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Clinical report

The combination of fludarabine and cyclophosphamide results in a high remission rate with moderate toxicity in low-grade non-Hodgkin's lymphomas

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We undertook a prospective study to evaluate the role of the combination of fludarabine and cyclophosphamide in patients with low-grade non-Hodgkin's lymphoma. Twenty-seven patients with low-grade non-Hodgkin's lymphoma were treated with i.v. fludarabine (30 mg/m^2) and cyclophosphamide (250 mg/m^2) on days 1–3. Cycles were given at 4-week intervals for a maximum of six courses. Fourteen patients (52%) were previously untreated, 13 patients (48%) had been treated with at least one chemotherapy regimen before. Of the 27 patients, 11 (41%) obtained a complete and 13 (48%) a partial remission, thus the overall response rate was 89%. The remission rate in untreated patients was slightly higher than in pretreated patients (93 versus 85%). The toxicity was mild, no treatment-related mortality occurred. Neutropenia was the most common side effect, grade 4 neutropenia of rather short duration was observed in less than 7% of the cycles. At the end of the treatment, the mean CD4$^+$ count was 155/μl and the mean CD8$^+$ count 204/μl. Severe infections did not occur. These results show that the combination of fludarabine and cyclophosphamide in the doses used in this study is an effective regimen with manageable toxicity in low-grade non-Hodgkin's lymphoma. [© 2002 Lippincott Williams & Wilkins.]

Key words: Chronic lymphocytic leukemia, cyclophosphamide, fludarabine, immunosuppression, non-Hodgkin's lymphoma, purine analogs.

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Introduction

Patients with low-grade non-Hodgkin's lymphoma cannot be cured with standard chemotherapy. With this approach a low fraction of patients achieve a complete remission (CR), and, even if remission is long lasting, they inevitably relapse. Therefore more effective treatments are urgently needed in this disease.

Fludarabine is a nucleoside analog which has been shown to be effective for untreated and pretreated patients with low-grade non-Hodgkin's lymphoma and chronic lymphocytic leukemia (CLL).[1–4] Combinations of fludarabine with other cytotoxic drugs were evaluated to increase the response rates. Cyclophosphamide is an effective alkylating agent in the treatment of low-grade non-Hodgkin's lymphomas and CLL, and this agent was shown to have synergistic effects with fludarabine in preclinical studies.[5,6] Furthermore, in clinical studies this combination has been shown to be effective in patients with CLL,[7–10] but revealed variable hematological toxicity depending on the applied dose intensity. In a phase II study, 48% of pretreated or untreated patients receiving fludarabine (30 mg/m^2/day for 3 days) and cyclophosphamide (300 mg/m^2/day for 3 days) experienced neutropenia below 500/μl, and pneumonia or sepsis occurred in 25% of patients.[9] Zaja *et al.* reported 86% severe neutropenia in pretreated patients with non-Hodgkin's lymphomas or CLL using a treatment schedule of fludarabine (30 mg/m^2/day) and

cyclophosphamide (300 mg/m^2/day) for 5 days.[7] In this study, we used fludarabine and cyclophosphamide in patients with low-grade non-Hodgkin's lymphomas with a modified dose regimen. Here we report our results with special regard to the follow-up of T cell depletion and to the hematological as well as non-hematological toxicity.

Patients and methods

Between 1997 and 2000, 27 patients were entered in this prospective phase II study. The criteria for entry included: diagnosis of low-grade non-Hodgkin's lymphoma based on REAL classification, stage III–IV disease as outlined by the Ann Arbor Conference or stage Binet B and C in CLL requiring therapy, normal hepatic, renal and cardiac function, HIV negativity, absence of autoimmune hemolytic anemia or autoimmune thrombocytopenia, and absence of pregnancy. The study was initiated after ethical approval in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients. CLL patients were included in this trial after the cessation of the recruitment for the CLL 2 trial and before the activation of the CLL 4 & 5 studies performed by the German CLL Study Group.

Patient characteristics

The median age of the 27 patients was 57 (range 40–71) years. Sixteen patients had follicular lymphoma, six patients had CLL, three immunocytoma, one marginal zone lymphoma and one had a low-grade Non-Hodgkin's lymphoma which could not be further classified. Among the six patients with CLL, five were in stage Binet B and one patient in stage Binet C. Among the remaining 21 lymphoma patients, eight had stage III disease according to the Ann Arbor classification and 13 patients were in stage IV. All patients included in the study had progressive disease requiring systemic treatment. Fourteen patients (52%) had not been previously treated when entering the study. Thirteen patients had been previously treated, six had one previous treatment, five had two or three previous treatments and two had more than three previous treatments.

Staging

Patients had a pretreatment evaluation including history and physical examination, blood counts, liver and renal function tests, chest X-ray, abdominal ultrasound and computed tomography scan, bone marrow aspiration, and biopsy and immunophenotyping of marrow and peripheral blood. After two cycles response evaluations were performed. Treatment was continued if a response was detectable. After four cycles treatment was continued if at least a partial response was achieved. All patients were evaluated after the completion of treatment, for a maximum of six cycles.

$CD4^+$ and $CD8^+$ lymphocyte counts and CD4/CD8 ratio were measured using immunophenotyping of peripheral blood before the initiation of the treatment, at the beginning of subsequent cycles of the chemotherapy and every 3 months after the end of the treatment.

Treatment schedule

Patients were treated with i.v. fludarabine (30 mg/m^2/day over 30 min) and cyclophosphamide (250 mg/m^2/day over 60 min) on days 1–3. Cycles were given at 4-week intervals for a total of six courses. Doses of fludarabine and cyclophosphamide were reduced in case of a relevant hematopoietic toxicity. If the nadir granulocyte count was less than 500/μl, or if the nadir platelet count was less than 50 000/μl, patients received a 25% dose reduction in the following cycles. In case of grade IV neutropenia, subsequent treatment was continued with granulocyte colony stimulating factor (G-CSF) support in order to prevent further dose reductions. All patients received prophylaxis for *Pneumocystis carinii* with cotrimoxazole (2 days per week) during the whole chemotherapy course and until the absolute $CD4^+$ lymphocyte count exceeded 200/μl after the end of treatment. Toxicity was evaluated by WHO criteria.

Response criteria

Response criteria defined by the National Cancer Institute working group were used in this study.[11,12] CR for low-grade non-Hodgkin's lymphomas requires the remission of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy. Partial remission (PR) requires 50% or more decrease of the sum of products of previous lymphoma manifestations. Stable disease was defined as less than a PR but no progressive disease.[12]

Statistical analyses

Overall survival was calculated according to the Kaplan–Meier method. The Mann–Whitney *U*-test was applied to compare data of unrelated groups. All statistical analyses were performed using SPSS software.

Results

Response to treatment

The overall remission rate of the patients in this phase II study treated with fludarabine and cyclophosphamide was 89%. Eleven patients (41%) achieved a CR and 13 (48%) patients a PR. In untreated patients the overall remission rate was slightly but not significantly higher than in pretreated patients (93 versus 85%; $p=0.72$). Previously untreated patients achieved a CR in 43% and a PR in 50% (Table 1). In the group of patients who had received at least one chemotherapy regimen before, the CR rate was 38% and the PR rate was 46%.

Follow-up

After a median follow-up of 25 months, 93% of previously untreated and 69% of pretreated patients were alive. The overall survival of the pretreated patients was lower than in untreated patients in our study, but the difference was not significant (Figure 1). Among the 11 patients who achieved a CR, six patients were still in CR without further treatment after a median follow-up of 25 months. Of the 13 patients who achieved a PR, seven patients were in continuous PR without further treatment.

Side effects

In total, 118 cycles of chemotherapy were delivered to 27 patients (Table 2). The major toxicity was myelosuppression, especially neutropenia. WHO grade 3 neutropenia occurred in 22 of 118 administered cycles (19%), grade 4 neutropenia (below 500/μl) in eight of 118 (7%). WHO grade 3 anemia and thrombocytopenia occurred each in two of 118 cycles only; no grade 4 anemia or thrombocytopenia was observed during the treatment (Table 2). Twenty-four episodes of infection or fever of unknown origin occurred in 24 of the 118 cycles (20%). Three patients experienced an infection WHO grade 3, all had pneumonia. Importantly, no patient had a grade 4 infection. Dose adjustments in subsequent cycles were mandatory for myelosuppression or occurrence of infection as previously described. Only one patient experienced vomiting evaluated as WHO grade 3 toxicity. Table 2 shows the rate of side effects referring to the number of administered cycles. The cumulative toxicity experienced by the patients is given in Table 3.

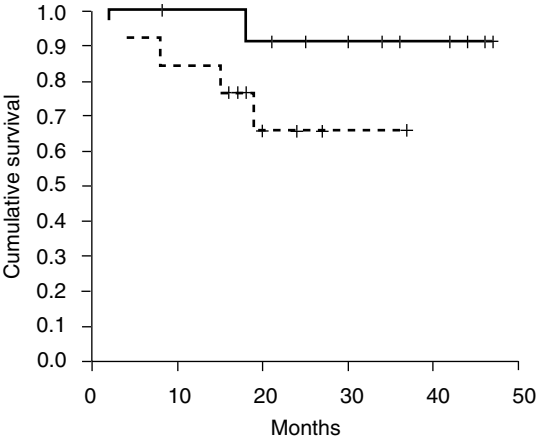

Figure 1. Kaplan–Meier curves of overall survival in the two groups of patients with previously untreated (solid line) and refractory/relapsed non-Hodgkin's lymphoma (dashed line).

Numbers of $CD4^+$ and $CD8^+$ lymphocytes

The median $CD4^+$ and $CD8^+$ lymphocyte counts decreased and the median CD4/CD8 ratio inverted during treatment. Before the initiation of treatment, the median $CD4^+$ and $CD8^+$ counts and the median

Table 1. Response to therapy in untreated and pretreated patients

Patients	Untreated		Relapsed/refractory		Overall	
		%		%		%
Remission rate	13/14	93	11/13	85	24/27	89
CR	6/14	43	5/13	38	11/27	41
PR	7/14	50	6/13	46	13/27	48

Table 2. Toxicity referring to the number of cycles (n=118)

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	82	26	8	2	0
Leukocytes	28	23	41	2	4
Granulocytes	33	23	32	22	8
Platelets	104	8	4	2	0
Gastrointestinal	68	41	8	1	0
Renal	106	12	0	0	0
Pulmonary	118	0	0	0	0
Allergic	118	0	0	0	0
Cutaneous	108	4	6	0	0
Infections	94	12	8	4	0
Cardiac	118	0	0	0	0
Neurotoxicity	111	7	0	0	0

Table 3. Cumulative toxicity of the treatment in 27 patients

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	14	7	4	2	0
Leukocytes	3	2	8	10	4
Granulocytes	3	1	7	9	7
Platelets	20	3	3	1	0
Gastrointestinal	3	18	5	1	0
Renal	20	7	0	0	0
Pulmonary	27	0	0	0	0
Allergic	27	0	0	0	0
Cutaneous	21	3	3	0	0
Infections	11	7	6	3	0
Cardiac	27	0	0	0	0
Neurotoxicity	23	4	0	0	0

CD4/CD8 ratio were 750/μl (range 163–2687/μl), 856/μl (range 70–2015/μl) and 1.2 (range 0.39–3.3), respectively. At the end of the treatment, the median $CD4^+$ count decreased to 155/μl (range 28–1145/μl), the median $CD8^+$ count to 204/μl (range 105–1987/μl) and the median CD4/CD8 ratio inverted to 0.69 (range 0.11–3.5). The T cell depletion was already apparent after the first cycle and reached a nadir for $CD4^+$ and $CD8^+$ lymphocytes after the sixth and for the CD4/CD8 ratio after the fifth cycle of the treatment (Figure 2). Two patients experienced an absolute $CD4^+$ count below 50/μl and 10 patients below 100/μl. The median $CD4^+$ and $CD8^+$ counts and the CD4/CD8 ratio increased significantly during the follow-up after treatment, but did not reach pretreatment values for 2 years after the end of the treatment.

Discussion

Fludarabine has demonstrated high efficacy in the treatment of low-grade non-Hodgkin's lymphomas and B-CLL.[3,13] Furthermore, preclinical data and some clinical studies indicated a higher activity when fludarabine was given in combination with suitable chemotherapeutic agents. Cyclophosphamide has shown a highly synergistic effect with fludarabine.[5–9,14] The effectiveness of this combination was attributed to the inhibition of DNA repair mechanisms in lymphoma cells. Cyclophosphamide as an alkylating agent induces DNA damage by intra- and interstrand cross-links, while fludarabine can inhibit the reversal of these interstrand cross-links and DNA repair. On the basis of these data we combined fludarabine with cyclophosphamide as a potentially effective treatment option in patients with low-grade non-Hodgkin's lymphomas.

A number of trials have been published using fludarabine-containing regimens, but results of only a few studies with fludarabine and cyclophosphamide are available in low-grade non-Hodgkin's lymphoma, with considerable differences in dose regimens.[4,7,15,16] Frewin *et al.* reported a phase II study with 17 patients (seven CLL and 10 non-Hodgkin's lymphomas) who received fludarabine and cyclophosphamide as salvage treatment.[4] None

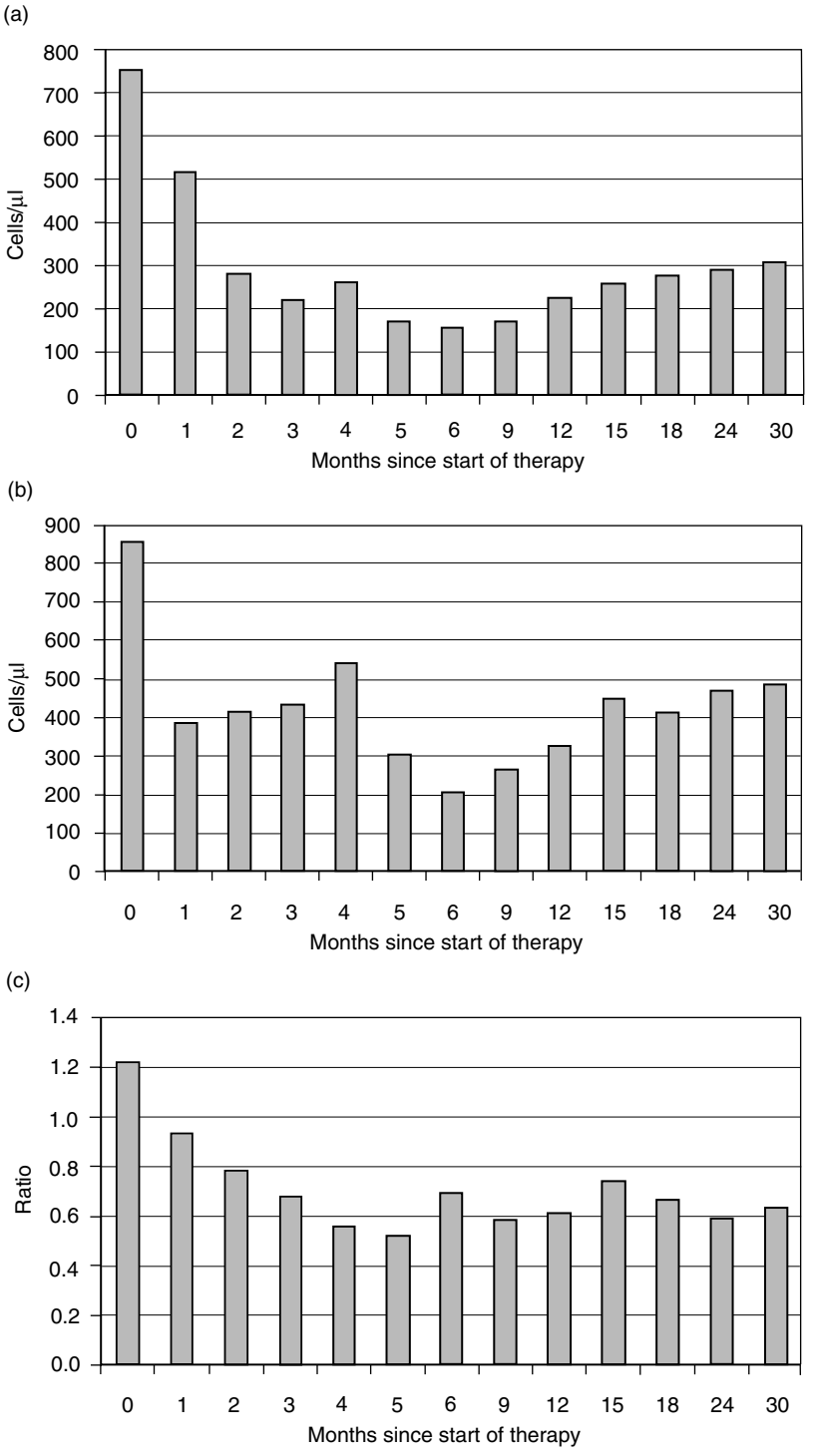

Figure 2. Changes in the median $CD4^+$ (a) and $CD8^+$ lymphocyte counts (b), and the CD4/CD8 ratio (c) before the chemotherapy cycles and in 3-month intervals after the end of the treatment.

of the non-Hodgkin's lymphoma patients achieved a CR and only 50% responded to treatment. In this trial the dose of fludarabine (25 mg/m^2 for 3 days) was lower than used in our study. Twelve of 17 patients had received purine analogs before and four of them had progressed during treatment. This patient selection may in part explain the inferior results found in this study.

In a phase I trial of previously untreated patients the hematological toxicity was high, even in dose level 3 (cyclophosphamide 1000 mg/m^2, day 1; fludarabine 20 mg/m^2, days 1–5) that was considered by the authors as the recommended dose for phase II trials.[17] Eight of 16 (50%) patients treated with dose level 3 experienced neutropenia grade 3 and 4. Furthermore, the incidence of grade 3 and 4 infections was 11%. Lazzarino *et al.* combined fludarabine (25 mg/m^2 for 3 days) with cyclophosphamide (350 mg/m^2 for 3 days) and dexamethasone (20 mg/day for 3 days) in 25 pretreated patients with low-grade and intermediate non-Hodgkin's lymphoma.[16] The toxicity was comparable to other studies mentioned above. Although they used a higher dose of cyclophosphamide, the overall response was 72% (CR 32% + PR 40%), comparable to that observed in our investigation. The combination of cyclophosphamide and fludarabine with dexamethasone seemed not to reveal a clear advantage, and dexamethasone may contribute to immunosuppression and infections in this setting. In a recently published study a group of 22 patients received the combination of fludarabine and cyclophosphamide, and another group consisting of 31 patients received the combination of cyclophosphamide and fludarabine plus mitoxantrone in a non-randomized fashion.[18] The overall response rate was 88% without significant differences between the two groups (95 versus 84%). Again, myelosuppression was the major toxicity, but no opportunistic infections occurred. The lack of opportunistic infections may be related to the use of G-CSF and antibiotic prophylaxis in this study. In our trial only seven patients experienced grade 4 neutropenia, which occurred in eight of 118 courses given (Tables 2 and 3). Moreover, the overall occurrence of fever of unknown origin or infection was low. Grade 3 infection only occurred in three patients and, most importantly, no treatment-related death was observed. The rate of infections in our trial is lower than that reported by O'Brien *et al.* in CLL patients treated with fludarabine and cyclophosphamide.[9] The lower rate of infections we observed may be related to the lower doses of cyclophosphamide we used or to differences in the group of patients treated.[9] Also in untreated or pretreated CLL patients who received the same schedule as in our trial, the hematotoxicity and the occurrence of infections were rather low.[10] Recently, preliminary data of a randomized study were published comparing the combination of fludarabine (20 mg/m^2, days 1–5) and cyclophosphamide (1000 mg/m^2, day 1) with the combination of cyclophosphamide (1000 mg/m^2, day 1), vincristine (1.4 mg/m^2, day 1) and prednisone (100 mg/m^2 for 5 days). This study conducted by ECOG and CALGB was terminated early because of an unacceptably high early mortality in the fludarabine/cyclophosphamide arm.[19] These results underline the importance of the schedule used with this combination, as the dosage used in our study resulted in moderate toxicity with a high CR rate.

Most opportunistic infections after treatment with fludarabine are due to *P. carinii* or are viral infections, i.e. varicella, herpes zoster, herpes simplex and cytomegalovirus infections.[13,20–22] Furthermore, listeriosis and mycobacterial infections were seen in patients following fludarabine treatment.[20] Immunologic effects on lymphocytes and other blood cells may occur following treatment with purine analogs.[23] Reduced total lymphocyte counts, decrease in B cells and transient reduction in NK cells had occasionally been described, but the reduction of $CD4^+$ and $CD8^+$ cells is considered as the major explanation for an increased risk of developing opportunistic infections.[13,21,22] Fenchel *et al.* reported on four cases of *P. carinii* pneumonia (PCP) in 77 patients treated with fludarabine.[13] All patients with lethal PCP had $CD4^+$ counts less than 50/μl. Although a significant reduction of $CD4^+$ lymphocytes was already detectable after the first course of chemotherapy, in our investigation only two patients reached a $CD4^+$ count of less than 50/μl and no PCP occurred using cotrimoxazole prophylaxis.

Conclusion

The present study showed a high activity of fludarabine and cyclophosphamide in previously untreated as well as pretreated patients with low-grade non-Hodgkin's lymphomas including CLL. It can be concluded that the non-hematological toxicity of this regimen is low and the hematological toxicity does not translate into a high infection rate. Nevertheless, higher doses of cyclophosphamide and fludarabine than used in this study seem to result in unacceptable high toxicity, and therefore should be avoided.

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