Fludarabine combined with cyclophosphamid is highly effective in the treatment of chronic lymphocytic leukemia*

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Combined treatment of fludarabine (FLU) with cyclophosphamide (CY) may increase the complete remission (CR) rate, decreased minimal residual disease (MRD) and, possibly, prolong survival in B-chronic lymphocytic leukemia patient's (B-CLL).

The aim of study was to evaluate the activity and toxicity of FLU in combination with CY, the FLU-CY schedule, in patients with previously untreated B-CLL.

From May 1999 to December 2002, 57 patients with advanced or progressive B-CLL received treatment with FLU at a dose of 30 mg/m^2 for three consecutive days and CY at a dose of 300 mg/m^2 for three days. The cycles were repeated at four week intervals or longer if severe myelosupression occurred.

Guidelines for the evalution of response and toxicity were those developed by the National Cancer Institute Sponsored Working Group. Minimal residual disease (MRD) was detected by immunophenotyping only in patients with CR by standard criteria.

In the analyzed group an overall response (OR) rate (CR+PR) of 89.5% (95% CI 80.6–94.7%) was achieved, including complete response in 29.8%. At the time of analysis 15 of 17 patients with CR are still in remission. Median duration of follow up in these is 12 (range 4–29.2) months. MRD was detected only in five out of 17 (29.4%) patients with CR. Grade III/IV thrombocytopenia was seen in 3 (5.2%) patients and grade III/IV neutropenia in 6 (10.5%). Severe infections were noted in 14 (24%) patients. Two (3.5%) patients died, one due to sepsis, one as a result of disease progression.

The FLU-CY regimen is highly effective combination in previously untreated CLL patients with acceptable toxicity. The efficacy of the regimen seems to be higher than that observed earlier after treatment with FLU alone.

Key words: Chronic lymphocytic leukemia, fludarabin, cyclophosphamid, activity, toxicity.

The purine nucleoside analogues (PA) – fludarabine (FLU), cladribine (2-CdA) and 2'deoxycoformycin (DCF) – represent a novel group of cytotoxic agents characterized by high activity in B-cell chronic lymphocytic leukemia (B-CLL). Alkylating agents are the best candidates for combined use with nucleoside analogues. Interference with DNA repair by purine analogues raises the possibility that there might be synergistic antitumor effects with cyclophosphamid (CY), which act mainly by cross linking of DNA [1, 2, 3, 21].

Combined use of PA with CY may increase the complete remission (CR) rate, decrease minimal residual disease (MRD) and, possibly, prolong survival in CLL patient's (pts) [10, 13, 15].

The aim of the study was to evaluate the activity and toxicity of FLU in combination with CY, the FLU-CY schedule in pts with previously untreated B-cell chronic lymphocytic leukemia.

Patients and methods

Patients. Between May 1999 and December 2002, 57 pts with progressive or symptomatic untreated B-CLL entered the study. The characteristics of the pts are represented in Table 1.

All pts fulfilled the National Cancer Institute-Sponsored Working Group diagnostic criteria for B-CLL. The clinical

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Table 1. Characteristics of B-CLL patients before treatment with fludarabine and cyclophosphamide (FLU-CY)

Characteristics	No of patients	Percentage
Total	57	100
Sex		
male	26	46
female	31	54
AGE (median, range)	59	(38–69)
RAI stage		
0	3	5
I	9	16
II	13	23
III	10	18
IV	22	38
Median disease duration in months (range)	3	(0–21)
Mean number of WBCx10^9/l (range)	96	(14–320)
Mean number of platelets x10^9/l (range)	140	(37–310)
Mean Hb concentration g/l (range)	110	(50–130)

WBC – leukocytes, Hb – hemoglobin.

stage was determined before starting (FLU-CY) treatment according to the RAI classification [17].

All pts with clinical stage III and IV disease were eligible for the treatment. Patients with stage 0, I and II (RAI) were eligible if they had evidence of active disease, including progressive lymphocytosis, massive splenomegaly or bulky lymphadenopathy, recurrent disease-related infections, weight loss greater than 10% in a six-months period, and temperature of 38 ˚C related to disease or extreme fatigue.

Patients with poor performance status (WHO scale 4), active infection were excluded from the study. Immunophenotypicaly, all pts were CD5, CD19, CD20 and CD23 positive and showed monoclonality for light chain immunoglobulin membrane surface receptors.

Treatment modality. The combine (FLU-CY) cytostatic regimen consisted of fludarabine given at a dose 30 mg/m^2 by 2-hours intravenous infusion for three consecutive days and cyclophosphamide at a doses of 300 mg/m^2 by i.v. infusion for three days.

The cycles were repeated every 28 days. In patients in whom FLU-CY treatment induced hematological complications (thrombocytopenia <50x10^9/l and/or neutrophils <1x10^9/l) or severe infections developed, the drugs were re-administered at time intervals longer than one month, ranging from two to three months, until recovery of hematological parameters. Patients were treated until they achieved maximal response or prohibitive toxicity. If no response or progression of the disease was observed after three courses, the treatment was discontinued.

Packed red cells were transfused for symptomatic anemia or prophylactically when the hemoglobin level was lower than 75 g/l. Platelets were administered prophylactically when the platelet count was less then 20x10^9/l. Blood products were irradiated. In order to prevent hyperuricemia, allopurinol (300 mg/daily) was given. Patients did not received antibiotics, antiviral agents, hematopoietic growth factors or antiemetic drugs prophylactically.

However, G-CSF was given if the absolute granulocyte count was less than 1.0x10^9/l and active infection was present.

Response criteria. Guidelines for response were those developed by the NCI-Sponsored Working Group [4, 5].

Complete response (CR) required the absence of symptoms and organomegaly and the return to a normal blood count, with granulocyte count greater than 1.5x10^9/l, platelet count >100x10^9/l, hemoglobin concentration >110 g/l, and bone marrow of normal cellularity, with less than 30% lymphocytes in the aspiration smear. Bone marrow biopsy was required two months after the evidence of clinical CR. Bone marrow biopsy and aspirate had to be at least normocellular and with <30% of nucleated cells being lymphocytes and an absence of lymphoid nodules. Patients fulfilling the criteria stated above but with persistent lymphoid nodules in bone marrow biopsy were classified as nodular partial response (nPR). Partial response (PR) was considered as 50% or greater decrease in the size of lymph nodes, liver and spleen and peripheral blood findings either identical to those of CR, or improved over pretherapy values by at least 50%. Patients who did not achieve CR or PR were classified as non-responders (NR).

Clinical relapse was defined according to Robertson et al [20] as increase in the absolute lymphocyte count above 10x10^9/l, more than 50% increase in the liver or spleen below the costal margin, new appearance of palpable hepatosplenomegaly or development of an aggressive lymphoma.

Evaluation of minimal residual disease. In pts who achieved CR, minimal residual disease (MRD) was evaluated by immunophenotyping on peripheral blood (PB) and bone marrow (BM) by flow cytometry using, a simultaneous dual color staining technique.

MRD was determined by co-expression of CD5/CD19 on B-lymphocytes in conjuction with monoclonality of surface light chain expression on CD5-positive B-cells. Phenotypic CR was considered when less than 10% of the total lymphocytic population were known to co-express CD19/CD5 with monotypic light-chain expression. A kappa:lambda or lambda:kappa ratio exceeding 3:1 was considered as monotypic light-chain expression [23].

Statistical analysis. Sample size was calculated using the level of significance 0,05 and assuming the power of the study at 80%. The objective was to increase the historical CR rate of 20% following FLU as first line therapy to 45% following FLU-CY. At least 40 pts were to be included.

The significance of differences was evaluated by the

Mann-Whitney test as the level of significance $p<0.05$ [5, 6]. Statistical analysis of the differences in % of pts response was evaluated by x^2 test. Ninety-five percent confidence intervals for response probability were calculated using the method described by DUFFY and SANTNER [6]. Progression-free survival (PFS) and overal survival (OS) curves were calculated using the method of KAPLAN and MEIER [12] and compared between groups using the log-rank test. PFS was calculated from the achievement of CR or PR after FLU-CY therapy to the time of relapse. The survival time (OS) was measured from the day of first treatment to death from any cause or to the day of last observation.

Results

Fifty-seven previously untreated pts with B-CLL entered the study and all of them were evaluatable. The median time from diagnosis to FLU-CY treatment was 3 months (range 0–21 months).

Response to treatment. A total of 253 courses of FLU-CY were given to the entire group. All pts received at least two FLU-CY courses. The mdian number of FLU-CY cycles was 4 (range 2–6). The results of treatment are presented in Table 2. The criteria for CR were fulfilled in 17 (29.8%) (95% CI 19.2–38.1%) and overall response rate was 89.5% (95% CI 80.6–94.7%). Six patients (10.5%) did not respond to FLU-CY. CR was achieved in 2 out 3 patients with stage RAI 0 and more frequently in the pts with stage I and II (36.3%) than stage III and IV (21.8%) (p=0.6). The OR rate in the same groups was 100%, 91% and 87.5%, respectively (p=0.3). The CRs were observed after a median of 4 cycles (range 3–6). At the time of analysis 15 of 17 pts with CR were still in remission. The median duration of observation in pts with CR was 16 months (range 8–26) months.

Median duration of observation in all responding pts was 12 months (range 4–29.2). Median PFS time has not been reached to the day of interim analysis. However, a significant diference in survival was seen between pts who did not respond to treatment and responders (Fig. 1). Surface immunophenotyping by flow cytometry using dual color staining on the peripheral blood and/or bone marrow was performed in 17 pts who achieved CR. MRD was demonstrated in five patients (29.4%).

Toxicity. The toxicity of FLU-CY regimen is presented in Tables 3 and 4. Myelosupression was the major side effect of FLU-CY therapy (Tab. 3). FLU-CY-induced neutropenia was observed only in 18 (31.5%) pts. However/grade III or IV neutropenia was observed only in 6 (10.5%) pts. G-CSF was given to support 5 cycles of therapy in seven pts.

Thrombocytopenia occured in 8 (14%) pts and after 19 (7.5%) cycles. Three patients required platelets transfusion.

Table 2. Results of the treatment of B-CLL patients with FLU-CY according to RAI stages

RAI stage	N_0 of patients	CR n (%)	nPR (%)	n PR n (%)	OR n (%)	NR n (%)
0	3	2(66.6)	1(33.3)	0	3 (100)	0
I+II	22	8(36.3)	10(45.4)	2(9.1)	20(91)	2(9.1)
III+IV	32	7(21.8)	9(28.1)	12(37.5)	28(87.5)	4(12.5)
Total	57	17(29.8)	20(35.1)	14(24.5)	51(89.5)	6(10.5)

n – number of patients, CR – complete response, nPR – nodular partial response, PR – partial response, NR – no response, OR – CR+PR.

Table 3. Hematological toxicity of FLU-CY therapy in 57 patients with B-CLL

Toxicity	Grade I and II n (%)	Grade III and IV n (%)	Total n (%)
neutropenia	12 (21)	6 (10.5)	18 (31.5)
thrombocytopenia	5 (8.7)	3 (5.2)	8 (14)
anemia	3 (5.2)	2 (3.5)	5 (8.7)

n – number of patients.

Table 4. Non-hematological side effects of FLU-CY programme in 57 patients with B-CLL

Side effect	n (%)	nc (%)
Infections		
total	14 (24)	11 (4.3)
pneumonia	5 (8.7)	3 (1.2)
herpes	2 (3.5)	4 (1.6)
urinary tract infections	4 (7.0)	2 (0.8)
FUO	3 (5.3)	2 (0.8)
Vomiting grade III or IV	2 (3.5)	3 (1.2)

n – number of patients, nc – number of FLU-CY courses. Total number of FLU-CY courses = 253.

Anemia was seen only in five pts (8.7%). In one of them autoimmune hemolytic anemia (AIHA) was noted after the third FLU-CY course. The pts had no clinical or laboratory symptoms of AIHA before FLU-CY treatment. Non-hematological side effects are in Table 4. Infections occured in 14 (24%) patients. Pneumonia occured in five (8.7%) patients, herpes zoster reactivation in 2 pts. Vomiting at grade III or IV according to the WHO classification was seen in 2 (3.5%) pts, after 3 courses. Febrility of unknown origin (FUO) had 3 pts (5.3%). Opportunistic infections were not observed.

Four (7%) patients died 2–8 months from the start of FLU-CY treatment. Two of these four pts were non-responders, two as a result of disease progression.

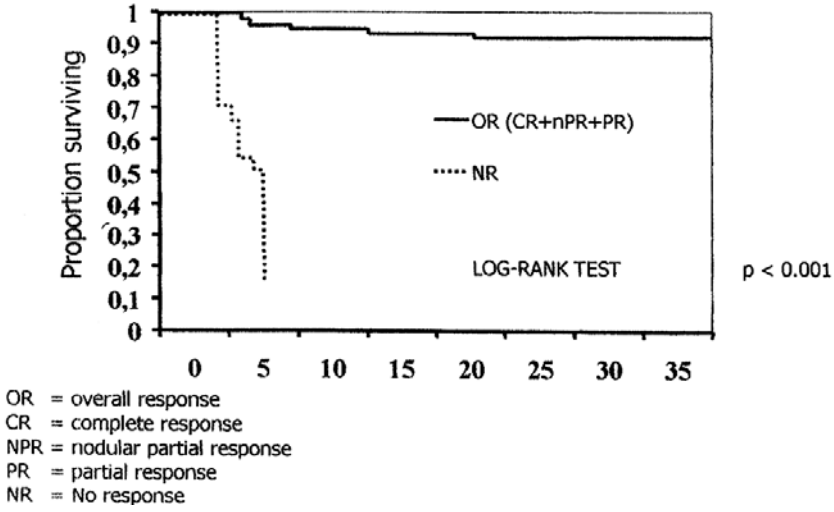

Figure 1. Survival of CLL patients treated with FLU-CY program according to response.

Discussion

The activity of purine nucleoside analogues, especially FLU and 2-CdA in CLL patients has been extensively studied for more than 10 years.

The results of randomized trials published recently indicate that treatment with these drugs results in a higher response rate and longer response duration than conventional treatment with chlorambucil or combination therapy such as the CVP or CAP regimen [8, 9]. However, despite their high activity, purine nucleoside analogues used as first line treatment do not prolong the survival time of CLL pts as compared to conventional therapy and so more effective treatment of CLL is still needed [7, 14, 20].

High activity of 2-CdA and cyclophosphamid was previously reported by Van Den Neste et al [22] in 13 pretreated CLL pts. They also observed an overall response rate of 62%, including 7% CR.

There are a few studies using FLU in combination with CY in untreated or previously treated CLL and low grade non Hodgkin's lymphoma (LG NHL) patients [9, 11, 16, 18, 19].

The aim of our study was to evaluate the activity and toxicity of a combination regimen consisting of FLU and CY (FLU-CY regimen) in previously umtreated B-CLL pts. We have observed a high overall response rate (89.5%), including high rate of complete response (29.8%).

O'Brien et al [15] gave the same dose of FLU for three days and CY at a dose of 300–500 mg/m^2 for three days as first or second line treatment in the group of 128 CLL pts. The effectiveness of this protocol seems to be similar to our FLU-CY regimen. The rate of OR in previously untreated pts in this study was 80%, including 38% CR [15]. An important aspect of our study was the evaluation of MRD by immunophenotyping. MRD was found in 5 (29.4%) out of 17 pts who fulfilled morphological criteria of CR. The percentage of pts with detectable MRD in our study is slightly higher than the 8% that observed by O'Brien et al [15]. Because of the short follow-up time of our pts it is impossible to evaluate the influence of MRD on the response duration and survival time. However, other studies indicate, that the elimination of MRD results in a prolonged response duration and overall survival time.

In the present study we have observed acceptable toxicity. Six pts (10.5%) suffered from neutropenia grade III or IV and grade III or IV thrombocytopenia was observed only in 3 pts (5.2%). However, infection occured in 14 (24%) pts, most often fever of unknown origin, pneumonia herpes zoster reactivation and urinary tract infections.

In conclusion, the FLU-CY regimen is a highly effective combination in previously untreated CLL pts and is associated with acceptable toxicity. The efficacy of the regimen seems to be higher than that observed earlier after treatment with FLU alone.

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