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Fludarabine in combination with cyclophosphamide or with cyclophosphamide plus mitoxantrone for relapsed or refractory low-grade non-Hodgkin's lymphoma

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Background and Objectives. We report the activity of two combinations of fludarabine (FLU), one with cyclophosphamide (FLU/CY) and the second with CY plus mitoxantrone (FLU/CY/MITO). The aim of the study was to evaluate the activity and toxicity of these two schedules in patients with non-Hodgkin's lymphoma (NHL).

Design and Methods. Twenty-two patients with recurrent low grade non-Hodgkin's lymphoma (LGL) received FLU/CY (FLU 25 mg/m^2 days 1 to 3, CY 300 mg/m^2 days 1 to 3), and 31 patients received FLU/CY/MITO (FLU 25 mg/m^2 days 1 to 3, CY 300 mg/m^2 days 1 to 3, mitoxantrone 10 mg/m^2 day 1). Patients received antibiotic oral prophylaxis during all treatments and growth factors (G-CSF) when grade III granulocytopenia (WHO scale) occurred.

Results. Of the 53 patients, 31 achieved complete remission (CR) (58%) and 16 partial remission (PR) (30%). Response was similar in both arms of the study. After 3 courses, 77% of patients who achieved CR showed a complete disappearance of disease. Seventy-nine per cent of patients experienced granulocytopenia. Few patients had fever, all without infection. One patient died with fever of unknown origin three months after completion of six courses of treatment.

Interpretation and Conclusions. Both treatments were seen to be effective in recurrent low-grade NHL. Antibiotic prophylaxis with G-CSF support seems to reduce treatment-related infection.

Key words: fludarabine, cyclophosphamide, mitoxantrone, low-grade non-Hodgkin's lymphoma

Low-grade non-Hodgkin's lymphomas (LGL), in spite of the high complete remission (CR) rate, are only occasionally cured. Most patients relapse[1] and need further treatment. Salvage regimens can only obtain an overall response rate of about 50%-60% with a CR rate of less than 30%, and response duration is expected to be short.[2] Fludarabine phosphate (FLU) used as a single agent has been shown to be effective in pre-treated patients with recurrent LGL. An overall response rate ranging from 52% to 65% has been reported.[3-5]

A 33% partial response (PR) rate was also reported in pre-treated mantle cell lymphoma,[6] suggesting a potential usefulness of fludarabine in this subset of patients. The associations of fludarabine with mitoxantrone (MITO) and steroids, with cyclophosphamide (CY), or with cyclophosphamide and epirubicin proved to be highly effective in pre-treated, relapsed and refractory LGL,[7,8-12] but granulocytopenia and infection occurred. Our aim was to evaluate fludarabine in combination with cyclophosphamide or with cyclophosphamide and mitoxantrone in patients with recurrent LGL, using appropriate antibiotic and growth-factor support. We report the activity and toxicity of these two combinations in pre-treated LGL.

Design and Methods

Eligibility criteria and treatments

Between June 1996 and August 1999, 53 consecutive patients with low-grade NHL as defined by the R.E.A.L. classification[13] entered two studies. Two centers treated patients with FLU/CY and 3 centers with FLU/CY/MITO. Eligibility criteria included: 1) nodal biopsy confirming diagnosis of LGL; 2) age ≤ 75 years; 3) no previous exposure to fludarabine or other purine analogs; 4) recurrent or refractory disease; 5) ECOG performance status 0-2; 6) normal cardiac, renal, pulmonary and hepatic function; 7) granulocyte count > 1,500/mcL and platelet count > 100,000/mcL, except in cases of bone marrow

(BM) involvement; 8) informed consent.

A) The fludarabine/cytoxan (FLU/CY) schedule was as follows: fludarabine 25 mg/m^2 i.v. days 1 to 3, cytoxan 300 mg/m^2 i.v. days 1 to 3. Courses were repeated every 28 days for a maximum of 6 cycles. B) The fludarabine/mitoxantrone/cytoxan (FLU/CY/MITO) schedule was as follows: fludarabine 25 mg/m^2 i.v. days 1 to 3, mitoxantrone 10 mg/m^2 i.v. day 1, cytoxan 300 mg/m^2 i.v. days 1 to 3. Courses were repeated every 28 days for a maximum of 6 cycles. Treatment was discontinued after 3 cycles in those patients in whom clinical and instrumental disappearance of disease was confirmed after 1 or 2 cycles, or in the case of persistent disease which remained stable for at least two cycles.

Twenty-two patients received FLU/CY, and 31 received FLU/CY/MITO.

Infection prophylaxis with ciprofloxacin (1 g per day) and fluconazole (200 mg per day) was given throughout treatment to all patients.

Granulocyte colony-stimulating factor (G-CSF) was provided if the granulocyte count dropped below 1,000/mcL. Leukocyte-free erythrocyte concentrates were administered if the hemoglobin dropped below 8 g/dL. All blood products were irradiated (20 Gy).

Patient characteristics

The fifty-three patients, 26 male and 27 female, had a median age of 56 years (range 35-75). According to the R.E.A.L. classification, 12/53 patients had small B-lymphocytic, 34 had follicular, 5 mantle cell, and 2 marginal zone non-Hodgkin's lymphoma.

Patients were evaluated by conventional chest X-ray, CAT and/or ultrasound scan, bone marrow aspiration with phenotype, and bilateral iliac crest biopsies. Five patients were in stage II (9%), 13 patients in stage III (25%) and 35 patients in stage IV (66%). Previous therapy included doxorubicin in all but five patients. Nine had received mitoxantrone over 12 months previously while none had received purine analogs. Patients had received a median of 2 previous regimens (range 1 to 5). Six patients in each treatment group had received peripheral blood progenitor cell (PBPC) transplantation and had relapsed before receiving fludarabine-containing regimens.

The patients' characteristics are reported in Table 1. Characteristics of the two groups were similar.

Complete re-staging was performed after 3 courses of chemotherapy and at the end of treatment. This re-staging included two posterior iliac crest biopsies, MRI scans, and radionuclide scans when required. Re-staging was carried out every three months during the first year after completion of therapy, and every 6 months in subsequent years. In addition, patients were carefully followed and all necessary tests were performed when clinically required.

Response criteria and statistical analysis

A complete remission (CR) was defined as the complete disappearance of the disease for at least 8 weeks. A partial remission (PR) was defined as a $\geq$ 50% reduction of all measurable lesions (including BM when involved by disease) maintained for at least 4 weeks. Non-response (NR) was defined as a less than 50% reduction in tumor masses and in BM infiltrate, and progressive disease (PD) as in increase in the size of disease by at least 25% or the appearance of new lesions.

Overall survival (OS) was measured from the beginning of administration of fludarabine combination regimens to the date of death or last follow-up evaluation. Failure-free survival (FFS) was calculated from the date of treatment to the date of relapse, progression, death, or the last follow-up evaluation. Survival analysis was performed according to the number of cycles of chemotherapy (3 vs 6), sex, histology, stage II + III vs. stage IV, lactate dehydrogenase (LDH) level ($\leq$1x vs. >1x normal value), BM involvement, spleen involvement, previous treatment. We used the Kaplan and Meier[14] method for univariate analysis, and assessed the difference between curves by the long-rank test. Cox's proportional hazards analysis[15] was used to calculate hazard ratios and 95% confidence intervals. Multivariate analysis by stepwise backward selection of independent

Table 1. Patient characteristics.

	Patients No.53 (%)
Sex	
Male	26 (49)
Female	27 (51)
Median age years (range)	56 (35-75)
Histology	
Lymphocytic	12 (23)
Follicular	34 (64)
Mantle cell	5 (9)
Marginal zone	2 (4)
Stage	
II	5 (9)
III	14 (27)
IV	34 (64)
Bulky disease (> 10 cm)	3 (6)
Status of disease at treatment	
Relapse after CR	20 (38)
PR in progression	21 (40)
Non-response	6 (11)
Progressive disease	6 (11)
LDH level	
$\leq$normal	40 (75)
> 1 $\times$ normal	13 (25)
BM involvement	28 (53)
Spleen involvement	6 (11)
Extra-nodal localization	9 (17)
Median interval from diagnosis to treatment in months (range)	40 (6-202)
Median number of prior regimens (range)	2 (1-5)

FLU/CY = fludarabine/cytoxan; FLU/CY/MITO = fludarabine/cytoxan/mitoxantrone.

Table 2. Response of 53 patients according to treatment and histology.

	All patients N° (%)	*FLU/CY N° (%)*	*FLU/CY/MITO N° (%)*
Patients	53	22	31
CR (%)	31 (58)	12 (54)	19 (61)
Lymphocytic	6/12 (50)	3/8 (37)	3 /4 (75)
Follicular	21/34 (62)	9/13 (69)	12/21 (57)
Mantle cell	2/5 (40)	–	2/4 (50)
Marginal zone	2/2 (100)	–	2/2 (100)
PR (%)	16 (30)	9 (41)	7 (23)
Lymphocytic	5/12 (42)	4/8 (50)	1/4 (25)
Follicular	11/34 (32)	4/13 (31)	6/21 (29)
Mantle cell	1/5 (20)	1/1 (100)	–
CR + PR (%)	47 (88)	21 (95)	26 (84)
NR/PD	6 (11)	1 (5)	5 (16)

FLU/CY = fludarabine/cytoxan; FLU/CY/MITO = fludarabine/cytoxan/mitoxantrone.

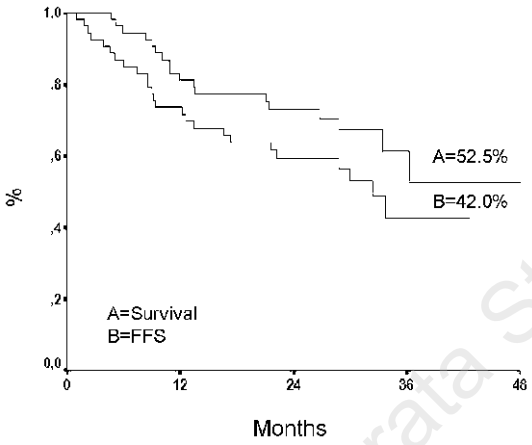

Figure 1. Overall survival (A) and failure-free survival (B) of patients treated with fludarabine-containing regimens.

variables based on the likelihood ratio was carried out. Statistical significance was set at $p < 0.05$.

Treatment toxicity was evaluated according to the World Health Organization (WHO) criteria.

Results

Response and survival

Analysis was based on status of disease in August 2000. All patients were evaluable for response. There were 31 CRs (58%) and 16 PRs (30%), for an overall response rate of 88% (95% with FLU/CY; 84% with FLU/CY/MITO). Six patients did not respond (NR) or proceeded to progression during or following treatment. Results relating complete remission to histology are reported in Table 2.

The median number of administered cycles was 6 (range 3 to 6), but 6 patients in the FLU/CY arm and 14 in the FLU/CY/MITO arm received 3 cycles. Seventy-seven per cent of patients (24/31) who achieved CR showed clinical and instrumental disappearance of disease after 3 cycles of treatment, 58% with FLU/CY and 90% with FLU/CY/MITO.

Nine CR patients (29%) relapsed. Relapse was not related to the number of chemotherapy courses received. The median time of relapse was 13 months (range 2 to 26). The disease in 10 patients with PR progressed after a median time of 4.5 months.

Eighteen patients (34%) died, 16 from progressive disease (6 NR and 10 PR in progression), one with fever of unknown origin (FUO) and one from lung cancer, both while in CR.

Twenty patients are still in CR at a median overall observation time of 26 months (range 8 to 32).

The median follow-up survival of the 53 patients was 31 months (range 5 to 49), with 52.5% (standard error ± 10.9%) of patients alive at 3 years (Figure 1). The 3-year survival was 42.8% (standard error ± 13.7%) in those receiving FLU/CY treatment and 71.1% (standard error ± 8.9%) in those receiving the FLU/CY/MITO combination. At 3 years, the overall FFS rate was 42.0% (standard error ± 9.0%) with an estimated probability of 31.1% (standard error ± 11.1%) for the FLU/CY group and 57.1% (standard error ± 11.5%) for the FLU/CY/MITO group. There was no difference in survival and FFS between those patients who had received 3 cycles and those who had received 6 cycles.

Prognostic factors for survival and FFS were studied using both univariate and multivariate analyses. Results of univariate analysis of survival, stage (p= 0.03) and BM involvement (p= 0.02) proved significant. Patients in stage II/III and with a normal BM had the best outcome. BM involvement remained significant (p= 0.01) after multivariate analysis. Similar results were observed in terms of FFS. BM involvement (p = 0.04) was significant after univariate analysis, maintaining its significance after multivariate analysis (p = 0.02).

Toxicity

The 53 patients received a total of 258 FLU/CY (114 administrations) and FLU/CY/MITO (144 administrations) courses. Myelosuppression was the most evident toxic effect. Other toxicity was very mild.

Complete assessment of hematologic toxicity was available for 189 courses of therapy, referring to 40 patients (20 in the FLU/CY and 20 in the FLU/CY/MITO schedule, respectively). Granulocytopenia was observed in 92 out of the 189 (48%) courses. The median nadir granulocyte count was 1,000/mcL and occurred at a median of day 11. Grade III-IV granulocytopenia was observed in 39 courses (21%). Six patients (11%) experienced a total of 9 brief episodes of fever during granulocytopenia, but no bacterial or opportunistic infections were confirmed. One patient died with FUO 105 days after the completion of 6 courses of FLU/CY treatment.

According to the trial design, 50% of patients received G-CSF administration at the dosage of 5 μg/kg/day until neutrophils reached > 1,500/mcL for 3 days. No dose reductions were applied. Mild thrombocytopenia (grades I and II) occurred in 37.5% of patients. Grade II and III anemia was observed in 10% of patients.

Five out of 53 patients (9%) required a delay in drug administration (median 7 days) because of persistent granulocytopenia. One of them, who had a delay of 30 days, died of progressive disease with persistent bone marrow hypoplasia after 3 courses of FLU/CY. This patient had previously received three courses of chemotherapy.

Non-hematologic toxicity was very mild and represented by grade I nausea and vomiting in two patients.

Discussion

The fludarabine combination regimens were seen to be effective in these groups of patients with recurrent low-grade NHL. The overall response rate in 53 patients was 88% (95% for FLU/CY; 84% for FLU/CY/MITO). Fifty-eight percent of patients achieved CR. Fludarabine as a single agent is capable of producing a response rate of about 50-60% in pre-treated LGL, with a CR rate ranging from about 20% to 35%.[3-5] Mitoxantrone was found to have a favorable impact in phase II studies on lymphoma.[16,17] The addition of this drug to fludarabine and steroids improved the response rate to over 80%, with a CR rate ranging from 35% to about 45%.[8,9] The clinical activity of cytoxan in NHL has been well established in the past.[18] Fludarabine/cytoxan regimens have been reported to produce an overall response rate of about 70%, with a CR rate ranging from 32% to 66% in LGL.[10,11] This background formed the basis of our treatments. Five patients had mantle cell lymphoma and two of them achieved CR following the FLU/CY/MITO regimen. This and similar observations reported by others[6,8,9,11,12] justify the use of fludarabine in this particular subset of patients, regardless of whether they are pre-treated or not.[19,20]

Projected actuarial curves show a 3-year probability of survival of 52.5% with a probability of failure-free survival of 42% (Figure 1). These results are similar to those previously observed with fludarabine in combination with epirubicin and cyclophosphamide[12] even if, in contrast to our group of recurrent, pre-treated patients, two thirds of the patients included in that study were untreated. In conclusion, the two fludarabine combinations compare favorably with previously reported data,[8-11] even though a median observation time of about 2.5 years is too short to draw any definitive conclusions. According to multivariate analysis, patients with BM involvement showed a poor 3-year probability of overall survival ($p = 0.01$) and of FFS ($p = 0.02$) when compared with patients without BM involvement.

This study cannot define the optimal duration of fludarabine in combination therapy. Most cases of complete disappearance of the disease (77%) were achieved after 3 courses of chemotherapy. According to our observation, discontinuing treatment after 3 courses did not increase relapse or affect probability of survival in these patients.

Non-hematologic toxicity was very mild in both groups of treatment and myelosuppression, as expected,[3-6,8-12] was the major toxic effect. Granulocytopenia was observed in 48% of administered courses of chemotherapy. However, the use of G-CSF allowed granulocyte recovery and maintenance of drug doses in all patients. Grade III-IV granulocytopenia was observed in 21% of courses and nine brief episodes of fever occurred, all without infection although one patient in CR died with FUO more than 3 months after completing six courses. If we consider this patient, the toxicity-related death rate is 2%. The lack of opportunistic infection was probably related to the concomitant use of G-CSF during granulocytopenia and to the antibiotic prophylaxis, as suggested by others.[8,9,20] The use of growth-factors and antibiotic prophylaxis seems to strengthen the feasibility of this approach.

In conclusion, this study confirms that fludarabine in combination with cytoxan or with cytoxan plus mitoxantrone provides an effective and safe treatment for recurrent low-grade NHL. The overall results achieved with these two different combinations are similar.

Contributions and Acknowledgments

All listed authors contributed to the conception and design of the study, and the final version of the text. GS was the principal investigator and contributed to the conception of the study, its design, data handling and interpretation and wrote the paper. MS, AG, DP, TC, RV, ER, DM and MG were involved in the recruitment of and day-to-day contact with patients and contributed to data handling and interpretation. AR performed the statistical analysis. MT performed the histologic revision. SN, AMC, MRS are responsible for the critical revision. We thank Anne Freckleton for her help in preparing the text and Simona Barozzi for data management.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Paolo G. Gobbi, who acted as an Associate Editor. The final decision to accept this paper was taken jointly by Dr. Gobbi and the Editors. Manuscript received September 25, 2000; accepted February 12, 2001.

Potential implications for clinical practice

- Fludarabine in combination with cytoxan or with cytoxan and mitoxantrone is active in relapsed or refractory indolent non-Hodgkin's lymphoma.
- The use of growth-factors and antibiotic prophylaxis seems to reduce treatment-related infection and make this approach feasible.

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