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ORIGINAL REPORT

Comparison of Cladribine Plus Cyclophosphamide With Fludarabine Plus Cyclophosphamide As First-Line Therapy for Chronic Lymphocytic Leukemia: A Phase III Randomized Study by the Polish Adult Leukemia Group (PALG-CLL3 Study)

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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ABSTRACT

Purpose
Little is known about comparison of the activity of different purine nucleoside analogs in chronic lymphocytic leukemia (CLL). We conducted a randomized phase III trial to compare efficacy and safety of cladribine and fludarabine, each combined with cyclophosphamide, in previously untreated progressive CLL.

Patients and Methods
Patients received cladribine at 0.12 mg/kg combined with cyclophosphamide at 250 mg/m^2 for 3 days intravenously (CC regimen) or fludarabine at 25 mg/m^2 combined with cyclophosphamide at 250 mg/m^2 for 3 days intravenously (FC regimen), every 28 days for up to six cycles. The primary end point was complete response (CR) rate. Secondary end points included overall response rate (ORR), progression-free survival (PFS), overall survival (OS), and treatment-related toxicity.

Results
Of 423 randomly assigned patients (211 to CC and 212 to FC), 395 were evaluated in the final analysis. The CR and ORR reached 47% and 88% in the CC arm and 46% and 82% in the FC arm ($P = .25$ and $P = .11$, respectively). The median PFS was 2.34 years with CC and 2.27 years with FC ($P = .51$). OS and grade 3/4 treatment-related toxicity were also comparable. Moreover, we did not observe any significant differences in CC and FC efficacy across different patient prognostic subgroups that included patients with 17p13 (*TP53* gene) deletion who had poor survival in both study arms.

Conclusion
Cladribine and fludarabine in combination with cyclophosphamide are equally effective and safe first-line regimens for progressive CLL. Both combinations have unsatisfactory activity in patients with 17p13 (*TP53* gene) deletion.

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a malignant proliferation of monoclonal, morphologically mature B cells that contribute significantly to morbidity and mortality from hematologic tumors in the elderly population.[1] In the majority of patients, CLL is progressive and treatment is necessary.[1] Recent clinical studies have demonstrated significant improvement regarding response rate and duration in CLL.[1-4] However, to date no randomized trial has shown that one therapy is superior to another with respect to prolongation of overall survival (OS).[2-4]

Purine nucleoside analogs—fludarabine (FA), cladribine (2-CDA; 2-chlorodeoxyadenosine), and pentostatin (DCF; 2′-deoxycoformycin)—are among the most active drugs against CLL that are used alone or combined with other agents.[5] Recently, several randomized trials have concordantly shown superiority of FA plus cyclophosphamide (FC) over FA alone; thus, FC has been proposed as a new standard first-line treatment in progressive CLL.[4,6,7] The Polish Adult Leukemia Group (PALG) has been actively developing 2-CDA–based combinations.[8,9] On the basis of results from a recent PALG randomized study, we established 2-CDA

plus cyclophosphamide (CC) as an first-line therapy for progressive CLL in Poland.[9]

However, little is known about the comparison of the activity of different purine nucleoside analogs in CLL. FA, 2-CDA, and DCF share a similar chemical structure and mechanism of action, but they also exhibit important differences, especially regarding interactions with enzymes involved in adenosine and deoxyadenosine metabolism and certain cytotoxicity pathways.[5] Interestingly, in vitro and in vivo studies reported only partial cross-resistance between 2-CDA, FA, and DCF.[10-12] Recent work using individualized tumor response testing revealed lower cross-resistance between DCF and 2-CDA or FA than between 2-CDA and FA.[12] Moreover, it has been suggested that 2-CDA and clofarabine (2-chloro-2′-arafluoroadenine), but not FA, can induce cell death by direct mitochondrial injury, which does not require p53 activity.[13] This is of potential importance because defective apoptotic response via the p53 pathway, which can be caused by deletions or mutations of *TP53* (locus in 17p13) or *ATM* (locus in 11q22) genes, is a leading cause of poor prognosis in CLL.[14] Interestingly, our preliminary observations on a small number of patients have suggested a relatively good response rate to 2-CDA in CLL with 17p13 deletion.[15]

Concerning the above data, we hypothesized that functional differences between 2-CDA and FA may translate into different activity of these drugs in CLL, or in specific patient subsets characterized by clinical, cytogenetic, or molecular features, such as dysfunction of p53. Therefore, in 2004, PALG initiated this phase III multicenter randomized study with the aim of directly comparing the efficacy and toxicity of CC and FC regimens in untreated patients with progressive CLL.

PATIENTS AND METHODS

Criteria for Eligibility

Patients age 18 years or older with previously untreated progressive CLL were enrolled onto the study. Diagnosis of CLL was established according to the National Cancer Institute–Sponsored Working Group (NCI-WG) criteria.[16] Staging was done according to Rai classification.[17] CLL was identified as progressive in patients with Rai stage III and IV or in patients with stage 0 to II with any of the following symptoms: progressive lymphocytosis (doubling time shorter than 6 months), massive splenomegaly or bulky lymphadenopathy, recurrent disease-related infections, weight loss above 10% over a 6-month period, disease-related fever of 38°C or higher, or extreme fatigue. Patients with poor performance status (WHO grade 4), autoimmune hemolytic anemia (AIHA), autoimmune thrombocytopenia, active infections, abnormal liver or renal function, Richter's syndrome, or concomitant neoplasm were not considered eligible.

The trial was conducted in accordance with the updated Declaration of Helsinki. The study protocol was approved by the local ethics committees at the participating institutions. Written informed consent was obtained for all participants.

Randomization Procedure and Treatment Modality

Central randomization was performed in the Department of Hematology, Medical University of Lodz, and the participating centers were informed about treatment assignment by phone or fax. Eligible patients were randomly assigned to receive either CC (2-CDA at 0.12 mg/kg intravenously over 30 minutes and cyclophosphamide at 250 mg/m^2 intravenously over 30 to 60 minutes, for cycle days 1, 2, and 3) or FC (FA at 25 mg/m^2 intravenously over 30 minutes and cyclophosphamide at 250 mg/m^2 intravenously over 30 to 60 minutes, for cycle days 1, 2, and 3). Both regimens were repeated every 28 days for up to six cycles. No routine prophylaxis with antibiotics, antiviral agents, or growth factors was planned. Therapy was interrupted if severe infections or progressive cytopenias developed until recovery from these complications. Patients with no response or progression after the first three cycles were to discontinue protocol treatment.

Study End Points, Response, and Toxicity Criteria

The primary study end point was complete response (CR) rate. The secondary end points included overall response rate, progression-free survival (PFS), OS, and treatment-related toxicity in the whole study population and within prognostic subgroups.

Treatment effects were monitored by physical examination, imaging techniques (chest x-ray and abdomen ultrasound or chest and abdomen computed tomography at the investigator' discretion), blood count evaluation, bone marrow aspiration, and biopsy. Response assessments were scheduled after administration of the initial three chemotherapy courses and then after treatment completion. Guidelines for response were those developed by the NCI-WG.[16] Patients who had not achieved CR or partial response were classified as nonresponders, including those who obtained less than three courses of chemotherapy due to disease progression or death.

OS was measured from random assignment to death from any cause or last contact. PFS was counted from random assignment to disease progression, death, or last contact. Disease progression was considered if at least one of the following occurred: an increase in the absolute lymphocyte count above 10×10^9/L, increase of $>$ 50% in new lymph nodes, increase of $>$ 50% in liver or spleen below the costal margin, the appearance of palpable hepatomegaly or splenomegaly, or development of an aggressive lymphoma. For nonresponders and progressive disease, the date of progression was when no response or progressive disease were recorded.

Hematologic toxicity was evaluated according to the NCI-WG criteria.[16] Treatment-induced anemia, neutropenia, and thrombocytopenia were diagnosed if a further decrease of hemoglobin, neutrophil, or platelet levels occurred after chemotherapy. Infections were considered therapy-related if they developed while on therapy or within 3 months of its completion. Direct and indirect Coombs tests were routinely performed at baseline and at the end of the treatment.

Interphase Cytogenetics and Immunophenotyping

Cytogenetic analysis was performed before the start of the study treatment using fluorescent in situ hybridization (FISH) assay. FISH was carried

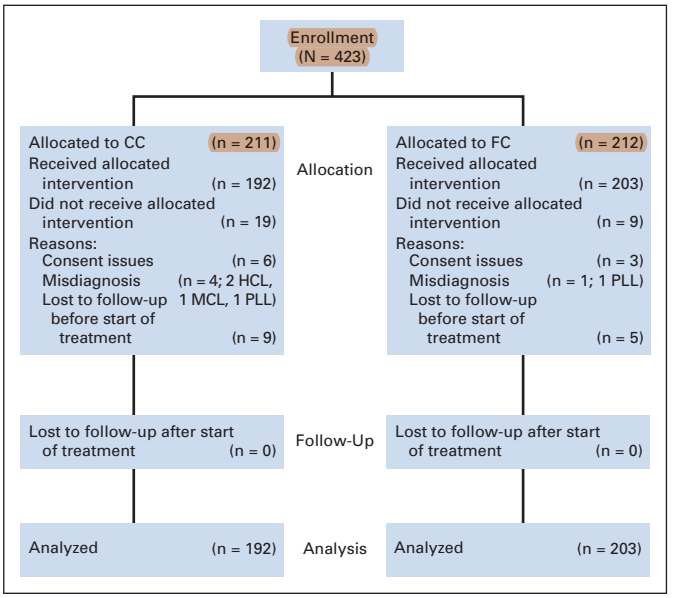

Fig 1. CONSORT diagram of the Polish Adult Leukemia Group Chronic Lymphocytic Leukemia 3 (PALG CLL-3) study. CC, cladribine + cyclophosphamide; HCL, hairy cell leukemia; MCL, mantle-cell lymphoma; PLL, prolymphocytic leukemia; FC, fludarabine + cyclophosphamide.

out on interphase nuclei of lymphocytes on blood smears as previously reported.[15] Four commercial probes were used (Vysis, Bergish-Gladbach, Germany), including the microsatellite chromosome 12 probe D12Z3 and the unique sequence or region-specific DNA probes: *TP53* (17p13.1.1 locus), *ATM* (11q22.3 locus), and D13S319 (13q14.3 locus). Signals were counted in at least 200 interphase nuclei for each sample. The cutoff level to define patients positive for tested cytogenetic abnormalities, including 13q14 deletion, 11q22 deletion, and 17p13 deletion, or trisomy 12, was set at 10% of analyzed cells. Given that some cytogenetic aberrations occur in combination, Döhner's hierarchical model was used to analyze their influence on each treatment effect.[14]

Table 1. Baseline Patient Characteristics and Prognostic Factors According to Treatment Arm

Characteristic	CC No.	CC %	FC No.	FC %	*P*
No. of patients randomly assigned	211		212		
No. of patients evaluated	192		203		
Age, years					
Median	58		59		.40
Range	37-81		27-81		
Male	122	63.5	138	68.0	.35
Leukocyte count, 10^9/L					
Median	86.8		93.8		
Range	6-630		9-681		.73
Hemoglobin, g/L					
Median	13.1		12.8		
Range	16-17		4-17		.60
Platelet count, 10^9/L					
Median	142		148		
Range	1-654		28-390		.61
Rai stage					.93
0	5	2.6	6	3.0	
I	39	20.3	45	22.2	
II	88	45.8	84	41.4	
III	23	12.0	25	12.3	
IV	37	19.3	43	21.2	
Time since diagnosis, months	1.1	0-208	1.6	0-263	
Hierarchical cytogenetics subgroup					.62
No. of patients evaluated	104		116		
Sole 13q deletion	24	23.1	25	21.6	
Normal	32	30.8	44	37.9	
Trisomy 12 (no 17p13 or 11q22 deletion)	8	7.7	9	7.8	
11q22 deletion (no 17p13 deletion)	25	24.0	19	16.4	
17p13 deletion	15	14.4	19	16.4	
β_2-microgrobulin level					
No. of patients evaluated	146		154		
Normal	41	28	45	29	
Elevated	105	72	109	71	
CD38 expression					.50
No. of patients evaluated	116		117		
High (≥ 30% cells)	49	42.2	44	37.3	
Low (> 30% cells)	67	57.8	73	62.4	
Bone marrow infiltration pattern					.80
No. of patients evaluated	97		115		
Nodular	8	8.2	12	10.4	
Mixed	17	17.5	22	19.1	
Diffuse	72	74.2	81	70.4	

Abbreviations: CC, cladribine + cyclophosphamide; FC, fludarabine + cyclophosphamide.

Flow cytometry immunophenotyping of peripheral blood and bone marrow aspirates was performed in all patients at baseline and after treatment to assess response.[18] Minimal residual disease in patients with CR was defined as the presence of > 10% of B lymphocytes coexpressing CD5/CD19 and CD5/CD20 with monotypic light-chain expression.[19]

Statistical Analysis

The planned sample size was 404 patients (202 patients per randomization arm) to allow detection of a 15% increase in CR rate from 25% CR rate expected with FC, with 90% power and $\alpha = .05$ for two-sided test.

Baseline patients' characteristics, response, and toxicity were compared between treatment arms using either the Kruskal-Wallis exact test or the Mann-Whitney *U* test for continuous data and the χ^2 or Fisher's exact test for categoric data. The influence of different variables, such as chemotherapy type, on the probability of achieving CR was analyzed by a step-wise logistic regression. PFS and OS curves were plotted using the Kaplan-Meier method.[20] The probabilities of PFS and OS were compared between groups by the log-rank test. A multivariate analysis of potential prognostic factors influencing PFS and OS was performed using a step-wise Cox regression. For each analysis, $P < .05$ was considered statistically significant.

Table 2. Factors Associated With Probability of CR Achievement, Shortened PFS, and Shortened OS in Univariate and Multivariate Analyses

Factor	OR	95% CI	*P*
Univariate analysis			
Normal β_2-microglobulin	1.7	1.0 to 2.8	.038
Low or intermediate clinical stage (Rai 0-II)	2.8	1.8 to 4.4	< .001
CD38 expression < 30%	2.0	1.2 to 3.5	.010
Lack of deletion 17p or 11q	2.8	1.6 to 5.1	.001
Female sex	1.5	1.0 to 2.3	.045
Non-diffuse bone marrow infiltration	2.6	1.4 to 4.7	.003
Multivariate analysis			
Lack of deletion 17p13 or 11q22	3.0	1.6 to 5.5	.001
Low or intermediate clinical stage (Rai 0-II)	1.4	1.4 to 5.1	.003
Female sex	2.5	1.4 to 4.6	.003
	HR		
Progression-free survival			
Univariate analyses			
Elevated β_2-microglobulin	1.5	1.1 to 2.0	.013
Advanced clinical stage (Rai III-IV)	1.5	1.2 to 2.0	.001
CD38 expression ≥ 30%	1.6	1.2 to 2.2	.004
Deletion 17p or 11q	1.5	1.1 to 2.1	.016
Diffuse bone marrow involvement	1.7	1.1 to 2.5	.008
Multivariate analysis			
Deletion 17p or 11q	2.0	1.2 to 3.1	.006
Diffuse bone marrow involvement	2.0	1.1 to 3.5	.015
Overall survival			
Univariate analysis			
Elevated β_2-microglobulin	1.9	1.2 to 3.2	.012
Advanced clinical stage (Rai III-IV)	1.8	1.3 to 2.6	.001
Age ≥ 60 years	1.6	1.1 to 2.3	.011
Deletion 17p or 11q	1.9	1.2 to 3.1	.005
Diffuse bone marrow involvement	2.8	1.5 to 5.3	.002
Multivariate analysis			
Deletion 17p or 11q	3.2	1.7 to 6.3	.001
Diffuse bone marrow involvement	2.4	1.0 to 5.9	.049
Advanced clinical stage (Rai III-IV)	2.0	1.0 to 3.8	.052

NOTE. Prognostic factors with significant impact on probability of complete remission achievement, shortened progression-free survival, and shortened overall survival derived from univariate and multivariate regression models in the total study population.

Abbreviations: CR, complete response; PFS, progression-free survival; OS, overall survival; OR, odds ratio; HR, hazard ratio.

RESULTS

Patient Characteristics

Between January 1, 2004, and May 31, 2007, 423 patients from 15 hematology centers were enrolled onto the study. A total of 211 individuals were randomly assigned to CC and 212 patients to FC treatment. Twenty-eight patients were excluded from final study analysis, including 19 patients in the CC arm and nine patients in the FC arm. The reasons for patients' exclusion are shown in the CONSORT diagram (Figure 1). Therefore, responses to therapy, survival, and toxicity were assessed for a total of 395 patients (192 in the CC arm and 203 in the FC arm). The patients' baseline characteristics are listed in Table 1. The treatment arms were well balanced in regard to patients' age, sex, CLL stage, blood counts, and prognostic factors (Table 1).

Clinical Response

The CR rate reached 47% in the CC arm and 46% in the FC arm ($P = .25$). Minimal residual disease was tested in 109 of 183 patients who obtained CR and was found negative in 33 of 48 patients treated with CC and in 44 of 61 patients treated with FC ($P = .70$). The overall response rates with both treatments were also comparable (88% in the CC arm *v* 82% in the FC arm; $P = .11$). The median numbers of administered chemotherapy courses were six in the CC arm and five in the FC arm ($P = .28$). All six planned courses were given to 98 (51%) of 192 patients receiving CC and to 98 (48%) of 203 patients receiving FC. Factors associated with probability of CR achievement in univariate and multivariate analyses are presented in Table 2.

Concerning the prognostic subgroups, no significant differences in CR rate to CC and FC were found in patients stratified according to age, sex, clinical stage, Döhner's cytogenetic group, CD38 expression, β_2-microglobulin level, and pattern of bone marrow infiltration (Table 3).

Interestingly, we observed a relatively important, though not statistically significant, difference in treatment effects in 34 patients with deletion 17p13. Six (40%) of 15 patients in the CC arm achieved CR compared with only three (16%) of 19 in the FC arm ($P = .112$). To further explain this difference, we analyzed the distribution of other prognosticators in this group of patients. We found that patients from the FC arm had significantly more advanced stage than patients from the CC arm. Rai stage III-IV was present in 13 (86%) of 15 such patients in the FC arm compared with only eight (42%) of 19 patients in the CC arm ($P = .008$). Other prognostic factors were proportionally distributed in both arms. Therefore, this random nonbalanced distribution of advanced stage in patients with deletion 17p13 could likely contribute to the observed response difference. In line with this, in the combined analysis of patients with deletion 17p13 and/or deletion 11q22, there was no significant difference between the CC arm (13 [32%] of 40 CRs) and the FC arm (10 [26%] of 38 CRs; $P = .55$).

Table 3. Comparison of Complete Response Rate and Progression-Free Survival Achieved With CC and FC Chemotherapy Protocols in Subgroups of Patients Stratified According to Specific Prognostic Factors

	Complete Responders					Progression-Free Survival (months)				
	CC (n = 192)*		FC (n = 203)*			CC (n = 192)*		FC (n = 203)*		
Prognostic Factor	No.	%	No.	%	*P*	Median	95% CI	Median	95% CI	*P*
Age group, years										
< 60	46	45	50	48	.67	26.5	21.9 to 31.2	28.5	23.0 to 33.9	.87
60-69	25	50	22	37	.18	24.6	19.3 to 29.8	25.9	15.2 to 36.6	.79
≥ 70	14	47	18	60	.44	28.3	19.4 to 38.3	21.1	9.5 to 32.6	.59
Sex										
Male	52	43	59	43	1.00	26.5	23.1 to 29.9	26.4	23.0 to 29.9	.37
Female	38	54	34	52	.86	30.3	21.1 to 39.4	30.4	21.7 to 39.3	.95
Rai clinical stage										
0-II	74	56	71	53	.62	30.2	7.5 to 32.9	29.0	25.6 to 32.4	.52
III-IV	16	27	22	32	.56	22.5	18.6 to 26.4	17.0	6.6 to 27.4	.82
Hierarchical FISH group										
Sole 13q deletion	13	54	15	60	.68	24.9	6.2 to 43.7	34.5	27.4 to 42.1	.14
Normal	20	62	22	50	.28	32.2	25.1 to 39.2	28.8	24.3 to 35.4	.46
Trisomy 12 (no 17p or 17q deletion)	4	50	4	44	.82	28.0	0 to 62.5	30.3	17.5 to 43.2	.36
11q deletion (no 17p deletion)	7	28	7	37	.53	25.1	14.3 to 36.0	25.5	7.3 to 43.7	.53
17p13.1 deletion	6	40	3	16	.11	25.3	14.1 to 36.5	9.2	5.1 to 13.3	.14
β_2-microglobulin										
Normal	24	58	24	53	.63	32.2	26.2 to 38.2	29.7	19.0 to 40.4	.29
Elevated	44	42	47	43	.86	23.0	20.0 to 26.1	27.3	22.8 to 31.7	.30
CD38 expression										
< 30%	40	60	37	51	.28	37.8	27.6 to 48.1	31.5	24.6 to 30.1	.35
≥ 30%	19	39	16	36	.81	24.6	20.8 to 28.4	24.1	18.1 to 30.1	.49
Bone marrow infiltration pattern										
Nondiffuse	25	55	33	41	.44	40.5	22.9 to 58.2	36.0	32.4 to 39.5	.87
Diffuse	16	64	20	59	.69	24.1	19.3 to 28.8	25.9	21.3 to 30.6	.97

Abbreviations: CC, cladribine + cyclophosphamide; FC, fludarabine + cyclophosphamide; FISH, fluorescent in situ hybridization.
*For some analyses, numbers of patients were smaller because the analyses were restricted to patients with known result of a given prognostic factor.

PFS

At the time of this analysis, 264 patients had reported either progression or death in the absence of progression. This included 123 patients treated with CC and 141 patients who received FC. For all study participants, the median PFS reached 2.27 years (95% CI, 2.09 to 2.46 years). Both tested regimens resulted in a comparable duration of PFS (Fig 2). The median PFS in the CC arm reached 2.34 years (95% CI, 2.03 to 2.64 years), while in the FC arm it reached 2.27 years (95% CI, 1.99 to 2.54 years; $P = .51$). The risk estimates for various factors that showed a significant influence on PFS in univariate and multivariate models are given in Table 2. Comparisons of PFS between patients treated with CC and FC performed in different prognostic subgroups did not reveal a significant impact of treatment type (Table 3).

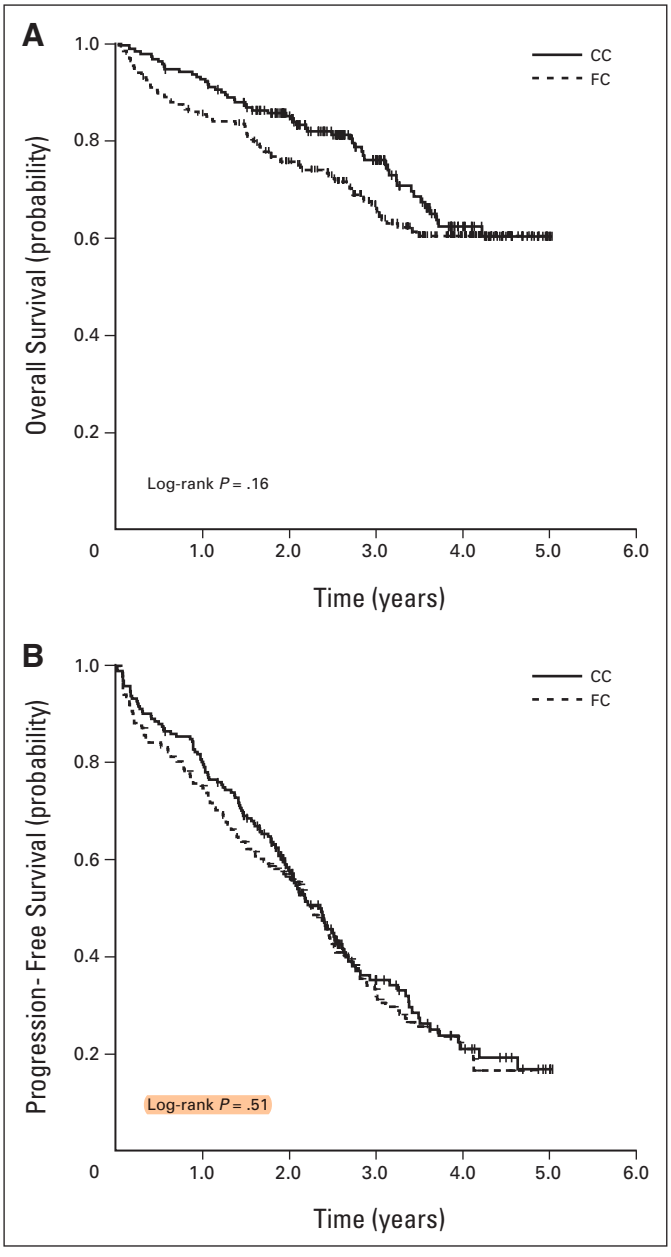

Fig 2. Comparison of probability of (A) overall survival and (B) progression-free survival in chronic lymphocytic leukemia patients treated according to cladribine plus cyclophosphamide (CC) or fludarabine plus cyclophosphamide (FC) protocol.

OS

At the time of this report, the median follow-up among the censored patients was 3 years 2 months. For all study participants, the median OS has not been reached, while the 4-year probability of survival was predicted at 61.7% (95% CI, 55.7% to 67.3%). In agreement with the results of PFS analysis, no significant difference was observed in OS between CC and FC arms (log-rank test, $P = .164$). Four-year OS probability was estimated as 62.4% (95% CI, 53.2% to 70.8%) for patients receiving CC and as 60.6% (95% CI, 52.9% to 67.8%) for patients treated with FC. The OS curves for CC and FC treatment arms are shown in Figure 2.

Importantly, the OS in patients with 17p13 was not significantly different in both studied treatment arms (log-rank test, $P = .198$). Similarly, the OS probability was comparable for patients with 11q22 deletion without deletion 17p13 ($P = .59$). Moreover, after adjustment for clinical stage, we observed similar influence of cytogenetics on probability of OS in patients treated with CC and FC (Fig 3). In both treatment arms, patients with deletion 17p13 had the worst outcome.

The factors associated with shortened OS in univariate and multivariate analyses are presented in Table 2. A multivariate model demonstrated that only the presence of deletion 17p13 or 11q22 (hazard ratio, 3.2; 95% CI, 1.7 to 6.3; $P = .001$) and diffuse bone marrow infiltration pattern (hazard ratio, 2.4; 95% CI, 1.0 to 5.9; $P = .049$) retained an independent negative impact on OS (Table 2).

Toxicity

The treatments were generally well tolerated in both study arms. Table 4 presents a comparison of grade 3 and 4 chemotherapy adverse effects stratified to regimen type. The most common grade 3/4 complications were infections (27% in the CC arm and 28% in the FC arm; $P = .84$), cytopenias, and AIHA (10% in the CC arm and 7% in the FC arm; $P = .30$). There were no significant differences in the proportion of specific acute hematologic and nonhematologic treatment adverse effects between study arms. Secondary tumors, including Richter's transformation, also seemed equally frequent (Table 4). However, a longer follow-up is needed regarding such relatively rare and late complications.

At the time of this report, 123 (31%) study patients had died (50 patients in the CC arm and 73 in the FC arm). The major causes of death included progression of CLL (34%) and severe infections (29%), while no significant differences in the rates of different causes of death were noted between study arms. Interestingly, in seven patients (four in the CC arm and three in the FC arm), AIHA was reported as the cause of death.

DISCUSSION

In this phase III study, we found that first-line treatment with the CC regimen gives similar response rate, response duration, survival, and toxicity to treatment with FC in patients with progressive CLL. Moreover, we have shown that the efficacy of CC and FC is comparable across different CLL patient subsets, including patients with 17p13 (*TP53*) or 11q22 (*ATM*) deletions.

Several large cooperative group studies comparing CC or FC to purine analog monotherapy have recently been reported.[4,6,7,9] In the

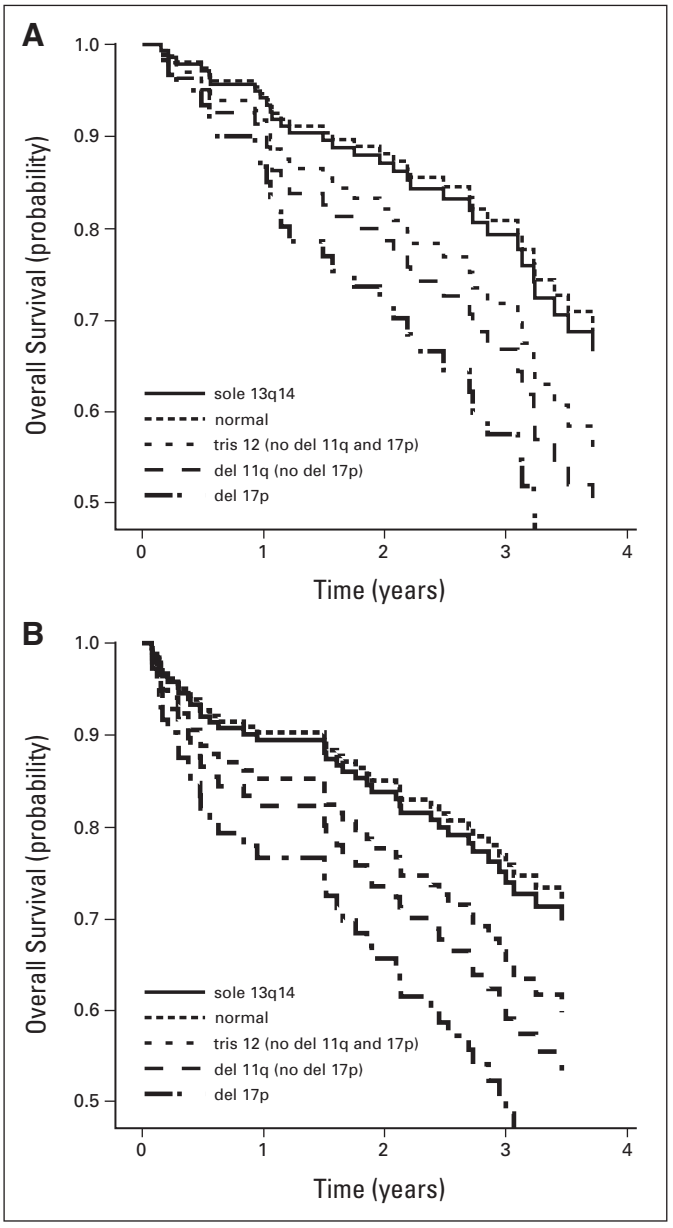

Fig 3. Probability of overall survival in (A) cladribine plus cyclophosphamide and (B) fludarabine plus cyclophosphamide treatment arms stratified by Döhner's hierarchical cytogenetic group and adjusted for Rai clinical stage. The plots show survival curves for different cytogenetic groups at the mean of the second covariate of the two-covariate Cox regression model, the Rai clinical stage.

Table 4. Grade 3/4 Hematologic and Nonhematologic Treatment Toxicities Stratified According to Randomization Group

Type of Toxicity/Hematologic Complication	CC (n = 192)		FC (n = 203)		
	No.	%	No.	%	*P*
Neutropenia	39	20	43	21	.81
Thrombocytopenia	24	12	22	11	.62
Anemia	21	11	18	9	.50
Infections	53	28	54	27	.84
Autoimmune complications					
AIHA	19	10	14	7	.30
ITP	14	7	8	4	.16
PRCA	1	0.5	1	0.5	.97
Secondary neoplasms	6	3	11	5	.26
Richter's syndrome	4		2		
Lung cancer	2		4		
Colon cancer	0		2		
Melanoma	0		2		
Renal carcinoma	0		1		
Other grade 3/4 complications	5	3	4	1	.67

Abbreviations: CC, cladribine + cyclophosphamide; FC, fludarabine + cyclophosphamide; AIHA, autoimmune hemolytic anemia; ITP, autoimmune thrombocytopenia; PRCA, pure red blood cell aplasia.

PALG-CLL2 trial, we compared 2-CDA monotherapy to the CC regimen and CMC regimen (cladribine, mitozantrone, and cyclophosphamide) in a total of 508 untreated progressive CLL patients.[9] We observed a CR rate of 29% and median PFS of 1.87 years in the CC treatment arm.[9] Somewhat better results with CC therapy in this study (CR rate of 47% and median PFS of 2.34 years) may be due to modification of the CC protocol, in which cyclophosphamide was administered for 3 days instead of 1 day and at a higher total dose.[9]

Regarding FC combination, the German Chronic Lymphocytic Leukemia Group trial compared FC or FA monotherapy in CLL patients younger than age 66 years and reported 24% CRs with a median PFS of 48 months with FC.[6] In the US Intergroup Trial E2997, which randomly assigned 278 patients to FC or FA, the CR rate in the FC arm reached 23% with a median PFS of 31.6 months.[7] The largest of these studies, the Leukemia Research Foundation Chronic Lymphocytic Leukemia 4 (LRF CLL4) Trial[4] assessed three first-line CLL treatments—chlorambucil, FA monotherapy, and FC—in 777 patients. The authors reported 38% CRs and median PFS of 3 years 7 months for patients receiving FC.[4] Interestingly, in these three trials and our study, the efficacy of FC varied significantly with CR rates between 23% and 46% and median PFS between 2.0 and 3.7 years.[4,6,7] It is likely that these differences are due to some alterations in FC protocol as well as different entry criteria for each of the trials. This further supports the need for direct randomized comparisons of treatments for CLL.

Most importantly, the results of our first randomized comparison of 2-CDA–based and FA-based combinations are not in line with a recent randomized comparison of 2-CDA and FA monotherapy by Karlsson et al.[21] They found significantly higher CR rate and longer PFS with 2-CDA than with FA or chlorambucil. The reason for this discrepancy is unclear, although it could be hypothesized that the increase of an antitumor effect after combination with cyclophosphamide is greater for FA than for 2-CDA.

We have not observed any differences between CC and FC efficacy across different prognostic subgroups of CLL patients. However, it should be noted that the prognostic factors, including FISH cytogenetics, were not available in a significant proportion of patients, which limited the power of our subanalyses. Despite our previous observations,[15] this trial has not indicated any significant difference between CC and FC in patients with 17p13 (*TP53*) deletion. In both treatment arms, 17p13 deletion was the most powerful predictor of poor outcome. Therefore, development of non-p53–dependent therapies, such as alemtuzumab, lenalidomide, flavopiridol, or nonmyeloablative stem-cell transplantation, seems crucial for improvement of outcome in this poor-prognosis setting.[22-27]

One of successful strategies for further improving the activity of CC and FC is incorporation of monoclonal antibodies.[28,29] As was

recently demonstrated,[28,29] the response to FC can be increased by the addition of rituximab, an anti-CD20 monoclonal antibody, in both chemotherapy-naïve and pretreated patients. Whether prolongation of patients' survival, which is the ultimate aim of CLL therapy, is attainable with the rituximab-based immunochemotherapy is not yet known.

In conclusion, in this randomized comparison of combinations of different purine nucleoside analogs in CLL, we have shown that 2-CDA and FA, when combined with cyclophosphamide, are equally effective and safe first-line treatments for progressive CLL. Both regimens have unsatisfactory activity in patients with p53 dysfunction; thus, further development of p53-independent agents and cytogenetic risk-adapted treatment strategy seem essential to improve CLL patients' survival.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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