<td>— (—)</td>
</tr>
<tr>
<td>Headache</td>
<td>5 (2)</td>
<td>— (—)</td>
</tr>
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</table>

Table 6. Incidence of Infections With FCR Therapy by NCIWG Response, Lowest Neutrophil Count per Course, and Course Number

<table>
<thead>
<tr>
<th>Infection</th>
<th>No. of Courses</th>
<th>Total No. of Infections</th>
<th>No. of Major Infections</th>
<th>Risk per Course†</th>
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</thead>
<tbody>
<tr>
<td>Response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>889</td>
<td>105</td>
<td>18</td>
<td>0.12</td>
</tr>
<tr>
<td>NPR</td>
<td>126</td>
<td>11</td>
<td>—</td>
<td>0.09</td>
</tr>
<tr>
<td>PR</td>
<td>165</td>
<td>26</td>
<td>11</td>
<td>0.16</td>
</tr>
<tr>
<td>NR</td>
<td>24</td>
<td>8</td>
<td>2</td>
<td>0.33</td>
</tr>
<tr>
<td>Nadir neutrophils‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 500 × 10⁹/L</td>
<td>257</td>
<td>23</td>
<td>5</td>
<td>0.09</td>
</tr>
<tr>
<td>500-999 × 10⁹/L</td>
<td>224</td>
<td>36</td>
<td>5</td>
<td>0.16</td>
</tr>
<tr>
<td>≥ 1,000 × 10⁹/L</td>
<td>442</td>
<td>46</td>
<td>9</td>
<td>0.10</td>
</tr>
<tr>
<td>Course number</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Courses 1 to 3</td>
<td>627</td>
<td>86</td>
<td>17</td>
<td>0.14</td>
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<tr>
<td>Courses 4 to 6</td>
<td>527</td>
<td>76</td>
<td>14</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Abbreviations: NCIWG, National Cancer Institute Working Group; FCR, fludarabine, cyclophosphamide, and rituximab; CR, complete remission; NPR, nodular partial remission; PR, partial remission; BM, bone marrow.

Table 4. Relapse by NCIWG and Flow Cytometry Responses at the End of FCR to Determine Residual Disease (CR + NPR + PR)

<table>
<thead>
<tr>
<th>Response Criteria</th>
<th>No. of Patients</th>
<th>No. of Patients</th>
<th>%</th>
<th>No. of Patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCIWG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>156</td>
<td>9</td>
<td>5.8*</td>
<td>2</td>
<td>1.3*</td>
</tr>
<tr>
<td>NPR</td>
<td>23</td>
<td>8</td>
<td>34.8</td>
<td>2</td>
<td>8.7</td>
</tr>
<tr>
<td>PR</td>
<td>34</td>
<td>9</td>
<td>26.5</td>
<td>9</td>
<td>26.5</td>
</tr>
<tr>
<td>CD5 + CD19 (BM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1%</td>
<td>138</td>
<td>5</td>
<td>3.61</td>
<td>4</td>
<td>2.81</td>
</tr>
<tr>
<td>1%-4.9%</td>
<td>41</td>
<td>8</td>
<td>19.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>≥ 5%</td>
<td>21</td>
<td>9</td>
<td>43</td>
<td>3</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Abbreviations: NCIWG, National Cancer Institute Working Group; FCR, fludarabine, cyclophosphamide, and rituximab; NR, nodular partial remission; PR, partial remission; BM, bone marrow.

*CR v NPR + PR, *P* < .001.
†< 1% v 1%-4.9% v ≥ 5%, *P* < .001.
‡Not available for all infections.

Fig 4. Time to Relapse by CD5 and CD19 flow cytometry response.
compared with three of 70 patients who did not receive prophylaxis. The incidence of fever and infection was no different in the first three courses of therapy compared with courses 4 to 6. There was no statistically significant association between the degree of neutropenia (≤1 × 10^9/L) and the incidence of major, minor, or herpetic infection (Table 6).

The Coomb’s (direct antibody) test was performed within 3 months of enrollment on the study in 101 patients and was positive in 13 patients. Autoimmune hemolysis developed in three of these 13 patients, and one patient had red cell aplasia. Autoimmune hemolytic anemia (AIHA) developed in seven (8%) of the 88 patients who were Coomb’s negative. In 123 patients without a Coomb’s test before therapy, there were six episodes (5%) of AIHA and one episode of red cell aplasia. A clear episode of immune thrombocytopenia was noticed in one patient.

Dose reductions were required in 35 patients. Nine of these patients had to discontinue therapy despite dose reductions. The incidence of dose reduction was 13% (21 of 156 patients) in patients who had a CR and 25% (14 of 57 patients) in patients who had a NPR or a PR. The incidence was significantly higher in patients older than 60 years and in patients with pretreatment Rai stage IV disease (P = .01).

Hypogammaglobulinemia was noted for IgG in 75 patients, IgA in 46 patients, and IgM in 50 patients. Sequential follow-up of Ig levels were not mandated in this study. There was no significant difference in the levels of IgG, IgA, or IgM between the starting level and the levels at the end of therapy (Table 7). Although some patients improved their IgG, IgA, and IgM levels after therapy, an equal number of patients decreased their level. No significant trend was noted with the Ig levels according to response.

However, patients who had low IgG, IgA, or IgM levels at the start of therapy; had achieved a CR; and had Ig levels measured at the end of therapy or at later times were found to be in the normal range in seven (18%) of 40 patients for IgG, five (9%) of 54 patients for IgA, and 14 (36%) of 39 patients for IgM. There was considerable oscillation in Ig levels during follow-up. There was a significant decrease in the mean and median distributions of IgG at the start of therapy and at 6 months after the end of treatment. Of the 224 patients, 105 (47%) had all three IgG measurements, 70 (31%) had two measurements, 45 (20%) had one measurement, and four (2%) had no IgG data. The general linear mixed model examined time in months as the only covariate. Because of skewed distributions, the IgG measurements were log transformed. The model revealed that there was a significant association (P < .001) between time and log IgG level, with a coefficient of −0.014 (SE = 0.003). Therefore the log IgG levels decreased 0.014 units per month over the 12 months of follow-up. There was no significant difference in the mean values for IgA and IgM, although values 6 months after the end of treatment were somewhat lower than at the start of therapy. The mean and median levels of Ig and the SEs and standard deviations are listed in Table 7.

### Comparison of the Current FCR Study and Historical Experience With FC

Thirty-four patients who had received FC as their initial therapy for CLL were previously reported. These patients received higher doses of fludarabine (30 mg/m^2 three times daily) and cyclophosphamide (300 mg/m^2 three times daily) than the doses administered with FCR. Only 14 (41%) of 34 patients treated with FC were able to complete all six prescribed courses. Twelve patients (35%) discontinued FC because of persistent cytopenia. Four patients had AIHA, and two of these patients had AIHA before starting FC. The clinical characteristics, response data, and comparison of survival, time to treatment failure, and TTP of responders are listed in Table 8. The CR rate, proportion of patients with CD5 and CD19 cells in their bone marrow less than 1%, time to treatment failure, TTP, and survival were significantly better with FCR than with FC.

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**Table 7. Levels of Serum IgG, IgA, and IgM at Various Time Points for FCR Patients**

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th></th>
<th>IgA</th>
<th></th>
<th>IgM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
<td>End of Rx</td>
<td>6 Months After Rx</td>
<td>Start</td>
<td>End of Rx</td>
<td>6 Months After Rx</td>
</tr>
<tr>
<td>Patients, No.</td>
<td>198</td>
<td>159</td>
<td>143</td>
<td>197</td>
<td>158</td>
<td>141</td>
</tr>
<tr>
<td>Levels, mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>855</td>
<td>747</td>
<td>688*</td>
<td>96</td>
<td>94</td>
<td>89</td>
</tr>
<tr>
<td>SE</td>
<td>38</td>
<td>27</td>
<td>28</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Median</td>
<td>749</td>
<td>690</td>
<td>636*</td>
<td>76</td>
<td>75</td>
<td>67</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>541</td>
<td>338</td>
<td>338</td>
<td>81</td>
<td>69</td>
<td>73</td>
</tr>
</tbody>
</table>

**Abbreviations:** Ig, immunoglobulin; FCR, fludarabine, cyclophosphamide, and rituximab; Rx, therapy.

*P < .05.
Table 8. Comparison of Patient Characteristics and Outcome Between FC and FCR

<table>
<thead>
<tr>
<th>Characteristic and Outcome</th>
<th>FC (n = 34)</th>
<th>FCR (n = 224)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>Median</td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>33-92</td>
</tr>
<tr>
<td></td>
<td>24-86</td>
<td></td>
</tr>
<tr>
<td>Sex, No.</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>195</td>
<td>69</td>
</tr>
<tr>
<td>Rai stage, No.</td>
<td>0-II</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>149</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III-IV</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>75</td>
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<tr>
<td>WBC, × 10^9/L</td>
<td>Median</td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>93.9</td>
<td>84.5</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>6.6-304</td>
</tr>
<tr>
<td></td>
<td>1.8-619.5</td>
<td></td>
</tr>
<tr>
<td>β2M, mg/dL</td>
<td>Median</td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>1.6-11.1</td>
</tr>
<tr>
<td></td>
<td>1.8-16.4</td>
<td></td>
</tr>
<tr>
<td>CR, %</td>
<td>35</td>
<td>70</td>
</tr>
<tr>
<td>NPR, %</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td>OR, %</td>
<td>89</td>
<td>95</td>
</tr>
<tr>
<td>CD5 + CD19; end of Rx, %</td>
<td>Median</td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>12.8</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>0.94-3</td>
<td>0.97-7</td>
</tr>
<tr>
<td>Major infections per course</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Minor infections per course</td>
<td>0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>Toxicity, %</td>
<td>Neutropenia</td>
<td>Grade 3</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Grade 4</td>
<td>28</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Grade 3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Grade 4</td>
<td>0</td>
</tr>
<tr>
<td>Median survival, months</td>
<td>73+</td>
<td>NR†</td>
</tr>
<tr>
<td>TTF, months</td>
<td>40</td>
<td>NR†</td>
</tr>
<tr>
<td>TTP, months</td>
<td>47</td>
<td>NR†</td>
</tr>
</tbody>
</table>

Abbreviations: FC, fludarabine and cyclophosphamide; FCR, fludarabine, cyclophosphamide, and rituximab; β2M, beta2-microglobulin; CR, complete remission; NPR, nodular partial remission; OR, overall response; Rx, therapy; TTF, time to treatment failure; NR, not reached; TTP, time to progression.

DISCUSSION

The use of fludarabine for initial treatment of CLL1-3 has increased the CR and OR rates compared with the rates seen with alkylating agents. Studies comparing fludarabine with cyclophosphamide, doxorubicin, and prednisone and chlorambucil1,3 confirmed the single-institution studies that showed high response rates.2 Combinations of FC have been associated with promising CR and OR rates.5-7 Rituximab, as a single agent, was more active in CLL when used in higher dose-intensity schedules than those recommended for follicular lymphoma and in previously untreated patients with CLL.12-15

The CR rate of 70% with FCR (using NCIWG criteria) is the highest rate reported for initial therapy for CLL, which supports the concept of additive or synergistic inter-

actions of these three agents.20,21 Preclinical data suggested that rituximab sensitized cells to both fludarabine and cyclophosphamide16-18 and that fludarabine may enhance one of rituximab's modes of action by downregulating complement-resistance proteins.19

The improvement in the CR rate with FC over fludara-

bline alone2,5 was modest (35% v 29%, respectively), suggest-

ing that the addition of rituximab was crucial to the success of this FCR regimen. A combination of fludarabine and rituximab has been investigated in the treatment of CLL in a Cancer and Leukemia Group B (CALGB) study that showed a higher CR rate when the two agents were admin-

istered simultaneously as opposed to sequentially.28 The CALGB study evaluated six 5-day courses of fludarabine either administered alone (51 patients) or combined with 375 mg/m² of rituximab administered on day 1 of each course and on day 5 of the first course (53 patients). Two months later, all stable or responding patients on both arms of the study received 4 weekly consolidation doses of rituximab. Several patients showed improved response with the consolidation phase. The OR and CR rates for the concurre-

ant arms were 90% and 47%, respectively. The OR and CR rates for the patients treated with sequential therapy were 77% and 28%, respectively. The CR rate after the six cycles of concurrent fludarabine and rituximab was 33% (approximately half the rate seen after six cycles of FCR). The early progression-free and overall survival results for the CALGB study are similar to those reported here to date. A comparison of the results of this study with the fludarabine-alone study population of the immediately preceding CALGB study showed a higher response rate and improved progression-free survival for the group receiving rituximab.29

The criteria for response in CLL have been formalized by the NCIWG.23 Application of these criteria has helped to compare responses in previous clinical studies. A clear relationship between response and both survival and TTP has been established.2,24 Additional criteria for response have been suggested. A decrease in the percentage of CD5- and CD19-coexpressing B cells has been associated with a pro-

longed TTP25 and is supported by this study. A recent study suggested that four-color flow cytometry to detect CD5/19/20/79b-expressing cells was more sensitive than polymerase chain reaction in detecting residual disease and predicting relapse.30 FCR therapy resulted in a marked decrease in the percentage of CD5- and CD19-coexpressing cells in the bone marrow to less than 1% in two thirds of responding patients. The median value for patients treated with FC alone was 12.8%.5 Thus, the addition of rituximab to FC is associated with significantly greater reductions in residual CLL cells in the marrow than the reductions seen with FC alone. In a study using single-agent fludarabine, only nine (39%) of 23 patients with NCIWG CR were in remission by flow cytometric criteria (CD5- and CD19-coexpressing


cells < 10%) compared with 120 (78%) of 153 patients with CD5- and CD19-coexpressing cells less than 1% with FCR. The follow-up time was not adequate to assess the effect of flow cytometry remissions as a surrogate end point of long-term prognosis.

The FCR regimen was well tolerated. Grade 3 to 4 symptoms of fever, chills, and hypotension with rituximab occurred in nine patients (6%), and 6% of first infusions were associated with dyspnea, but the infusions were completed in all patients on the first day. The frequency of nausea and vomiting with FCR was similar to that seen with FC alone and was usually easily controlled. Only three patients chose to discontinue therapy after three courses because of toxicity.

Despite the addition of a B-lymphocyte-depleting agent to FC, no substantial difference between the infection profiles of FCR and FC was noted. Neutropenia was more common with FCR than with FC, although the mechanisms for this observation remain uncertain. A higher incidence of neutropenia was noted when rituximab was administered with fludarabine (76%) compared with the same dose of fludarabine administered alone (39%) in the CALGB study. Thrombocytopenia was rare. AIHA and red cell aplasia occurred in 18 patients (8%), which is comparable with other fludarabine-based regimens.32 Tumor lysis was not observed in the FCR patients who received allopurinol, which should be used prophylactically.

Several patients developed prolonged myelosuppression (> 6 weeks), which was more common in older patients with Rai stage III and IV disease. Myelosuppression support in patients with previously untreated indolent lymphoid malignancies. Blood 96:71-75, 2000
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The combination of two chemotherapy drugs, FC, with the monoclonal antibody rituximab was associated with a high CR rate and evidence of a high degree of clearing of malignant cells in the bone marrow. A similar high CR rate has been reported for the fludarabine, cyclophosphamide, and mitoxantrone regimen.33 The effect of this increase in high-quality CR (morphologic, molecular, and by flow cytometric analysis) raises the possibility that the use of such regimens may translate into an improved prognosis.

Comparative studies are needed to confirm the effect of the addition of rituximab to FC. Long-term follow-up of the effect of flow cytometric–measured remissions will help to establish the role of these measurements as a surrogate for improved time to relapse or survival.

Authors’ Disclosures of Potential Conflicts of Interest
The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Acted as a consultant within the last 2 years: Michael Keating, Berlex, Genentech. Received more than $2,000 a year from a company for either of the last 2 years: Michael Keating, Berlex, Genentech.

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