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Fludarabine, ara-C, novantrone and dexamethasone (FAND) in previously treated chronic lymphocytic leukemia patients

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Background and Objectives. The objective of improving the quality of responses of chronic lymphocytic leukemia (CLL) patients has led to the design of protocols that combine fludarabine (FDR) with synergistic drugs. We evaluated the efficacy and toxicity of a schedule that includes fludarabine, ara-C, novantrone and dexamethasone (FAND) for the management of previously treated CLL patients under 60 years old.

Design and Methods. Thirty-one patients underwent FAND treatment. Twenty-three patients had active disease (relapsed patients: 9; unresponsive to prior therapy: 14). Eight patients had a partial response (PR) to prior therapy and were treated with the aim of further reducing residual disease. The FAND schedule included fludarabine (25 mg/m^2 i.v. days 1-3), ara-C (1 g/m^2 i.v. day 1: 8 patients; days 1-2: 23 patients), novantrone (10 mg/m^2 i.v. day 1) and dexamethasone (20 mg i.v. days 1-3). Infection prophylaxis consisted of fluconazole, acyclovir, trimethoprim/sulfamethoxasole and granulocyte colony-stimulating factor (G-CSF) in the presence of severe neutropenia.

Results. A response was observed in 7/14 refractory patients (complete response-CR: 29%), in all 9 relapsed patients (CR: 78%) and in 7/8 patients (CR: 87.5%) treated in PR. Taken together, 18 CRs were obtained and in 14 (78%) this was associated with a flow cytometric remission (CD5$^+$/CD20^{weak+} PB lymphocytes: <10%). Severe granulocytopenia occurred after 86 of the 124 administered courses (69%), but only after 10/86 courses (12%) were major infections recorded. In 10/15 mobilized patients (cyclophosphamide + G-CSF: 6 patients; FAND + G-CSF: 9 patients) after FAND ≥ 2×10^6/kg CD34$^+$ cells were collected. Nine patients were autografted in CR and showed a longer response duration than the 9 patients in CR who did not receive further therapy after FAND (53 vs 30% at 41 months; p=0.05).

Interpretation and Conclusions. FAND associated with extensive infection prophylaxis and G-CSF support is a highly cytoreductive and well-tolerated treatment for CLL patients and in most cases does not hamper subsequent stem cell mobilization.

Key words: chronic lymphocytic leukemia, treatment, fludarabine, mitoxantrone, cytarabine, dexamethasone.

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Over the last decades treatment has not modified the overall survival of patients with chronic lymphocytic leukemia (CLL). Patients requiring therapy show a survival probability that diminishes as the number of relapses increases and may be dramatically short – about 1 year – for patients unresponsive to therapy.[1-3]

At present fludarabine is considered the best therapeutic option as front-line treatment of patients with advanced CLL. As compared to those treated with other drugs, CLL patients treated with fludarabine as first-line therapy display a higher rate of clinical complete responses (CR) and a delayed time to retreatment.[4,5] However, the long-term results show that, when compared to CHOP or CAP, fludarabine treatment is not associated with a survival benefit.[5]

Fludarabine is less effective in previously treated patients in whom a response is reported in about half of cases and a CR in only 13% of cases.[6] The current therapeutic strategy is aimed at reaching better quality responses since patients achieving a CR show a more prolonged time to progression.[7-9] The achievement of a CR also represents an advantage for patients who are candidates for transplant procedures, since a better outcome has been observed in patients transplanted in CR.[10]

The objective of improving the quality of responses has led to the design of cytoreductive protocols that combine fludarabine with synergistic drugs, as determined by pharmacologic and clinical findings, particularly for the young patients who represent 20-30% of cases of CLL.[11] Different schedules based on fludarabine combined with one or more active

drugs, such as cytarabine,[12-14] cyclophosphamide,[15,16] cisplatinum,[14] anthracyclines[13,17–20] and dexamethasone, have been investigated in patients with chronic lymphoproliferative diseases. It has recently been reported that such combinations may be effective in both untreated, as well as in previously treated CLL patients.[15] Based on these findings, we designed a schedule that includes fludarabine combined with ara-C, novantrone and dexamethasone (FAND) for the management of previously treated CLL patients. The rationale of this schedule is based on the high intracellular concentrations of ara-CTP obtained when fludarabine is administered before cytarabine infusion,[12] on the inhibition of DNA repair enzymes by fludarabine and mitoxantrone,[21,22] and on the clinical activity of the association of fludarabine and dexamethasone observed in low-grade lymphoproliferative diseases.[19] The schedule was designed as salvage therapy in an attempt to improve the quality of response in patients with poor prognosis CLL. We report the efficacy and toxicity of this combination therapy administered to 31 previously treated CLL patients aged less than 60 years who were managed in a single institution.

Design and Methods

Patients

Between 1995-1999, 31 consecutive patients with CLL entered the study. Study entry required a diagnosis of CLL according to National Cancer Institute (NCI) criteria,[23] one or more previous treatments, the absence of other clinically significant diseases and that the patient was 60 or less years of age. CLL stage was defined according to the classifications proposed by Rai[24] and Binet.[25] Written informed consent was obtained from all patients included in the study. The median age of the patients was 54 years (range: 22-60); 29 patients were males and 2 females; the median duration of CLL was 53 months (range: 6-114) and the median follow-up of patients treated with FAND was 34 months (range: 6-68).

Two different subsets of patients were treated. The first group was formed of 23 patients with active disease (clinical stage C/III-IV: 11 patients), 9 of whom were in relapse and 14 unresponsive to previous therapy. A median number of 2 treatments (range: 1-4) had been administered prior to FAND and included fludarabine in 14 cases (8 relapsed patients and 6 refractory patients) (Table 1). In the latter group, resistance to fludarabine was defined as follows: stable disease after 2 cycles of treatment in 4 patients and an early relapse within 2 months of fludarabine discontinuation in the remaining 2 patients. The second group included 8 patients in partial response (PR) after prior therapy (fludarabine: 6 patients) in whom FAND treatment was given with the aim of further reducing residual disease. The latter was defined as the presence of more than 30% lymphocytes infiltrating the bone marrow and/or by the presence of enlarged nodes (>2 cm of diameter) and/or splenomegaly (>2 cm below the costal margin). The clinical characteristics of the entire case series are reported in Table 1.

Therapy schedule

The combination regimen was designed as follows:

- Fludarabine 25 mg/m^2 i.v. daily at 0, 24 and 48 hours;
- Ara-C 1 g/m^2 i.v. at 4 hours in the first 8 patients, and at 4 and at 28 hours in the remaining 23 patients;
- Novantrone 10 mg/m^2 i.v. at 6 hours;
- Dexamethasone 20 mg i.v. daily on days 1 to 3.

Courses were repeated every 4 weeks. The median number of administered courses was 4 (range 2-6).

Supportive care

Infection prophylaxis, consisting of daily administration of fluconazole 50 mg orally, acyclovir 200 mg orally every 8 hours and trimethoprim/sulfamethoxasole orally three times a week, was given to the majority of patients (26 patients). Granulocyte-colony stimulating factor (lenograstim) was administered in the presence of severe neutropenia (<500 PMN/μL) during the second part of the study (21 patients). Ondansetron was given prior to the administration of cytarabine. Patients requiring blood transfusions were given irradiated products. Treatment was administered on an outpatient basis.

Response evaluation

Clinical evaluation

Response was assessed according to the NCI criteria.[23] The restaging evaluation included physical examination, complete blood count, peripheral blood (PB) morphology, immunophenotypic evaluation, bone marrow (BM) histology and radiographic examination (computerized tomography scans, ultrasounds).

BM histology

At the time of treatment initiation and at the end of therapy, BM biopsies were taken to evaluate the degree and pattern of lymphocyte infiltra-

tion. A morphologic evaluation of the percentage of lymphocyte infiltration was always performed by the same pathologist. At least two BM specimens were analyzed and in each at least 500 cells were counted. According to Rywlin *et al.*[26] four patterns of lymphocyte infiltration were considered: nodular, interstitial, mixed and diffuse. Details of the patients' BM pattern of lymphocyte infiltration BM are reported in Tables 1 and 2.

Immunologic evaluation

PB and BM samples were analyzed by flow cytometry using a simultaneous dual color staining technique, as described elsewhere.[27] Briefly, a minimum of 100,000 ungated cells for each measurement was acquired by FACScalibur (Becton-Dickinson, Mountain View, CA, USA) and data analyses were performed using the Cell Quest or the Paint-a-gate Becton-Dickinson software.

Samples were stained with monoclonal antibodies (MoAb) directed against CD5, CD20 (both purchased from Becton-Dickinson) surface immunoglobulins (Sig) κ and λ (Dako A/S, Copenhagen, Denmark). The quantification of immunologically detectable residual disease was evaluated in all patients achieving a clinical CR. A flow cytometric remission was defined by the presence of less than 10% residual $CD5^+/CD20^{+weak}$ double positive lymphocytes in the presence of a κ/λ ratio not exceeding 3:1.[8,28] A sensitivity of at least 1×10^{-4} was achieved in all cases.

Molecular evaluation

The molecular evaluation of residual disease in the PB and/or BM was investigated according to the presence or absence of a heavy chain Ig gene region rearrangement by polymerase chain reaction (PCR) analysis, as described by Nizet *et al.*[29] and as previously reported.[27]

Peripheral blood stem cell (PBSC) mobilization

After FAND therapy, 15 responsive patients eligible for a post-remission autograft were submitted to PB stem cell mobilization. During the study two different mobilization regimens were carried out; initially, cyclophosphamide (7 g/m^2) followed by the daily administration of G-CSF (lenograstim: 5 μg/kg/sc/day) starting the first day after cyclophosphamide (6 patients) and, thereafter, G-CSF alone, at the same dose, given from day + 10 after the last course of FAND (9 patients).

Leukapheresis procedure

Collections were started when the white blood cell counts exceeded 1.0×10^9/L and $CD34^+$ cells

Table 1. Clinical features of patients treated with active disease and in PR after prior therapy.

CLL features before FAND	*Unresponsive patients (14)*	*Relapsed patients (9)*	*Patients treated in PR (8)*
Gender M/F	13/1	8/1	8/0
Median age (range)	55 (43-60)	55 (46-60)	43 (22-58)
Median PB lymphocytes $\times10^9$/L (range)	47 (2-190)	9 (5-123)	3.2[1] (1.0 – 6.0)
Lymphadenopathies	7 (50%)	5 (56%)	4 (50%)
Splenomegaly	10 (71%)	4 (44%)	5 (62%)
Stage			
B/II	7 (50%)	5 (56%)	NA
C/III-IV	7 (50%)	4 (44%)	NA
Median n. of prior regimens (range)	2 (1-3)	2 (1-4)	1 (1– 2)
Previous therapy			
Fludarabine ± other regimens	6	8	6 (1)
Alkylating agents ± other regimens	3	–	1
CHOP ± other regimens	5	1	1

Pattern of BM lymphocyte infiltration prior to FAND: Unresponsive patients: diffuse in all cases. Relapsed patients: diffuse in 3 cases, interstitial in 5, nodular/interstitial in 1. Patients treated in PR: interstitial in 6 cases, interstitial with nodules in 2 (nPR).

Table 2. Response to FAND by disease state of patients.

	Patients with active disease (23 patients)		*Patients in PR (8 patients)*	*All patients (31 patients)*
	Unresponsive (14 patients)	*Relapsed (9 patients)*		
Median n. of FAND courses (range)	4 (2-6)	4 (3-6)	4 (2-4)	4 (2-6)
Overall responses (CR+PR)	7 (50%)	9 (100%)	7 (87.5%)	23 (70%)
95% CI	24-76	–	65-100	54-86
CR	4 (29%)	7 (78%)	7 (87.5%)	18 (60%)[2]
PR	3 (21%)	2 (22%)	–	5 (16%)[3]
NA[1]	–	–	1 (12.5%)	1 (3%)[4]
Responses in patients previously treated with FD	3/6 (50%)[5]	8/8 (100%)	5/6 (83%)	16/20 (80%)

[1]NA: not applicable, 1 patient treated in PR had no further reduction of the disease and was still in PR after FAND; pattern of BM lymphocyte infiltration after FAND; [2]patients in CR: lymphocyte infiltration < 30%, interstitial in all cases; [3]patients in PR: interstitial in 2 cases, interstitial/nodular 3 patients (nPR); [4]one patient treated in nPR had no further reduction of BM lymphocyte nodules and was still in nPR after FAND; [5]CR: 2 patients; PR: 1 patient.

>10/μL. The procedure was then continued until the minimum target number of ≥2×10⁶/kg CD34⁺ cells had been reached.

The COBE Spectra (COBE BCT Laboratories, Lakewood, CO, USA) was used for all procedures. Unmanipulated leukapheresis products were cryopreserved and stored in liquid nitrogen until use.[30]

Analysis of CD34 cells by flow cytometry and contaminating leukemic cells in the leukapheresis bags

CD34⁺ cells were enumerated in unseparated PB and in the collected bags during leukapheresis and at the end of the procedure by incubating 3×10⁵ cells with a FITC-conjugated anti-CD34 (HPCA-2; Becton Dickinson) MoAb (Becton-Dickinson). Isotype and fluorochrome-matched irrelevant MoAb were used as controls. After incubation, cells were washed twice in PBS-NaN_3 and residual red cells were lysed with isomolar NH_4Cl buffer for 10 min at 4°C. Flow cytometry was performed on a FACScalibur (Becton-Dickinson). A minimum of 30,000 ungated cells were acquired for each measurement; for patients showing less than 0.1-0.2% of CD34⁺ cells, a minimum of 200 CD34⁺ cells were acquired in a CD34-fluorescence/SSC live gate and analyzed using Lysis (Becton-Dickinson) or FlowMate (Dako A/S) software. The presence of residual leukemic cells in the leukapheresis bags was evaluated by flow cytometry for CD5⁺/CD20^{+weak} double staining and κ/λ clonal excess, as well as by molecular analysis.

Statistical analysis

The corrected χ^2 test was applied to compare groups. Survival curves were calculated according to Kaplan and Meier,[31] and compared with the log-rank test.[32] Survival curves were calculated from the start of FAND to death. Time to progression was measured from the achievement of a response after FAND to the occurrence of clinical and hematologic signs of disease progression.

Results

Response to therapy

All 31 CLL patients entered into the study were assessed for response to therapy.

Patients with active disease

Seven of the 14 patients (50%) who had been unresponsive to previous therapy obtained a response that was a CR in 4 (29%) and a PR in 3 (21%). At the time of the analysis, 2 patients with no signs of response and 5 patients with only a transient (≤3 months) reduction of their initial disease were considered as non-responders. All 9 relapsed patients showed a response that was a CR in 7 cases (78%). Taken together, 11 out of 14 patients (78%) previously treated with fludarabine (8 relapsed, 3 refractory) achieved a response (Table 2).

Patients in PR after previous therapy

Seven of the 8 patients treated in PR after prior therapy (87.5%) obtained a further reduction of their residual disease and reached a CR according to NCI criteria, while no further improvement was observed in 1 patient treated in nodular (n) PR who had no further reduction of BM lymphocyte nodules (Table 2).

Residual disease in patients achieving a CR

Collectively, 18 patients achieved a CR according to NCI criteria. The pattern of residual lymphocyte infiltration of the BM was interstitial in all cases with a median proportion of residual lymphocytes of 12.5% (range: 5-30%).

While the median rate of residual CD5⁺/CD20^{+weak} in the BM aspirates was 3% (range: 1-18%), the median number of CD5⁺/CD20^{+weak} PB lymphocytes was 0.28×10⁹/L (range: 0.06-0.436×10⁹/L), with 14/18 patients (78%) in flow cytometric remission (CD5⁺/CD20^{+weak} PB lymphocytes <10% and no κ/λ clonal excess). In 5 patients (16%), IgH gene rearrangement analysis showed the absence of detectable disease.

PBSC mobilization

After FAND therapy, 15 responsive patients (CR: 11 patients; PR: 4 patients) were submitted to PBSC mobilization. In 10 of these 15 mobilized patients (67%), ≥2×10⁶/kg CD34⁺ cells were collected: in 4/6 (67%) mobilized with cyclophosphamide followed by G-CSF (median time from the last course of FAND and mobilization: 74 days; range: 55-130 days) and in 6/9 (67%) with G-CSF given at day +10 after the last course of FAND, who then proceeded to leukapheresis. Cells were not harvested from the 5 patients who showed an insufficient increase in CD34⁺ cells. The median number of CD34⁺ cell collected was 3.5×10⁶/kg (range: 2-11.3 ×10⁶). The time interval, ≤2 months (6 patients) or >2 months (4 patients), between the last course of FAND and the start of mobilization did not influence the rate of patients with an adequate increase in CD34⁺ cells, but did have a statistically significant effect on the number of cells collected: patients undergoing early mobilization yielded a lower CD34⁺ cell harvest (≤2 months vs >2 months: 2.9 vs 3.9 CD34⁺ cells ×10⁶/kg; p=0.05). Other parameters, such as the type of mobilization regimen

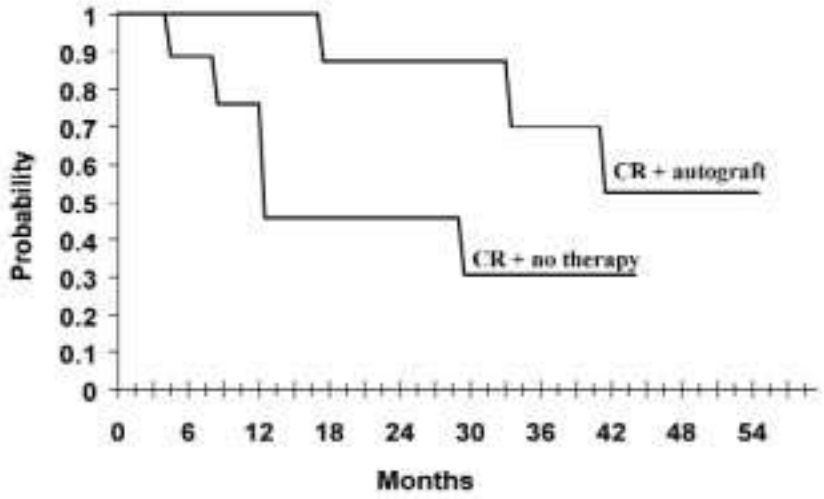

Figure 1. Actuarial time to progression probability in CR patients. CR+autograft (9 pts) vs CR+ no therapy (9 pts): $p<0.05$.

(cyclophosphamide + G-CSF vs FAND + G-CSF) and previous treatment (fludarabine vs fludarabine + other treatments), did not influence the $CD34^+$ cell increase or the number of stem cells collected. The 5 patients with an inadequate increase in $CD34^+$ cells showed a higher, though not significantly so, number of residual $CD5^+/CD20^{+weak}$ PB lymphocytes (351×10^9/L) than patients in whom the $CD34^+$ cell increase was adequate. The median percentage of $CD5^+/CD20^{+weak}$ lymphocytes in the leukapheresis products was 0.7% (range 0.1-5%).

Autologous transplantation was performed in 9 patients. All patients engrafted, with neutrophils > 0.5×10^9/L at a median time of 12 days and platelets > 20×10^9/L at a median time of 15 days. The median time to recover normal ranges of CD4 lymphocytes was 60 days.

Remission duration and survival

Taken together, 23 patients achieved a response after FAND. The actuarial median time to progression was 28 months and the actuarial overall survival at 67 months was 68%. Nine patients, all in CR, underwent an autologous transplantation, 1 in PR received an allogeneic transplant and 13 (9 in CR and 4 in PR) did not receive post-remission treatment. The outcome of three groups of patients could be analyzed: the first group included the 9 patients in CR who were autografted, the second one the 9 patients in CR who received no further treatment and the last group the 5 patients in PR who also received no further therapy. When the two groups of patients in CR were compared, autografted patients showed a statistically significant higher probability of a longer actuarial time to progression: 53 vs 30% at 41 months, $p<0.05$ (Figure 1). To date, no patient in either of the two groups has died and the patients of both groups are projected to be alive at 67 months.

A worse outcome was shown by the group of 5 patients who achieved a PR; for these patients, the median response duration was 9 months (range: 4-30 months) and the median survival duration 16 months (range: 6-36 months).

Toxicity

At the time of this report, 7 patients have died at a median time of 16 months (range: 12-23 months) after the start of FAND, 5 because of a new disease progression and 2 because of infection. Myelosuppression was the main toxicity observed during treatment. Severe granulocytopenia (WHO grade 3) occurred after 86 of the 124 administered courses (69%). However, only 10 of the 86 courses of therapy (12%) followed by granulocytopenia were characterized by the occurrence of major infections (pneumonia diagnosed on clinical basis: 7 patients; Gram-positive septicemias: 2 patients; peri-anal abscess: 1 patient) requiring parental antibiotics. During the first part of the study (10 patients), no G-CSF support was given in the presence of severe neutropenia that occurred after 16 courses and 3 cases of pneumonia were observed (3/16: 19%). During the second part of the study (21 patients), G-CSF support was given after 70 courses characterized by severe neutropenia and 7 cases of pneumonia were observed (7/70: 10%). Herpetic infections (dermatomal herpes-varicella zoster: 1 patient; herpes simplex: 4 patients) were observed in the first 5 patients enrolled in the study. No further cases of herpetic infection were recorded after the introduction of acyclovir prophylaxis. One patient devel-

oped a severe hepatitis B virus infection after FAND discontinuation. Severe anemia and/or thrombocytopenia (WHO grade ≥ 3) were recorded mainly in patients with marrow failure before the start of FAND (8/11 patients). Alopecia and non-hematologic toxicity were never observed. No nausea or vomiting was recorded. In all cases therapy could be administered on an out-patient basis, the only inconvenience being the 6-hour administration time required on days 1 and 2 of the protocol.

Discussion

Our results confirm the activity of schedules based on fludarabine combined with synergistic drugs, as observed in larger trials in which fludarabine has been combined with cyclophosphamide alone[15,16] or with cyclophosphamide and other drugs such as anthracyclines.[18,21] The FAND schedule which includes fludarabine, cytarabine, mitoxantrone and dexamethasone also proved active in patients with refractory CLL, a very poor prognostic subset among whom a low response rate to subsequent salvage regimens is usually observed.[2,3] A response was achieved by half of the patients previously unresponsive to fludarabine alone. This result is in agreement with the response rate of about 40% observed by O'Brien *et al.* in CLL patients refractory to fludarabine and treated with fludarabine + cyclophosphamide[15] and with fludarabine + doxorubicin[17] and further points to the superiority of therapeutic combinations including fludarabine and synergistic drugs.

As expected, in the group of relapsed patients a high rate of responses and in particular, a high rate of CR, (7/9 cases) was recorded. The CR rate achieved in our study is higher than rates reported in larger series: 12% after fludarabine + cyclophosphamide[15] and 8% after fludarabine + epirubicin given in first relapse.[20]

In the last group of patients, in PR after a prior therapy that was fludarabine in the majority of cases, treatment with FAND was aimed at further reducing residual disease and at improving the likelihood of a longer remission duration. A CR was obtained by all patients but one, suggesting that FAND administered after a previous treatment with fludarabine alone could enable an additional reduction of residual disease.

A similar reduction of residual disease with an improvement of the quality of response has also been reported to have occurred following high-dose cyclophosphamide given to CLL patients who had previously achieved a response to fludarabine.

The majority of patients treated with FAND (78%) who achieved a CR according to NCI criteria[23] showed <10% of residual $CD5^+/CD20^{+weak}$ lymphocytes and in 5 patients (16%) no molecular disease could be detected. A similar rate of molecular CR was reported by Bosch *et al.* in previously treated CLL patients after a schedule including fludarabine, cyclophosphamide and mitoxantrone (FCM).[18]

The achievement of a *good* CR with low residual disease and the capacity to mobilize PBSC after fludarabine-combining therapies is important for patients who may potentially benefit from a post-remission autograft. Controversial information has been reported on PBSC collection after fludarabine. Some authors have suggested that when a PBSC autotransplantation is planned, a prior fludarabine-containing regimen is unsuitable because of a subsequent low rate of successful stem cell collections.[34-36] This observation is not supported by the present study in which a successful harvest was obtained in 10/15 patients or by the European Blood and Marrow Transplantation (EBMT) study in which all CLL patients mobilized after fludarabine underwent subsequent PBSC collection which was of at least 2×10^6 $CD34^+$ cells/kg in 52% of cases.[37]

In our study, different patients were capable of mobilizing stem cells after G-CSF given after the last course of FAND. However, as previously observed by Michallet *et al.*,[37] patients who underwent a PBSC collection within the first two months after the last course of FAND had a lower $CD34^+$ cell harvest, suggesting that an interval of more than 2 months after the last course of therapy may increase the number of $CD34^+$ cells that can be harvested.

The presence of residual disease could have an additional unfavorable effect on the ability to mobilize stem cells.[38,39] In our study, the 5 patients with an inadequate increase in $CD34^+$ cell numbers showed a high rate of residual circulating $CD20^+/CD5^{+weak}$ leukemic lymphocytes.

Patients in CR after FAND who were subsequently autografted had a significantly longer response duration than patients who received no further post-remission therapy. Prospective and controlled studies are currently in progress to evaluate the therapeutic benefit of high-dose chemotherapy followed by PBSC transplantation in CLL. Preliminary results suggest that autotransplantation may not cure CLL, but can induce long-lasting remissions.[40] FAND was given on an out-patient basis and was well tolerated. Despite the high rate of mortality due to infections after fludarabine that has been reported to occur in heavily pretreated

patients,[41] the inclusion of steroids in the FAND schedule and the frequent occurrence of severe granulocytopenia, the rate of infectious complications in our patients was relatively low. These data confirm the importance of extended infection prophylaxis in fludarabine-treated patients.[42] The benefit of G-CSF administration in reducing the infection rate reported by O'Brien *et al.*[43] is confirmed in this study in which a lower rate of pneumonia was observed in neutropenic patients receiving G-CSF. Taken together, the results of this study indicate that FAND, a schedule that combines more DNA damaging agents, associated with extended infection prophylaxis, is a highly cytoreductive and well-tolerated protocol that in most cases of CLL does not hamper subsequent PBSC mobilization. Further studies are required to define the optimal combination of fludarabine-containing schedules and their benefit as initial treatment for CLL patients who are candidates for therapeutic approaches with potential curative intent.

Contributions and Acknowledgments

FRM designed the study and, with MG and EG, was responsible for the care of patients and data collection. GM was responsible for the care of autotransplanted patients. PdF and MSDP were responsible for the immunophenotypic characterizations. MCR was responsible for the molecular evaluations. DG was responsible for the statistical analyses.

RF and FM contributed to revising the manuscript. The authors are listed according to a criterion of decreasing individual contribution to the work, with the exception of the last author who had a major role as senior author in interpreting the data.

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Disclosures

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Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Emili Montserrat, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Professor Montserrat and the Editors. Manuscript received March 24, 2002; accepted June 18, 2002.

What is already known on this topic

The central role that for many years chlorambucil held in the treatment of patients with CLL is now being occupied by purine analogs, particularly fludarabine.

What this study adds

Treatment with fludarabine alone does not result in an improved survival compared to that achieved by chlorambucil. Because of this and given the synergism of fludarabine with other cytotoxic agents, there is an increasing tendency to use fludarabine combined with other drugs.

Potential implications for clinical practice

In pilot studies, as the one reported in this paper, this strategy offers promising results. The role of fludarabine-based combination chemotherapy regimens in the treatment of CLL should be determined in prospective randomized trials.

Emili Montserrat, Associate Editor