American Journal of Hematology 70:181–185 (2002)

Phase II Trial of Sequential Therapy With Fludarabine Followed by Cyclophosphamide, Mitoxantrone, Vincristine, and Prednisone for Low-Grade Follicular Lymphomas

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Advanced follicular lymphomas, grades I and II, are indolent tumors but are not considered curable with standard therapy. Fludarabine has the highest single-agent response rates in this disease. However, fludarabine-based combination chemotherapy regimens have been associated with significant myelotoxicity. Data exist suggesting that the best way to combine partially non-cross-resistant agents may be to use them sequentially. Patients with bulky stage II, stage III, or stage IV follicular lymphoma (grade I or II) were entered on this protocol. Patients were treated with 3 cycles of fludarabine followed by 6–8 cycles of cyclophosphamide, mitoxantrone, vincristine, and prednisone (CNOP). Response was assessed after the 3rd cycle of fludarabine and after the 4th, 6th, and 8th cycles of CNOP. Twenty-seven patients were entered on the protocol. Median follow-up was 50 months. Eighteen patients (67%) attained a complete response (CR), and eight patients (30%) attained a partial response (PR), for an overall response rate of 97%. Median relapse-free survival was 34 months, and median overall survival was not reached for the entire cohort. While all patients who achieved only PR progressed, more than half of those in CR remain free of progression at 39–84 months of follow-up. The regimen was well tolerated. The sequential combination of fludarabine and CNOP appears to be active and well tolerated in patients with grade I and II follicular lymphoma. Patients who achieve CR fare best, and many remain disease-free long term. While these results are encouraging, the addition of other active agents such as rituximab to this regimen may further enhance efficacy and is under investigation. Am. J. Hematol. 70:181–185, 2002.

Key words: follicular lymphoma; fludarabine; sequential chemotherapy

INTRODUCTION

Follicular lymphomas, follicular grade I (small cleaved cell) and grade II (mixed small and large cell), are low-grade lymphomas that originate from the germinal follicle center cell [1]. These lymphomas are characterized by the t(14;18) translocation that places the *Bcl-2* oncogene next to the immunoglobulin heavy-chain locus, resulting in the over-expression of *Bcl-2*. As *Bcl-2* is an anti-apoptotic protein, over-expression results in the inability of affected lymphocytes to undergo the normal process of cell death and leads to their accumulation. Follicular lymphomas account for 22% of all non-Hodgkin's lymphoma (NHL) in North America [2]. Although some patients with early-stage disease are curable with local therapy such as radiation [3], the majority of patients have advanced disease at the time of diagnosis [2,4]. While these patients often respond to chemotherapy and some live many years, they are not presently considered curable with standard therapy [5,6].

Fludarabine phosphate is a purine analogue that is ex-

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Received for publication 13 August 2000; Accepted 15 March 2002

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ajh.10137

tremely active as a monotherapy in this disease, producing a 65–70% overall response rate in previously untreated patients, with 35–40% complete responses [7], and up to a 50% response rate in those previously treated, with 10–15% complete responses [8]. These response rates can be increased to 80–100% in the first-line setting by combining this drug with other active agents, such as cyclophosphamide and mitoxantrone [9–11]. However, in spite of a 40% reduction in the dose of fludarabine in most of these studies, both immunosuppression and hematological toxicity have been common and prolonged. Prophylaxis against *Pneumocystis carinii* pneumonia and routine use of growth factors such as granulocyte-colony stimulating factor have been included in many of these protocols. As the majority of these patients are elderly and may have multiple co-morbidities, and many have bone marrow dysfunction secondary to lymphomatous involvement, such aggressive therapy may not be well tolerated.

A less toxic alternative to using effective, non-cross-resistant drugs in combination is to use them sequentially, a concept first proposed by Day in 1986 [12]. The first confirmation that there may be a therapeutic benefit of sequencing drug regimens was shown in patients with high-risk stage II breast cancer receiving adjuvant systemic therapy [13]. These investigators studied sequential versus alternating doxorubicin and cyclophosphamide, methotrexate, 5-fluorouracil (CMF) in the adjuvant treatment of patients with resectable breast cancer with more than three positive axillary lymph nodes. They reported a significantly superior treatment outcome at a median follow-up of 59 months for patients receiving sequential chemotherapy over the alternating regimen.

The goal of our trial was to cytoreduce patients with previously untreated advanced follicular lymphoma, follicular grade I and II, with three full courses of fludarabine, followed in sequence with 6–8 courses of full-dose combination therapy with cyclophosphamide, mitoxantrone, vincristine, and prednisone (F-CNOP). We chose mitoxantrone over doxorubicin because it has often been used in combination regimens for follicular lymphomas [11,14,15]. Additionally, it appears to be as active as doxorubicin in intermediate- and high-grade disease [16]. Our aim was to determine response rates (complete and partial), duration of response, and survival with this sequential regimen.

PATIENTS AND METHODS

Between April 1994 and January 1998, patients with biopsy-proven and previously untreated follicular lymphoma, follicular grade I and II, were entered onto this protocol. All patients were fully staged at the time of entry and evaluation included a serum chemistry profile, bilateral bone marrow biopsies, and CT scans of the neck, chest, abdomen, and pelvis. Patients >18 years of age with measurable stage II–IV disease and a performance status of <2 by Southwest Oncology Group criteria were considered eligible. The eligibility requirements also included adequate bone marrow function (granulocyte count >1,500/μL and platelet count >100,000/μL), serum bilirubin level <2.0 mg/dL, serum creatinine <1.6 mg/dL, and cardiac ejection fraction of 50% or more. Patients with known central nervous system involvement or those who were HIV positive were excluded from the trial. The protocol was approved by the Institutional Review Boards of the treating hospitals, and all patients provided informed consent.

Patients received fludarabine 25 mg/m^2 daily for 5 days each month for 3 months followed by 6–8 courses (2 courses beyond complete response, maximum 8 cycles) of monthly cyclophosphamide 750 mg/m^2 intravenously on day 1, mitoxantrone 12 mg/m^2 intravenously on day 1, vincristine 2 mg intravenously on day 1, and prednisone 100 mg orally for 5 days. The drug dosages were reduced by 50% for grade 3 and 4 toxicities. Patients were pretreated prior to fludarabine with 10 mg of prochlorperazine and prior to CNOP with 1 mg of granisetron and 10 mg of dexamethasone. Patients received 300 mg of allopurinol for 7 days of each cycle of therapy, and 300 or 480 μg of G-CSF (depending on body weight) starting 24 hr after completion of chemotherapy and continued until ANC > 20,000.

Response was monitored with CT scans of the chest, abdomen, and pelvis as well as bone marrow biopsy (if abnormal prior to treatment) at the end of the 3 cycles of fludarabine and after the completion of 4, 6, and 8 courses of CNOP. Subsequently, patients were followed clinically every 2 months for 2 years and then every 6 months. CT scans were repeated every 6 months. On progression, further therapy was at the discretion of the treating physician.

Complete response was defined as the disappearance of all clinical evidence of disease for a minimum of 8 weeks. Partial response was defined as greater than or equal to a 50% decrease in the sum of the products of the diameters of all measured lesions for at least 4 weeks. No lesions could increase in size, and there could be no new lesions. Patients who had unacceptable toxicity or those who progressed during therapy were taken off protocol.

Time to progression and survival were analyzed by the Kaplan–Meier method. The log-rank (Mantel–Cox) test was used to test for significance.

RESULTS

Twenty-seven patients were entered onto this protocol. Patient characteristics are shown in Table I. All patients had stage III, stage IV, or bulky stage II disease. Fifteen of the 27 patients had stage IV disease defined by bone marrow involvement. The median age was 48 years (range, 23–73 years), with 15 men and 12 women. Eigh-

TABLE I. Patient Characteristics and Responses

Characteristics	No. of patients	No. of responses[a]		
		CR	PR	NR
All patients	27	18	8	1
Sex				
Male	15	11	3	1
Female	12	7	5	
Age (yrs)				
<50	14	11	2	1
50–69	11	7	4	
>70	2		2	
Histologic subtype[b]				
Grade I	18	14	4	
Grade II	9	4	4	1
Stage				
Bulky II	4	3	1	
III	8	6	1	1
IV	15	9	6	

[a]CR, complete response; PR, partial response; NR, no response.
[b]Histologic subtypes: grade I, ≤5 large cells per HPF; grade II, 6–15 large cells per HPF.

teen patients had follicular lymphoma, follicular grade I (small cell), and nine had follicular lymphoma, follicular grade II (mixed small and large cell).

Twenty-five patients completed 3 cycles of fludarabine, and 2 patients completed 2 cycles; 23 partial responses were recorded. There were 3 complete responses. One patient progressed and was taken off the protocol but was included in the analysis. One patient could not receive further chemotherapy after 2 cycles of fludarabine due to severe pancytopenia. Nineteen patients completed 6–8 courses of CNOP. Six did not complete CNOP due to toxicity or disease progression (3 discontinued for toxicity, and 3 for progression). Following CNOP, there were a total of 18 complete responses (67%) and 8 partial responses (30%), for an overall response rate of 97%. Table I shows responses by patient characteristics.

Table II shows toxicity data for this regimen. Overall, the therapy was well tolerated. One patient developed a fatal case of disseminated herpes zoster while on CNOP, and 2 elderly patients on CNOP were discontinued for bone marrow toxicity. One patient, who had a partial remission to F-CNOP followed by abdominal radiation therapy, died from acute myelogenous leukemia assumed to be secondary to therapy.

Median follow-up for the cohort was 50 months (range 7–84 months). Relapse-free and overall survival curves are shown in Figs. 1 and 2, respectively. For the entire cohort, median relapse-free survival (RFS) was 34 months and median overall survival (OS) was not reached. For patients whose best response to F-CNOP was a partial response (PR), the median RFS was 8 months and the median OS was 22 months. For patients who had a complete response (CR), both of these endpoints were not reached. Sixty percent of this group remains free of recurrence after their initial therapy.

TABLE II. Toxicity Data

Grade 3 and 4 toxicities	Fludarabine (no. of episodes)	CNOP (no. of episodes)
Neutropenia	3	12
Nausea/vomiting	1	1
Anemia	0	3
Thrombocytopenia	0	6
Infection	0	3
Mucositis	0	2

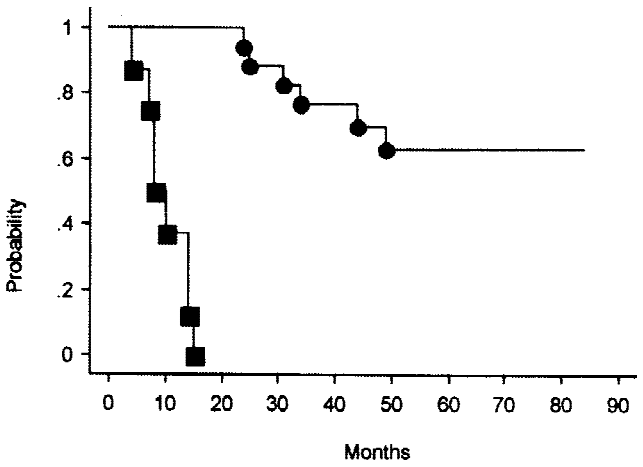

Fig. 1. Relapse-free survival in patients with a complete response (●) or partial response (■) to F-CNOP ($P < 0.0001$, log-rank test).

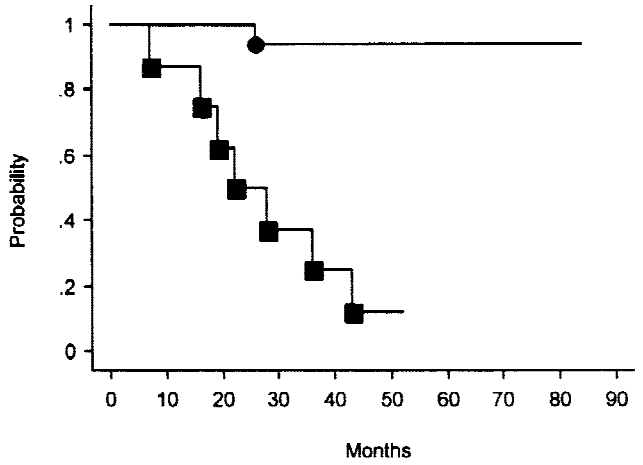

Fig. 2. Overall survival in patients with a complete response (●) or partial response (■) to F-CNOP ($P < 0.0001$, log-rank test).

DISCUSSION

Follicular lymphomas are indolent lymphomas that usually present at an advanced stage and are rarely curable with standard therapy [5,6]. The use of fludarabine, a purine nucleotide analogue, has been a significant step forward in the treatment of this disease as it produces response rates similar to multi-agent chemotherapy with fewer side effects [7].

In order to further increase response rates, investigators have combined this drug with other effective agents. Because purine analogues work by interfering with DNA repair, combining them with DNA-damaging agents such as cyclophosphamide or mitoxantrone could reasonably be expected to enhance efficacy. Indeed, such combinations have been associated with high overall and complete response rates in phase II clinical studies [9–11,14,15]. However, although highly active, the use of these combinations is limited by prolonged myelo- and immunosuppression.

Since patients who fail alkylator-based combination chemotherapy often respond (~50% response rate) to fludarabine monotherapy in the salvage setting [8], these regimens are at least partially non-cross-resistant. Evidence exists, both from mathematical modeling as well as clinical studies [12,13,17], to suggest that using such partially non-cross-resistant regimens in sequential fashion rather than in combination may be optimal. Such sequencing could be expected to provide all of the benefits of combining these regimens while minimizing the overlapping toxicities. This was the hypothesis we sought to test in the present study.

One of the problems in designing a therapeutic study of follicular lymphomas is the difficulty in ensuring that enrolled patients indeed have low-grade (grade I or II) lymphoma and have not transformed to an intermediate- or high-grade lymphoma. Such transformation is very likely to have occurred in patients who progress rapidly on fludarabine, even if histology is low grade. This is because discordance occurs in up to one-third of lymphomas, leading to sampling errors [18]. Although it is an excellent drug for indolent lymphomas, fludarabine has a much lower response rate in higher-grade disease [8]. Two of the 27 patients enrolled in our study progressed relatively rapidly (within 4 months), and it is possible that these were actually undetected high-grade transformations.

As the name suggests, indolent lymphomas tend to have a relatively long natural history. The median follow-up in this study was just over 4 years, sufficient to enable some meaningful conclusions to be drawn.

The high overall response rate of 97% achieved on this protocol is similar to that obtained in studies combining fludarabine concurrently with other drugs such as cyclophosphamide, mitoxantrone, and/or steroids in first-line therapy [9–11]. The CR rate of 67% seen with the F-CNOP regimen in our study is also similar to that obtained with these combinations. This validates the notion that using active agents in sequence is as efficacious in terms of response as combining them.

Clearly patients who had a complete response (CR) to F-CNOP fared significantly better than those who only attained a partial response (PR). While patients in the latter group all progressed within 15 months (median of 8 months), 11 of the 18 patients who achieved a CR (all of whom completed the full F-CNOP regimen) remain free of disease progression at 39–84 months of follow-up. Thus many patients who are able to complete the regimen and achieve a CR seem to have durable responses. As low-grade lymphomas have a long natural history with a median survival of 8–10 years, further follow-up is required to determine if these remissions are maintained, resulting in a cure.

Even though the numbers in each subgroup are small and statistical analysis would not be meaningful, it appears that younger patients, those with lower stage of disease, and those with grade I as opposed to grade II follicular lymphoma are more likely to achieve a CR. The first two parameters are prognostic factors of known importance in follicular lymphoma [19].

Toxicity of the F-CNOP regimen was acceptable overall, with only four patients discontinuing therapy for this reason. Two of these patients were elderly and were not able to tolerate CNOP. One patient died of disseminated herpes zoster, and another, from a presumed secondary acute myelogenous leukemia. The latter patient had received abdominal radiation following a partial response to F-CNOP.

The response rate reported in our study is clearly superior to single-agent therapy in previously untreated patients, as well as many fludarabine-based combination regimens. One exception is a trial of combination fludarabine (20 mg/m^2, days 1–5) with an escalating dose of cyclophosphamide (600–1,000 mg/m^2, day 1) in 27 previously untreated patients (FC regimen). In this trial, a 100% response rate with 89% complete responses is reported. At a median follow-up of 61 months, 12 of these patients remain disease-free, and the median overall survival has not been reached [9]. Although myelo- and immunosuppression were significant initially, these problems could be ameliorated by increasing the interval between cycles as well as the use of prophylactic G-CSF and antibiotics. However, another study [10] using this regimen for low-grade lymphomas showed response rates similar to ours in the subset of patients with follicular lymphoma. Given that the median time to progression with fludarabine alone as initial therapy for these lymphomas is only 13.6 months [7], the use of either the F-CNOP or FC regimen would appear to be advantageous if toxicity were acceptable.

Rituximab (Rituxan, Genentech, Inc., South San Francisco, CA, and IDEC Pharmaceutical Corporation, San Diego, CA) is a humanized, murine monoclonal antibody directed against the CD20 antigen expressed on the surface of B-lymphocytes, which are the cell of origin of follicular lymphoma. As a single agent, rituximab has a 69% overall response rate in previously untreated patients with a low tumor burden [20] and a 50% overall response rate in recurrent or relapsed disease [21]. Be-

cause its toxicities are minimal and do not overlap those of standard chemotherapy, it can be used in combination with chemotherapy. Studies in both low- and high-grade lymphomas have suggested that it enhances the efficacy of CHOP chemotherapy [22,23] as well as fludarabine [24]. Adding this agent to the F-CNOP combination may improve efficacy without incurring additional toxicity, and such a study is being planned.

In conclusion, sequential combination chemotherapy that includes fludarabine followed by CNOP is well tolerated and very active in follicular lymphomas. Many patients who achieve a CR can expect long-term failure-free survival. The inclusion of rituximab in this regimen may be of additional benefit and is being studied in a phase II study. Whether any combination is capable of curing this disease remains to be seen.

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