Sequential Therapy With Fludarabine, High-Dose Cyclophosphamide, and Rituximab in Previously Untreated Patients With Chronic Lymphocytic Leukemia Produces High-Quality Responses: Molecular Remissions Predict for Durable Complete Responses

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ABSTRACT

Purpose

Modern combination strategies are active in chronic lymphocytic leukemia (CLL) but can have significant myelosuppression and immunosuppression that may require dose attenuation for safety. We explored a sequential treatment strategy to allow safe delivery of active agents at full doses. Previously, we studied sequential therapy with fludarabine followed by cyclophosphamide (F→C). In that study, cyclophosphamide consolidation improved the frequency of complete response (CR) four-fold. Subsequently, rituximab was added to this regimen (F→C→R).

Patients and Methods

Thirty-six previously untreated CLL patients received therapy with fludarabine 25 mg/m² on days 1 through 5 every 4 weeks for six cycles, followed by consolidation with cyclophosphamide 3,000 mg/m² administered every 3 weeks for three cycles, followed by consolidation with weekly rituximab 375 mg/m² for four cycles. Evaluation for minimal residual disease included flow cytometry and a highly sensitive clonotypic polymerase chain reaction (PCR). The median age was 59 years (range, 37 to 71 years), 61% of patients had high-risk disease, and 58% had unmutated *IgV_H* genes.

Results

There were 32 responses (89%), including 22 CRs (61%). Consolidation with cyclophosphamide improved responses in 13 patients (36%); nine patients (25%) further improved their response with rituximab. Twenty patients (56%) achieved flow cytometric CRs, and 12 patients (33%) achieved a molecular CR (PCR negative). Patients achieving molecular CRs had an excellent prognosis with a plateau in the response duration curve, and 90% remain in clinical CR at 5 years. For the entire group, 5-year survival rate is 71% compared with a rate of 48% with our prior F→C regimen ($P = .10$).

Conclusion

Sequential therapy with F→C→R yields improvement in quality of response, with many patients achieving a PCR-negative state.

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INTRODUCTION

The introduction of purine analogs has changed treatment options for patients with chronic lymphocytic leukemia (CLL). In a prospective randomized study, fludarabine was demonstrated to produce a superior frequency of response compared with chlorambucil, including more complete responses (CRs). Unfortunately, fludarabine produced CRs in only a minority of patients (20%) and did not convey a survival advantage.¹ To improve the frequency of CR, investigators previously evalu-

ated combination therapy, and trials of fludarabine combined with corticosteroids² or chlorambucil^{3,4} were conducted. The results of these initial combinations were disappointing, with increased toxicity limiting dose-intensity and without clear-cut improvement in responses. More recently, combinations of fludarabine with cyclophosphamide ± rituximab have been administered to patients, but such regimens require careful attention to dosing because this synergistic combination has potent immunosuppressive and myelosuppressive effects leading to a substantial risk of infection.⁵

To take advantage of the activity of these agents without sacrificing dose-intensity, we avoided concomitant administration and, instead, combined these agents using a sequential treatment program. We previously reported that induction therapy with fludarabine followed by consolidation with high-dose cyclophosphamide markedly improves the frequency of CR compared with treatment with fludarabine alone (CR in 38% of patients after consolidation with high-dose cyclophosphamide compared with 8% of patients after single-agent fludarabine).⁶ Given those encouraging results, we added rituximab as a non-cross-resistant second consolidation to create the sequential fludarabine, cyclophosphamide, and rituximab regimen (F→C→R) and now report the results of that trial and compare it with our prior fludarabine followed by cyclophosphamide (F→C) treatment.

PATIENTS AND METHODS

Patients were required to have Rai intermediate- or high-risk CLL and to have active disease as defined by the National Cancer Institute (NCI) Working Group.⁷ All patients gave written informed consent. This study was reviewed and approved by the Institutional Review Board of Memorial Hospital.

Trial Design

Patients received induction with fludarabine 25 mg/m²/d intravenously for 5 days every 4 weeks. All patients received sulfamethoxazole-trimethoprim or alternate for *Pneumocystis carinii* pneumonia prophylaxis and acyclovir for herpes zoster prophylaxis. Filgrastim was not administered before protocol therapy and was only administered to patients who were neutropenic or developed neutropenia after fludarabine therapy. Patients with no response after three cycles of fludarabine went directly to consolidation with high-dose cyclophosphamide; all other patients received six cycles of fludarabine. Four to 6 weeks after completing fludarabine treatment, patients received the first consolidation with intravenous cyclophosphamide 3,000 mg/m² every 3 weeks for three doses. Patients received aggressive hydration to prevent hemorrhagic cystitis and prophylactic filgrastim and ciprofloxacin. Approximately 4 weeks after completing cyclophosphamide, patients received the second consolidation with rituximab 375 mg/m² once weekly for four doses.

Evaluation Criteria

Pretreatment evaluation included a history, physical examination, CBC, comprehensive profile, lactate dehydrogenase, uric acid, phosphorus, immunofixation, quantitative immunoglobulins, β_2 -microglobulin, and immunophenotyping of blood and bone marrow by flow cytometry. Blood or bone marrow samples were also assessed for trisomy 12 by fluorescent in situ hybridization (FISH) using a centromeric probe for chromosome 12.⁸ Radiographic studies were not required, but if performed at baseline, they were repeated to assess for response after each phase of therapy.

Responses were graded according to the NCI Working Group criteria.⁷ In addition to standard testing, peripheral blood and/or bone marrow samples were analyzed by flow cytometry using a bright CD45 (lymphocyte) gate for CD5/CD19 dual staining and κ/λ clonal excess.⁹ These evaluations were performed at baseline, before the fourth cycle of fludarabine, before cyclophosphamide treatment, before rituximab treatment, and 4 to 6 weeks after completion of rituximab.

Patients with trisomy 12 by FISH analysis had subsequent analyses for minimal residual disease (MRD). Because this trial opened to accrual in 1998, more than 2 years before the landmark study published by Döhner et al¹⁰ in December 2000, we did not require evaluation for other chromosomal abnormalities.

Flow cytometric analysis was evaluated separately as an indication of MRD but was not included in the definition of response. Flow cytometric CR was defined as no clonal excess and a normal number of CD5/CD19 dual staining cells (< 5% of the lymphocyte gate).⁶

Using pretreatment samples obtained from blood and/or bone marrow, patient/tumor-specific oligonucleotides were generated for the subsequent

detection of MRD. The immunoglobulin heavy-chain locus (IgV_H) containing clone-specific complementarity determining region III (CDR-III) was amplified by polymerase chain reaction (PCR) and sequenced. On the basis of the clone-specific sequence, semi-nested oligonucleotide primers were constructed for each patient/tumor. These primers were then used in a two-step semi-nested clonotypic PCR to test subsequent blood/bone marrow samples for MRD. This technique can detect one malignant cell in a background of 100,000 polyclonal cells. A molecular CR was defined as patients who had no evidence of disease by PCR. A more thorough review of this PCR technique is described elsewhere.¹¹

Toxicity

Hematologic toxicity was graded according to the NCI Working Group guidelines.⁷ Nonhematologic toxicity was assessed according to the NCI Common Toxicity Criteria.¹²

Statistical Methods

Response duration, time to treatment failure, and overall survival were calculated according to the Kaplan-Meier method. Response duration was measured from time of maximum response until disease progression or relapse. Survival was measured from start of protocol therapy to death. The log-rank test was used to compare differences in response duration and overall survival for F→C and F→C→R. To determine whether tumor response was associated with improved survival, a landmark analysis was performed in which survival time was defined as the time from 1 month after initiating treatment (approximately time of first response assessment) to the date of death or last follow-up. This was done to correct for patients who died before they had an opportunity to be assessed for tumor response.

RESULTS

Patient Characteristics

Thirty-nine patients were registered between September 1998 and September 2004. The median follow-up time is 60 months. Three patients were unassessable and withdrew consent from protocol therapy before receiving consolidation with high-dose cyclophosphamide. One patient with recurrent prostate cancer withdrew consent after five cycles of fludarabine (partial response [PR] at time of withdrawal). The second patient withdrew consent after five cycles of fludarabine seeking alternative therapy (PR at time of withdrawal). The third patient withdrew consent after not returning for follow-up after two cycles of fludarabine. No patient withdrew consent from therapy during the cyclophosphamide or rituximab phases of treatment. Of the 36 assessable patients, 14 (39%) had Rai intermediate-risk disease, and 22 (61%) had high-risk disease. IgV_H mutational status is available for all 36 patients, and 21 patients (58%) were unmutated. Other patient characteristics are listed in Table 1.

Response

Thirty-two (89%) of 36 assessable patients achieved a response. After completion of all therapy, there were 22 CRs (61%), two nodular PRs (nPRs; 6%), eight PRs (22%), and four treatment failures (11%). One patient classified as having a PR had mild thrombocytopenia with increased megakaryocytes in the marrow. This patient had no detectable disease on physical examination or blood/bone marrow by histology, flow cytometry, or PCR. Although we believe this patient's abnormalities were caused by immune-mediated thrombocytopenia (ITP) and not persistent leukemia, we have categorized him as PR in accordance with the formal criteria. Four patients were considered as having treatment failure after fludarabine and were removed from the study; two of these patients developed severe immune cytopenias

Table 1. Patient Characteristics

Characteristic	No. of Patients (N = 36)	%
Sex		
Male	28	78
Female	8	22
Age, years		
Median	59	
Range	37-71	
Rai stage		
Intermediate risk	14	39
High risk	22	61
Anemia (hemoglobin < 11 g/dL)	9	25
Thrombocytopenia (< 100,000/ μ L)	11	31
Anemia and thrombocytopenia	2	6
IgV _H mutational status		
Mutated	15	42
Unmutated	21	58
Blood counts		
WBC, $\times 1,000/\mu$ L		
Median	99	
Range	6.2-531.6	
Hemoglobin, g/dL		
Median	12	
Range	7.5-15.7	
Platelets, $\times 1,000/\mu$ L		
Median	134	
Range	58-366	
Other characteristics		
β_2 -microglobulin > 3 mg/L	19	53
Trisomy 12	7	19

requiring alternate therapy, and the other two patients developed (Richter's) transformed disease necessitating a change in therapy. Two patients who experienced treatment failure with fludarabine proceeded to consolidation after three cycles. All responding patients received six cycles of fludarabine.

Responses after each phase of therapy are shown in Table 2. After completing fludarabine, four patients (11%) achieved a CR, and 32 patients (89%) did not. Of the 32 patients who did not achieve a CR after fludarabine, consolidation with high-dose cyclophosphamide improved the response in 13 (41%). After consolidation with high-dose cyclophosphamide, 14 patients had a CR (39%), and 22 patients had lesser responses. Of these patients, nine (41%) with PRs further

improved their response when consolidated with rituximab (eight achieved a CR and one achieved an nPR).

MRD

After treatment, 20 patients (56%) achieved a flow cytometric CR. Flow cytometric CR correlated with CR except in three patients who achieved a CR but had MRD by flow cytometry and the one patient with a flow cytometric CR who was characterized as a PR (because of ITP).

The 22 patients who achieved a CR and the one patient who was classified as a PR because of ITP but had no other evidence of residual morphologic disease were evaluated for molecular response. Of these patients, 22 had assessable samples after induction therapy with fludarabine, and all but one patient had detectable disease. Twenty-two patients had assessable samples after completion of consolidation with high-dose cyclophosphamide, and seven patients (19% of all patients and 32% of those tested) had no detectable disease by clonotypic PCR. After completion of all therapy, patients were tested by PCR a median of three times (range, one to seven times) at various time points up to 45 months after treatment. Twelve patients (33% of all patients and 52% of those tested) had at least transient eradication of PCR-detectable disease.

FISH Response for Patients With Trisomy 12

Seven (19%) of 36 patients had trisomy 12 by FISH analysis. All seven of these patients achieved a CR and also tested negative for trisomy 12 at the completion of therapy.

Response by Mutational Status

In 21 (58%) of 36 patients, the IgV_H gene was unmutated. For these patients, the overall response frequency was 81%; 10 patients (48%) achieved a CR, one patient (5%) achieved an nPR, and six patients (28%) achieved a PR. In 15 patients (42%), the IgV_H gene was mutated; all of these patients responded, with 12 patients (80%) achieving a CR, one patient (7%) achieving an nPR (7%), and two patients (13%) achieving a PR.

Response Duration and Survival

The median response duration was 43 months (Fig 1). Patients with high-quality responses (CR/nPR) had superior overall survival compared with the remaining patients, with a Kaplan-Meier survival rate of 92% at 5 years (median survival not reached) compared with 33% survival at 5 years (median, 35 months) for the

Table 2. Patient Responses After Each Phase of Therapy

Response	Fludarabine (N = 36)		High-Dose Cyclophosphamide (N = 36)		All Treatment (N = 36)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%
Complete response	4	11	14	39	22	61
Nodular partial response	1	3	1	3	2	6
Partial response	25	69	17	47	8	22
Failure	6	17	4	11	4	11
Flow cytometric complete response	6	17	15	42	20	56
Molecular (PCR) complete response	1	3	7	19	12	33

Abbreviation: PCR, polymerase chain reaction.

Fig 1. Response duration for the 32 responding patients was 43 months. Tick mark indicates last follow-up. CR, complete response; NR, nodular response; PR, partial response.

other patients ($P = .0008$; Fig 2). When patients are categorized by stage of disease, those who achieved a high-quality response had superior survival compared with their counterparts who did not regardless of disease stage at enrollment. The Kaplan-Meier estimated survival rate at 5 years was 93.3% for patients with Rai high-risk disease who achieved a CR/nPR compared with an estimated 14.3% survival rate at 5 years for all others with high-risk disease (Fig 3). Patients with mutated IgV_H had a median response duration of 46 months compared with 35 months for unmutated patients ($P =$ not significant).

The 12 patients who achieved a CR and were MRD negative by clonotypic PCR had a longer response duration than the 11 patients who achieved CR who were PCR positive (median response duration, not reached v 34.9 months, respectively; $P = .007$). The response duration curve for the PCR-negative patients seems to plateau, with 90% of patients still in morphologic CR at 5 years (Fig 4).

Fig 2. Overall survival of patients with high-quality responses (complete response [CR] + nodular partial response [nPR], $n = 24$) compared with the remaining patients (partial response [PR] + nonresponders, $n = 12$). Survival was measured from the start of protocol treatment. Tick mark indicates last follow-up.

Fig 3. Overall survival stratified by Rai stage and response: Rai high risk and complete response (CR)/nodular partial response (nPR) compared with Rai high risk and partial response (PR)/nonresponders compared with Rai intermediate risk and CR/nPR compared with Rai intermediate risk and PR/nonresponders. Survival was measured from the start of protocol treatment. Tick mark indicates last follow-up.

Toxicity

There were no treatment-related deaths. Grade 3 or 4 anemia occurred in six patients (17%) including one who developed autoimmune hemolytic anemia during treatment with fludarabine. Neutropenia occurred in 32 patients (89%), and thrombocytopenia occurred in 28 patients (78%). Grade 3 or 4 infectious complications occurred in only five patients (14%); two patients had pneumonia, one patient had colitis complicated by catheter-related bacteremia, and two patients had skin infections.

Comparison to F→C

Compared with our prior F→C regimen, we note that the patient characteristics of the two groups are comparable in terms of age, sex, disease stage, and β_2 -microglobulin.⁶ Table 3 compares the results of

Fig 4. Patients achieving a complete response (CR) stratified by molecular polymerase chain reaction (PCR)-negative ($n = 12$) versus PCR-positive ($n = 11$) status. Tick mark indicates last follow-up.

Table 3. F→C v F→C→R: Response in CLL Patients

Response	F→C (N = 25)		F→C→R (N = 36)	
	No. of Patients	%	No. of Patients	%
Total responses	19	76	32	89
Complete response	8	38	22	61
Nodular partial response	4	12	2	6
Partial response	7	33	8	21
Treatment failures	6	24	4	11
Median response duration, months	15		43	
Survival				
Median, months	58		Not reached	
5-year survival rate	48		71	
Flow cytometric complete response	9	36	20	56
Molecular PCR complete response	3	12	12	33

Abbreviations: F→C, sequential fludarabine and cyclophosphamide; F→C→R, sequential fludarabine, cyclophosphamide, and rituximab; CLL, chronic lymphocytic leukemia; PCR, polymerase chain reaction.

the sequential F→C→R regimen to our prior two-drug combination (F→C). The addition of rituximab increased the frequency of CR (61% with F→C→R v 38% with F→C). Survival at 5 years was 71% (median not yet reached) with F→C→R compared with 48% (median, 58 months) for F→C (Fig 5). There was also an improvement in flow cytometric CR (56% with F→C→R v 36% with F→C). Molecular CRs were seen in 33% of patients on F→C→R compared with 12% of patients on F→C. Despite the use of high-dose cyclophosphamide in the 61 patients treated on these sequential programs, there have been no cases of myelodysplastic syndrome/secondary acute myeloid leukemia.

DISCUSSION

The results of this study using sequential F→C→R yield similar findings to two other reported combination regimens used as initial therapy for CLL.^{13,14} There are obvious limitations in comparing phase II trials, and the apparent differences in the frequency of CR may

be a result of several factors including schedule and dosing of agents administered and patient selection. The M. D. Anderson Cancer Center group reported that therapy with concurrent FCR achieved a CR in 70% of patients (95% CI, 63% to 76%).¹³ Myelosuppression was the major toxicity, with 26% of patients unable to complete six courses of therapy mainly because of persistent cytopenias; in addition, one third of the patients had more than one episode of infection, and an additional 10% had fever of unknown origin. In a Cancer and Leukemia Group B study of two different regimens combining fludarabine and rituximab, there was a higher frequency of CR for concurrent administration compared with sequential therapy (47% v 28%, respectively); however, rituximab dosing in the two groups was not similar.¹⁴ The concurrent arm received 11 doses (total dose = 4,125 mg/m²) compared with four doses (total dose = 1,500 mg/m²) in the sequential group. Despite more CRs in the concurrent arm, there was no difference in progression-free survival or overall survival between these groups, and in later analyses, these groups were combined.¹⁵

We chose a sequential program to take advantage of the activity of these agents without sacrificing dose-intensity with the goal of inducing high-quality sustained responses. We anticipated that this would allow patients to complete the planned therapy with less toxicity than concurrent strategies. Despite grade 3 or 4 neutropenia occurring in 50% of patients during fludarabine treatment and in 67% of patients during high-dose cyclophosphamide, only five patients (14%) experienced grade 3 or 4 infectious complications. There were no early withdrawals because of treatment-related toxicity. With the exception of the four patients who experienced treatment failure and were removed from study, all patients completed the entire sequential program. The median age of patients was 59 years (similar to previously published studies) with all patients having either Rai intermediate-risk (39%) or high-risk (61%) disease. Four patients (11%) achieved a CR after single-agent fludarabine. This is similar to the 6% to 20% CR frequency reported by others when single-agent fludarabine is used as initial therapy.^{1,6,14,16-20} With the subsequent consolidations, most patients improved the quality of their response, with 39% and 61% achieving a CR after high-dose cyclophosphamide and rituximab, respectively. As shown in Figure 2, the quality of response correlates significantly with survival. Patients who achieve a CR/nPR have a longer survival compared with patients who only

Fig 5. Overall survival of patients treated with sequential fludarabine and cyclophosphamide (F→C; n = 25) compared with sequential fludarabine, cyclophosphamide, and rituximab (F→C→R; n = 32). Tick mark indicates last follow-up.

achieve a PR or who experience treatment failure (5-year survival, 92% v 33%, respectively; $P = .0008$). Importantly, achieving a high-quality response overcomes the prognostic significance of high-risk disease because patients with Rai high-risk disease who achieve a CR/nPR have an overall survival comparable to intermediate-risk patients achieving a CR/nPR (5-year survival, 89% v 93%, respectively; Fig 3).

Compared with our previously reported F→C regimen, the addition of rituximab may convey a survival advantage (F→C: median overall survival, 58 months; F→C→R: median overall survival has not been reached; $P = .10$). The finding that rituximab, when added to purine analog-based therapy for CLL, prolongs survival has been reported in at least three prior settings.^{13,15,21} Although none of these trials are prospective/randomized, the consistency of this finding across different regimens supports the concept that rituximab may prolong survival in CLL.

In addition, we used a highly sensitive PCR to assess MRD in patients who achieved a CR after treatment with this regimen. Nineteen percent of all patients (32% of those tested) were PCR negative after high-dose cyclophosphamide, and this further increased to 33% (52% of those tested) after completion of the entire regimen. Comparable MRD testing has not been routinely reported after chemoimmunotherapy. In patients who achieved a CR by NCI criteria, we find that a PCR-negative state imparts an excellent prognosis, with 90% still in remission at 5 years compared with 0% of the patients who were PCR positive. Patients in CR who are PCR positive have an excellent overall survival but a much shorter response duration. This finding further reinforces the concept that, in CLL, achieving high-quality responses predicts for longer response duration, longer time to next chemotherapy, and likely prolonged survival.^{1,2,6,18,21}

The observation that CR and nPR correlate with prolonged survival has suggested to some that aggressive combination strategies (which yield more CRs than monotherapy) will lead to prolonged survival and should therefore be offered to most patients. However, we do not yet know whether achieving a CR is what permits patients to live longer or whether it is a biologic marker that identifies patients with sensitive disease who would do well in the salvage setting regardless of initial therapy. In line with this concern are the results of prospective randomized trials that indicate that more aggressive treatments yield more CRs but do not improve overall survival.^{1,18,19} This is important because combination regimens tend to be more toxic than monotherapy and most of the published literature for such regimens treat a group of patients 10 to 15 years younger than the median age for CLL. Furthermore, because these current strategies are not curative, it is necessary to balance the benefits of therapy against the risks, particularly in older individuals.

Patients treated on this regimen achieved high-quality responses that improved after each phase of therapy, including eradication of MRD as measured by flow cytometry and a highly sensitive PCR. The clinical benefit of attaining such high-quality

responses and the plateau seen in the PCR-negative response duration curve is encouraging as we improve our therapies for patients with CLL.

Currently, there are two reported approaches to administering fludarabine, cyclophosphamide, and rituximab to previously untreated patients with CLL—the concomitant approach¹³ reported by M. D. Anderson and our sequential treatment program. Although both regimens produce long-lasting CRs in the majority of treated patients and either regimen can be considered state of the art therapy for selected patients, it is currently not possible to know which (if either) of these regimens is superior in terms of response and toxicity. Only a prospective randomized comparison of these regimens can answer this question.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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