

Cladribine combined with cyclophosphamide is highly effective in the treatment of chronic lymphocytic leukemia

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The aim of the study was to evaluate the activity and toxicity of cladribine (2-CdA) in combination with cyclophosphamide (CY), the CC schedule, in patients with previously untreated B-cell chronic lymphocytic leukemia (B-CLL). From November 1998 to May 2002 82 patients with advanced or progressive B-CLL received treatment with 2-CdA at a dose of 0.12 mg/kg for three consecutive days and CY at a dose of 650 mg/m² on day 1. The cycles were repeated at four week intervals or longer if severe myelosuppression occurred. Guidelines for the evaluation 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 analysed group an overall response (OR) rate (CR+PR) of 87.8% (95% CI 80.7–94.9%) was achieved, including complete response (CR) in 29.3% patients (95% CI 19.4–39.1%). Twenty-two of 24 patients with CR and 39 of 48 patients with PR are still in remission. Median duration of follow-up in these patients is 11.8 months (range 3–25.4). MRD was only detected in six out of 24 (25%) patients with CR. Grade III/IV thrombocytopenia was seen in four patients (4.9%) and neutropenia in 10 (12.2%). Severe infections were noted in 21 (25.6%) patients. Thirteen patients died, including seven with treatment related toxicity, one because of CLL progression and five because of reasons not related to CLL. In conclusion, the CC schedule is a highly active regimen in previously untreated B-CLL, with acceptable toxicity. The efficacy of the regimen seems to be higher than observed earlier after treatment with 2-CdA alone. A randomized clinical trial is in progress in our institutions.

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Introduction

The purine nucleoside analogues – fludarabine (FAMP), cladribine (2-chlorodeoxyadenosine 2-CdA) and 2′deoxycoformycin (DCF) – represent a novel group of cytotoxic agents with high activity in B-cell chronic lymphocytic leukemia (B-CLL).^{1–4} 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 antitumour effects with cyclophosphamide (CY) or chlorambucil, which act mainly by cross linking of DNA.^{5,6}

In a previous experimental study we have shown synergistic action of 2-CdA and CY against murine leukaemias L1210 and P388.⁷ Furthermore, it has been demonstrated *in vitro* that 2-CdA and FAMP increase the antitumour effect of CY derivatives in B-CLL cells.^{8,9} Combined use of nucleoside analogues with alkylating agents may increase the CR rate, decrease minimal residual disease (MRD) and, possibly, prolong survival in CLL patients. Early clinical trials evaluating combinations of FAMP or 2-CdA with CY have demonstrated significant activity of such combinations in pre-treated and untreated patients with low-grade lymphoproliferative malignancies.^{10–13} In this study, we present our experience with the combination of 2-CdA with cyclophosphamide (CC regimen) in previously untreated patients with B-CLL.

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Patients and methods

Patients

Between November 1998 and May 2002, 82 patients with progressive or symptomatic B-CLL entered the study. All of them were previously untreated. The characteristics of the patients are presented in Table 1. All patients fulfilled the National Cancer Institute–Sponsored Working Group diagnostic criteria for CLL.¹⁴ The clinical stage was determined before starting CC treatment according to the Rai classification.¹⁵ Patients with stage 0, I and II were eligible if they had evidence of active disease, including progressive lymphocytosis (lymphocyte doubling time ≤ 6 months), massive splenomegaly or bulky lymphadenopathy, recurrent disease-related infections, weight loss greater than 10% in a six-month period, and temperature of 38°C related to disease or extreme fatigue. All patients with clinical stage III and IV disease were eligible for the treatment. Patients with poor performance status (WHO scale 4), active infection, abnormal liver or renal function, and Richter syndrome were excluded from the study. Immunologically, all patients were CD5, CD19, CD20 and CD23 positive and showed monoclonality for light chain immunoglobulin membrane surface receptors. We used monoclonal antibodies manufactured by DAKO and 2-color flow cytometry (Coulter, Hialeach, FL, USA).

The study was conducted in accordance with the updated Declaration of Helsinki. It was approved by the local ethical committee and all patients gave written informed consent.

Treatment modality

The doses and schedule of treatment agents were based on previous studies.^{4,16,17} 2-CdA (Biodrybin) was synthesized according to the method of Kazimierzczuk *et al.*¹⁸ and was commercially available from the

Institute of Biotechnology and Antibiotics–Bioton (Warsaw, Poland). The CC regimen consisted of 2-CdA given at a dose 0.12 mg/kg by 2-h intravenous infusion for three consecutive days and cyclophosphamide 650 mg/m² i.v. on day 1. The cycles were repeated every 28 days. In patients in whom CC treatment induced hematological complications (thrombocytopenia $< 50 \times 10^9/l$ and/or neutrophils $< 1 \times 10^9/l$) or severe infections developed, the drugs were re-administered at time intervals longer than one month, ranging from two to four months, until recovery of hematological parameters or experimental from infections occurred. 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 7.0 g/dl. Platelets were administered prophylactically when the platelet count was less than $15 \times 10^9/l$. Blood products were irradiated. In order to prevent hyperuricemia, allopurinol (300 mg/daily) was given. No patients received antibiotics, antiviral agents, hematopoietic growth factors or antiemetic drugs prophylactically. However, G-CSF was given if the absolute granulocyte count was less than $1.0 \times 10^9/l$ and active infection was present.

Response criteria

Guidelines for response were those developed by the NCI–Sponsored Working Group.¹⁴ Complete response (CR) required the absence of symptoms and organomegaly and the return to a normal blood count, with granulocyte count greater than $1.5 \times 10^9/l$, platelet count $> 100 \times 10^9/l$, hemoglobin concentration > 11.0 g/dl, 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 pre-therapy 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.*¹⁹ as increase in the absolute lymphocyte count above $10 \times 10^9/l$, more than 50% increase in the sum of the sizes of at least two lymph nodes, appearance of new lymph nodes, more than 50% increase in the liver or spleen below the costal margin, new appearance of palpable hepatosplenomegaly or development of an aggressive lymphoma.

Table 1 Characteristics of B-CLL patients before treatment with cladribine and cyclophosphamide (CC)

Characteristics	No. of patients	Percentage
Total	82	100
Sex		
Male	35	43
Female	47	57
Age (median range)	61 (28–78)	
Rai stage		
0	4	5
I	13	16
II	32	39
III	18	22
IV	15	18
Median disease duration in months (range)	4 (0–175)	
Mean number of WBC $\times 10^9/l$ (range)	110.4 (5–555)	
Mean Hb concentration g/dl (range)	11.4 (5–15)	
Mean number of platelets $\times 10^9/l$ (range)	147.4 (30–286)	

Evaluation of minimal residual disease

In patients who achieved CR, minimal residual disease (MRD) was evaluated by immunophenotyping on peripheral blood and bone marrow by flow cytometry using a simultaneous dual color staining technique. Residual disease was determined by co-expression of CD5/CD19 on B lymphocytes in conjunction 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 $k:\lambda$ or $\lambda:\kappa$ ratio exceeding 3:1 was considered as monotypic light-chain expression.²⁰

Toxicity monitoring

Hematological toxicity was evaluated according to NCI Sponsored Working Group criteria.¹⁴ Drug induced anemia, thrombocytopenia and neutropenia were diagnosed if after any treatment course a further decrease of erythrocytes, platelet and granulocyte numbers were observed. Non hematological side effects were assessed according to WHO criteria.²¹ The blood count, creatinine, bilirubin, GOT, GPT, ECG, urinalysis and general physical examination were serially evaluated and recorded.

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 2-CdA as first line therapy to 45% following CC. At least 60 patients were to be included.

The significance of differences was evaluated by the Mann-Whitney test at the level of significance $P < 0.05$. Statistical analysis of the differences in percentages of patients response was evaluated by the χ^2 test. Ninety-five per cent confidence intervals for response probability were calculated using the method described by Duffy and Santner.²² Progression-free survival and overall survival curves were calculated using the method of Kaplan and Meier²³ and compared between groups using the log-rank test. Progression free survival (PFS) was calculated from the achievement of CR or PR after CC therapy to the time of relapse. The survival time was measured from the day of first treatment to death from any cause or to the day of last observation.

Results

Eighty-two previously untreated patients with B-CLL entered the study and all of them were evaluable. The median time from the diagnosis to CC treatment was 4 months (range 0–175 months).

Response to treatment

A total of 330 courses of CC were given to the entire group. All patients received at least two CC courses. The median number of CC cycles was 3 (range 3–6). The results of treatment are presented in Table 2. The criteria for CR were fulfilled in 24 (29.3%) (95% CI 19.4–39.1%) and overall response rate was 87.8% (95% CI 80.7–94.9%). Ten patients (12.2%) did not respond to CC. CR was achieved in 2 out of 4 patients with stage Rai 0 and more frequently in the patients with stage I and II (33%) than stage III and IV (21%) ($P = 0.6$). The OR rate in the same groups was 100, 91 and 82%, respectively ($P = 0.3$). The CRs were observed after a median of 3 cycles (range 3–6). At the time of analysis 22 of 24 patients with CR and 39 out of 48 in PR were still in remission. The median duration of observation in patients with CR was 12.8 (range 4–25.4) months and in patients with PR 10.9 (3–24.9) months. Median duration of observation in all responding patients was 11.8 months (range 3–25.4). Median progression free survival time has not been reached to the day of interim analysis. However, a significant difference in survival was seen between patients who did not respond to treatment and responders (Figure 1).

Surface immunophenotyping by flow cytometry using dual color staining on the peripheral blood and/or bone marrow was performed in 24 patients who achieved CR. MRD was demonstrated in six patients (25%).

Toxicity

The toxicity of CC programme is presented in Tables 3 and 4. Myelosuppression was the major side effect of CC therapy (Table 3). CC-induced neutropenia was observed in 25 (30.5%) patients and after 50 (15.2%) courses. However, grade III or IV neutropenia was observed only in 10 patients. G-CSF was given to support 10 cycles of therapy in eight patients.

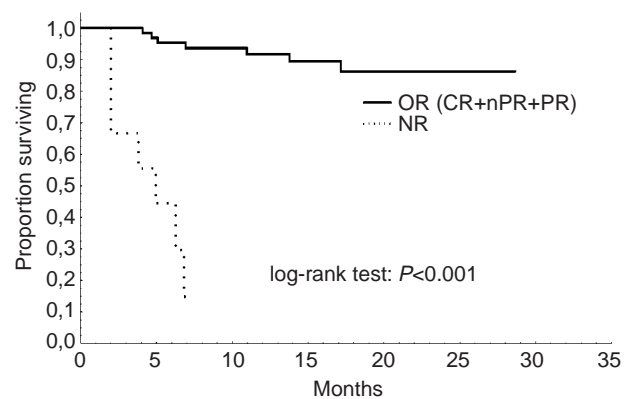


Figure 1 Survival of CLL patients treated with CC program according to response. OR = overall response; CR = complete response; nPR = nodular partial response; PR = partial response; NR = no response.

Table 2 Results of the treatment of B-CLL patients with CC according to Rai stages

Rai stage	No. of patients	CR n (%)	nPR n (%)	PR n (%)	OR n (%)	NR n (%)
0	4	2 (50)	1 (25)	1 (25)	4 (100)	0
I+II	45	15 (33.3)	10 (22.2)	16 (35.5)	41 (91.1)	4 (8.8)
III+IV	33	7 (21.3)	9 (27.2)	11 (33.3)	27 (81.8)	6 (18.2)
Total	82	24 (29.3)	20 (24.4)	28 (34.1)	72 (87.8)	10 (12.2)

n = number of patients; CR = complete response; nPR = nodular partial response; PR = partial response; NR = no response.

Table 3 Hematological toxicity of CC programme in 82 patients with B-CLL

Toxicity	Grade I and II		Grade III and IV		Total	
	n (%)	n1 (%)	n (%)	n1 (%)	n (%)	n1 (%)
Neutropenia	15 (18.3)	28 (8.5)	10 (12.2)	22 (6.7)	25 (30.5)	50 (15.2)
Thrombocytopenia	12 (14.6)	26 (7.9)	4 (4.9)	5 (1.5)	16 (19.5)	31 (9.4)
Anemia	4 (4.8)	6 (1.8)	2 (2.4)	5 (1.5)	6 (7.3)	11 (3.3)

n = number of patients, *n1* = number of courses (total number of CC courses = 330).

Table 4 Non-hematological side effects of CC programme in 82 patients with B-CLL

Side effect	Number of patients (%)	Number of courses ^a (%)
Infections		
Total	21 (25.6)	31 (9.4)
Pneumonia	10 (12.1)	20 (6.1)
Urinary tract infections	1 (1.2)	1 (0.3)
Thrombophlebitis	1 (1.2)	1 (0.3)
Herpes	9 (11.0)	9 (2.7)
Vomiting grade III or IV	4 (4.8)	4 (1.2)
Creatinine increased	1 (1.2)	1 (0.3)
DIC	1 (1.2)	1 (0.3)

^aTotal number of CC courses = 330.

Thrombocytopenia occurred in 16 (19.5%) patients and after 31 (9.4%) courses. Grade III or IV thrombocytopenia was observed in four patients. Four patients required platelets transfusions.

Moderate (grade II) anemia was seen only in four patients. In one of them autoimmune hemolytic anemia (AIHA) was noted after the third CC course. The patient had no clinical or laboratory symptoms of AIHA before CC treatment.

Infections occurred in 21 (25.6%) patients, after 31 (9.4%) courses. Pneumonia occurred in 10 (12.1%) patients and herpes zoster reactivation in nine (11.0%) patients. Opportunistic infections were not observed. Vomiting at grade III or IV according to the WHO classification was seen in four (4.8%) patients, after four (1.2%) courses. Disseminated intravascular coagulation syndrome developed in one case. Secondary malignancies comprised one case of breast cancer and one case of skin cancer. Richter syndrome manifested as Hodgkin disease was diagnosed in one patient.

Thirteen (18%) patients died 1.5–7.0 months (mean 3.5) from the start of CC treatment. Ten out of these 13 patients were non-responders. Four patients died because of sepsis, three because of pneumonia, one due to intestinal tract bleeding, one as a result of disease progression and four because of causes unrelated to CLL.

Discussion

The activity of purine nucleoside analogues, especially FAMP 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 consignment therapy such as the CAP regimen.^{3,4,24} However, despite their high activity, purine nucleoside analogues used as first line treatment do not prolong the survival time of CLL patients as compared to conventional therapy and so more effective treatment of CLL is still needed.

The aim of our phase II study was to evaluate of the activity and toxicity of a combination regimen consisting of 2-CdA and cyclophosphamide (CC regimen) in previously untreated B-CLL patients. We have observed a high overall response rate (87.8%) including high rate of complete response (29.3%). The efficacy of the CC regimen was similar to that reported earlier in CLL patients treated with 2-CdA alone or combined with prednisone (82.5 and 45.4% overall response rates, respectively) but the response criteria used in those studies were less restrictive.¹⁶ In our previous studies we used earlier the criteria proposed by NCI sponsored Working Group, which were based only on bone marrow cytology, without trephine biopsy and histology examination.²⁵ By these criteria the patients with nodular PR were probably diagnosed as CR patients, which could explain the high CR rate observed.¹⁴

High activity of 2-CdA and cyclophosphamide was previously reported by Van den Neste *et al.*¹¹ in 13 pretreated CLL patients. They also observed an overall response rate of 62%, including 7% CR.

There are a few studies using FAMP in combination with cyclophosphamide in untreated or previously treated CLL and low grade non Hodgkin's lymphoma (LG NHL) patients.^{10,13,26,30,31} The results of these studies are difficult to compare with ours because of heterogeneity of patients and differences in doses and schedules of the cytotoxic agents. O'Brien *et al.*¹³ gave

FAMP at a dose of 30 mg/m² for three days and cyclophosphamide at a dose of 300–500 mg/m² for three days as first or second line treatment in the group of 128 CLL patients.¹³ The effectiveness of this protocol seems to be similar to our CC regimen. The rate of overall responses in previously untreated patients in this study was 80%, including 38% CR.

An important aspect of our study was the evaluation of MRD by immunophenotyping. MRD was found in six (25%) out of 24 patients who fulfilled morphological criteria of CR. A similar analysis performed earlier in the group of B-CLL patients treated by us with 2-CdA alone, revealed MRD in nearly 30% of CR patients.³² The percentage of patients with detectable MRD in our present study is slightly higher than the 8% that observed by O'Brien *et al.*¹³ in previously untreated CLL patients who were administered FAMP and cyclophosphamide.¹³ Because of the short follow-up of our patients 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 study by O'Brien *et al.*¹³ median time to disease progression has not been reached after a median follow-up of 41 months. Moreover, it is known that the elimination of MRD, evaluated by molecular methods is a positive prognostic factor in CLL patients after autologous and allogeneic stem cell transplantation.³³

In the present study we have observed acceptable toxicity. Ten patients (12.2%) suffered from neutropenia grade III or IV and grade III or IV thrombocytopenia was observed only in four patients (4.9%). However, infection occurred in 21 (25.6%) patients, most often pneumonia or fever of unknown origin (FUO) and herpes zoster reactivation. A strong myelosuppressive and immunosuppressive effect results in the high incidence of infections in patients treated with purine nucleoside analogues.^{33–35} Earlier studies have shown that the combination of FAMP or 2-CdA with other cytotoxic agents resulted in increased myelotoxicity and a higher infection rate as compared to FAMP or 2-CdA alone. In a randomized study comparing chlorambucil with FAMP in monotherapy

and with FAMP combined with chlorambucil the latter arm was stopped because of high toxicity associated with the combined regimen.³ Moreover, combination therapy with FAMP plus chlorambucil resulted in significantly more infections than treatment with either single agent.³⁶ In our previous studies of untreated CLL patients treated with the CMC regimen (cyclophosphamide, 2-CdA and mitoxantrone), we have observed significant myelotoxicity and a higher incidence of infections, especially when 2-CdA was given for five days.¹⁷ The relatively lower toxicity of CC regimen may be explained by a significant reduction of the dose of 2-CdA. Although the daily dose of this drug (0.12 mg/kg), remained the same as in our previous studies,^{4,16} the duration of treatment has been shortened from five to three days. A similar reduction of the dose of purine nucleoside analogues combined with cyclophosphamide was used by others.^{12,13} Betticher *et al.*³³ found that the reduction of the dose of 2-CdA from 0.7 mg/kg per cycle to 0.5 mg/kg per cycle (29%) in pre-treated patients with malignant lymphomas did not decrease the activity of this compound, but greatly reduced the incidence of infections. Similarly, Karlson *et al.*³⁸ reported less major infections in CLL patients treated with oral 2-CdA for three days than in patients who received this agent in five day schedule.

In conclusion, the CC regimen is a highly effective combination in previously untreated CLL patients and is associated with acceptable toxicity. The efficacy of the regimen seems to be higher than that observed earlier after treatment with 2-CdA alone. MRD was demonstrated in only six out of 24 patients who achieved CR. A randomized study comparing the efficacy of the CC regimen with 2-CdA alone is being undertaken by our group.

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References

- 1 Tallman MS, Hakimian D. Purine nucleoside analogs: emerging roles in indolent lymphoproliferative disorders. *Blood* 1995; **86**: 2463–2472.
- 2 Robak T, Kasznicki M. Alkylating agents and nucleoside analogues in the treatment of B cell chronic lymphocytic leukemia. *Leukemia* 2002; **16**: 1015–1027.
- 3 Rai KR, Peterson BL, Appelbaum FR, Kolitz J, Elias L, Shepherd L, *et al.* Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. *N Engl J Med* 2000; **343**: 1750–1757.
- 4 Robak T, Błoński JZ, Kasznicki M, Błasińska-Morawiec M, Krykowski E, Dmoszyńska A, *et al.* Cladribine with prednisone versus chlorambucil with prednisone as first-line therapy in chronic lymphocytic leukemia: report of a prospective, randomized, multicenter trial. *Blood* 2000; **96**: 2723–2729.
- 5 Plunkett W. Fludarabine: pharmacokinetics, mechanism of action, and rationales for combination therapies. *Semin Oncol* 1993; **20**: 2–12.

- 6 Johnston JB, Verburg L, Shore T, Williams M, Israels LG, Begleiter A. Combination therapy with nucleoside analogs and alkylating agents. *Leukemia* 1994; **8**: (suppl 1), 140–143.
- 7 Góra-Tybor J, Robak T. Synergistic action of 2-chlorodeoxyadenosine and cyclophosphamide on murine leukemia L1210 and P388. *Acta Haematol Pol* 1993; **24**: 177–182.
- 8 Van den Neste E, Bontemps F, Delacauw A, Cardoen S, Louviaux I, Scheiff JM, et al. Potentiation of antitumor effects of cyclophosphamide derivatives in B-chronic lymphocytic leukemia cells by 2-chloro-2'-deoxyadenosine. *Leukemia* 1999; **13**: 918–925.
- 9 Bellosillo B, Villamor N, Colomer D, Pons G, Montserrat E, Gill J. In vitro evaluation of fludarabine in combination with cyclophosphamide and/or mitoxantrone in B-cell chronic lymphocytic leukemia. *Blood* 1999; **94**: 2836–2843.
- 10 Frewin R, Turner D, Tighe M, Davies S, Rule S, Johnson S. Combination therapy with fludarabine and cyclophosphamide as salvage treatment in lymphoproliferative disorders. *Br J Haematol* 1999; **104**: 612–613.
- 11 Van Den Neste E, Loviaux I, Michaux JL, Dellanoy A, Michaux L, Sonet A, et al. Phase I/II study of 2-chloro-2'-deoxyadenosine with cyclophosphamide in patients with pretreated B-cell chronic lymphocytic leukemia and indolent non-Hodgkin's lymphoma. *Leukemia* 2000; **14**: 1136–1142.
- 12 Laurencet FM, Zulian GB, Guetty-Alberto M, Iten PA, Betticher DC, Alberto P. Cladribine with cyclophosphamide and prednisone in the management of low-grade lymphoproliferative malignancies. *Br J Cancer* 1999; **79**: 1215–1219.
- 13 O'Brien SM, Kantarjian HM, Cortes J, Beran M, Koller CA, Giles FJ, et al. Results of fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. *J Clin Oncol* 2001; **19**: 1414–1420.
- 14 Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O'Brien S, Rai KR. National Cancer Institute-Sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood* 1996; **87**: 4990–4997.
- 15 Rai KR, Sawitsky A, Cronkite E, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. *Blood* 1975; **46**: 219–234.
- 16 Robak T, Błoński JZ, Kasznicki M, Konopka L, Ceglarek B, Dmoszyńska A, et al. Cladribine with or without prednisone in the treatment of previously treated and untreated B-cell chronic lymphocytic leukaemia – updated results of the multicenter study of 378 patients. *Br J Haematol* 2000; **108**: 357–368.
- 17 Robak T, Błoński JZ, Kasznicki M, Góra-Tybor J, Dwilewicz-Trojaczek J, Boguradzki P, et al. Cladribine combined with cyclophosphamide and mitoxantrone as front-line therapy in chronic lymphocytic leukemia. *Leukemia* 2001; **15**: 1510–1516.
- 18 Kazimierzczuk Z, Cottam HB, Ravanliar GR, Robins RK. Synthesis of 2'-deoxytubercidin, 2'-deoxyadenosin and related 2'-dexynucleosides via a novel direct stereospecific serum salt glycosylation procedure. *J Amer Chem Society* 1984; **106**: 6379–6382.
- 19 Robertson LE, Huh YO, Butler IJ, Pugh WC, Hirsch-Ginsberg C, Stass S, et al. Response assessment in chronic lymphocytic leukemia after fludarabine plus prednisone: clinical, pathologic, immunophenotypic and molecular analysis. *Blood* 1992; **80**: 29–36.
- 20 Brugiattelli M, Claisse JF, Lenormand B, Marabito F, Callea V, Malloum K, et al. Long term clinical outcome of B-cell chronic lymphocytic leukaemia patients in clinical remission phase evaluated at phenotypic level. *Br J Haematol* 1997; **97**: 113–118.
- 21 Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981; **47**: 207–214.
- 22 Duffy DR, Santner TJ. Confidence intervals for binomial parameter based on multistage tests. *Biometrics* 1987; **43**: 81–93.
- 23 Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1993; **53**: 457–481.
- 24 French Cooperative Group on CLL, Johnson S, Smith AG, Loffer H, Osby E, Juliusson G, Emmerich B, et al. Multicenter prospective randomized trial of fludarabine versus cyclophosphamide, doxorubicin and prednisone (CAP) for treatment of advanced-stage chronic lymphocytic leukemia. *Lancet* 1996; **377**: 1432–1438.
- 25 Cheson BD, Bennett JM, Rai KR, Grever MR, Kay NE, Schiffer CA, et al. Guidelines for clinical protocols for chronic lymphocytic leukemia: recommendations of the National Cancer Institute-Sponsored Working Group. *Am J Hematol* 1989; **29**: 152–163.
- 26 Flinn W, Byrd J, Morrison C, Jannison J, Diehl LF, Murphy T, et al. Fludarabine and cyclophosphamide with filgrastim support in patients with previously untreated indolent lymphoid malignancies. *Blood* 2000; **96**: 71–75.
- 27 Zaja F, Rogata A, Russo D, Mann, Silvestri F, Baccarani M. Combined therapy with fludarabine and cyclophosphamide in relapsed/resistant patients with B-cell chronic lymphocytic leukaemia and non-Hodgkin's lymphomas. *Eur J Haematol* 1997; **59**: 327–328.
- 28 Lazarino M, Orlandi A., Baldanti F, Furione M, Pagnucco G, Astori C, et al. The immunosuppression and potential for EBV reactivation of fludarabine combined with cyclophosphamide and dexamethasone in patients with lymphoproliferative disorders. *Br J Haematol* 1999; **107**: 877–882.
- 29 Scarisbrick JJ, Child FJ, Clift A, Sabroe R, Whittaker SJ, Spittle M, Russel-Jones R. A trial of fludarabine and cyclophosphamide combination chemotherapy in the treatment of advanced refractory primary cutaneous T-cell lymphoma. *Br J Dermatol* 2001; **144**: 1010–1015.
- 30 Hallek M, Schmitt B, Wilhelm M, Busch R, Kröber A, Fostitsch HM, et al. Fludarabine plus cyclophosphamide is an efficient treatment for advanced chronic lymphocytic leukemia (CLL): results of a phase II study of the German CLL Study Group. *Br J Haematol* 2001; **114**: 342–348.
- 31 Marotta G, Bigazzi C, Lenoci M, Tozzi M, Bocchia M, Lauria F. Low-dose fludarabine and cyclophosphamide in elderly patients with B-cell chronic lymphocytic leukemia refractory to conventional therapy. *Haematologica* 2000; **85**: 1268–1270.
- 32 Robak T, Błoński JZ, Urbańska-Ryś H, Błańska-Morawiec M, Skotnicki AB. 2-Chlorodeoxyadenosine (Cladribine) in the treatment of patients with chronic lymphocytic leukemia 55 years old and younger. *Leukemia* 1999; **13**: 518–523.

- 33 Esteve J, Villamor N, Colomer D, Cervantes F, Campo E, Carreras E, Monsterrat E. Stem cell transplantation for chronic lymphocytic leukemia: different outcomes after autologous and allogeneic transplantation and correlation with minimal residual disease status. *Leukemia* 2001; **15**: 445–441.
- 34 Anaissie EJ, Konotoyiannis DP, O'Brien S, Kantarjian H, Robertson L, Lerner S, Keating MJ. Infections in patients with chronic lymphocytic leukemia treated with fludarabine. *Ann Intern Med* 1998; **129**: 559–556.
- 35 Van den Neste E, Delannoy A, Vandercam B, Bosly A, Ferrant A, Mineur P, *et al*. Infections complications after 2-chlorodeoxyadenosine therapy. *Eur J Haematol* **56**, 235–240.
- 36 Morrison VA, Rai KR, Peterson BL, Kolitz JE, Elias L, Appelbaum FR, *et al*. Impact of therapy with chlorambucil, fludarabine or fludarabine plus chlorambucil on infections in patients with chronic lymphocytic leukemia: Intergroup Study Cancer and Leukemia Group B 9011. *J Clin Oncol* 2001; **19**: 3611–3621.
- 37 Betticher DC, Ratschiller D, Hsu Schmitz SF, von Rohr A, Hess U, Zulian G, *et al*. Reduced dose of subcutaneous cladribine induces identical response rates but decreased toxicity in pretreated chronic lymphocytic leukaemia. *Ann Oncol* 1998; **9**: 721–726.
- 38 Karlson K, Strömberg M, Liliemark J, Dellannoy A, Johnson SAN, Porwit A, *et al*. Oral cladribine for B-cell chronic lymphocytic leukemia: report of a phase II trial with a 3-d, 3-weekly schedule in untreated and pretreated patients, and a long-term follow-up of 126 previously untreated patients. *Br J Haematol* 2002; **116**: 538–548.