Fludarabine + prednisone ± α-interferon followed or not by α-interferon maintenance therapy for previously untreated patients with chronic lymphocytic leukemia: long term results of a randomized study

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Background and Objectives. Fludarabine is an effective therapy for patients with chronic lymphocytic leukemia (CLL) and interferon-α (IFN-α) has been reported to have anti-leukemic activity in CLL patients. A randomized study was designed to evaluate whether the addition of IFN-α to a first-line treatment with fludarabine and prednisone could increase the response rate in patients with advanced CLL and whether IFN-α given as maintenance therapy could improve the duration of response.

Design and Methods. One hundred and thirty-three patients were randomized to receive fludarabine (25 mg/m²/i.v, days 9-13) and prednisone (20 mg/m², days 1, 3, 5, 7 and 14 and 40 mg/m², days 9-13) (arm A: 66 patients) or in addition to the same schedule, IFN-α (2 MUI/sc, days 1, 3, 5, 7, 9, 11, 13 and 15) (arm B: 67 patients). Seventy-eight patients responsive to therapy entered the post-remission phase of the study in which 41 patients were randomized to receive IFN-α (3 MUI three times a week) and 37 to clinical observation.

Results. A similar response rate (complete responses + partial responses) was observed in the 2 arms: 86% for arm A and 84% for arm B ($p = 0.4$). A longer response duration was observed in patients who achieved a complete response ($p = 0.001$) and in patients who received maintenance therapy with IFN-α ($p < 0.05$). However, the quality of response was the only significant and independent factor influencing response duration ($p < 0.01$). No benefits in terms of infection-related mortality and morbidity could be ascribed to IFN-α administration.

Interpretation and Conclusions. In previously untreated CLL patients with advanced disease a high response rate is obtained from first-line fludarabine and prednisone and no benefit is derived from the addition of IFN-α to this regimen. The achievement of a good quality response to therapy was the only independent predictor of a prolonged response.

Key words: chronic lymphocytic leukemia, treatment, fludarabine, α-interferon.

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During the last 10 years three prospective randomized studies have demonstrated that fludarabine therapy is superior to CAP (cyclophosphamide, doxorubicin, and prednisone),[1] CHOP (cyclophosphamide, vincristine, doxorubicin, and prednisone)[2] and chlorambucil[3] as first-line treatment of patients with chronic lymphocytic leukemia (CLL). However, most patients relapse after 2-4 years suggesting that fludarabine has a very effective debulking activity, but that it is not curative: residual disease represents the main cause of relapse.

Interferon-α (IFN-α) is a biological agent with multiple properties including inhibition of cell proliferation and modulation of cellular immunity.[4-5] IFN-α has been shown to have an anti-tumor effect in different hematologic malignancies, in particular multiple myeloma, non-Hodgkin's lymphoma and hairy cell leukemia.[6-12] During the late eighties, several clinical trials evaluated the anti-tumor activity of IFN-α, given at variable doses and for different durations, as a single agent in CLL patients with different clinical pictures. While only limited activity was recorded in patients previously treated and with advanced disease,[13-16] a higher response rate was observed in untreated patients with early disease.[17-22] Furthermore, some authors reported a therapeutic benefit of IFN-α given as post-remission therapy.[23,24]

A synergistic effect of IFN-α with several cytotoxic drugs has been documented *in vitro*[25] and favorable clinical results in patients with low grade lymphoma have been described.[26] Positive clinical results were reported by Mandelli *et al*.[27] and by Molica *et al*.[28] in small series of CLL patients with advanced disease treated with a regimen including chlorambucil, prednisone and IFN-α.

On the basis of the anti-leukemic activity shown by IFN-α given alone or in combination with cytotoxic drugs and considering its potential immuno-modulatory properties, a multicenter study was designed to evaluate the therapeutic effect of IFN-α in previously untreated CLL patients with advanced disease. The study was characterized by two randomized phases. In the first phase, the activity and toxicity of two induction schedules, fludarabine and prednisone with or without IFN-α, were evaluated. The primary objective of this first part of the study, which included 134 CLL patients, was to evaluate whether the addition of IFN-α could increase the response rate. In the second phase, patients who achieved a response to induction therapy were randomized to receive IFN-α as maintenance therapy or no treatment. The primary objective of this second part of

the study, which included 78 CLL patients, was to evaluate whether the administration of IFN-α as maintenance therapy could improve the response duration. Herein, we report, the results of this study.

Design and Methods

Patients

One hundred and thirty-four patients with untreated progressive or advanced CLL were prospectively enrolled into a randomized clinical trial between March 1993 and December 1998. Patients were recruited from three Italian hematologic centers: Dipartimento di Biotecnologie Cellulari ed Ematologia, University "La Sapienza" of Rome (74 patients), Istituto di Ematologia ed Oncologia "L. & A. Seragnoli", University of Bologna (32 patients), and Istituto di Ematologia, University of Udine (28 patients).

Pre-study evaluation and eligibility criteria

All patients fulfilled the National Cancer Institute-Sponsored Working-Group diagnostic criteria for CLL.[29] At baseline, peripheral blood lymphocytes were characterized by immunophenotyping and morphology as previously described.[30] The stage of the disease was assessed according to Rai's classification.[31] Pre-treatment work-up included a medical history, physical examination, complete peripheral blood (PB) cell count with differential, bone marrow (BM) aspiration and biopsy, Ig quantification, liver and renal function tests and radiographic examination (computerized tomography scans, ultrasounds).

Eligibility criteria included: age 65 years or less, no prior treatment, advanced stage (III-IV) or intermediate stage (I-II) with one or more clinical signs of active disease. Exclusion criteria included: prior treatment; autoimmune cytopenia; history of other malignancies within 2 years prior to study entry (except for adequately treated carcinoma *in situ* of the cervix; basal or squamous cell skin cancer); active infection requiring systemic therapy, human immunodeficiency virus infection, hepatitis B or C; history of uncontrolled hypertension, severe cardiac, pulmonary, or neurological disease; uncontrolled metabolic disorder; any co-existing medical or psychological condition that would preclude participation in the study or compromise ability to give informed consent.

The protocol was approved by the local ethics committee. All patients gave written informed consent before enrollment into the study which was carried out in accordance with the Good Clinical Practice precepts.

Treatment plan

Eligible patients were centrally randomized to one of the two induction treatments (arm A or arm B). Randomization was balanced for each participating center and stratified according to the following factors: institution, stage and bone marrow histology.

Arm A: fludarabine (Fludara®; kindly given by Schering SpA) associated with prednisone.
Fludarabine: 25 mg/m^2 i.v. for 5 consecutive days, on days 9 to 13;
Prednisone: 20 mg/m^2 orally on days 1, 3, 5, 7 and 14.
Prednisone: 40 mg/m^2 orally from day 9 to day 13.

Arm B: fludarabine associated with prednisone as in arm A with the addition of IFN-α as follows:
IFN-α: (lymphoblastoid IFN-α; Wellferon®, kindly given by Glaxo-Wellcome) 2 MUI on days 1, 3, 5, 7, 9, 11, 13 and 15.

Both regimens were administered every 4 weeks for a total number of 6 courses. Patients achieving a complete or partial response (CR or PR) according to NCI criteria were considered for the second randomized part of the study, the post-remission phase. Patients were randomized to two post-remission approaches (arm C or arm D).

Arm C: IFN-α: 3 MUI three times a week until disease progression

Arm D: no therapy.

Randomization was stratified according to 3 factors: institution, prior induction therapy and quality of response. All study treatments were carried out on an outpatient basis and IFN-α was self-administered subcutaneously.

Dose modifications

In the presence of severe (III-IV grade according to WHO criteria) hematologic toxicity, the start of the subsequent course was delayed. In the case of severe cytopenias persisting for more than 2 weeks, treatment was discontinued, while, in the presence of moderate cytopenias, fludarabine and IFN-α doses were reduced by 50%. Treatment was withheld in the presence of grade III-IV non-hematologic WHO toxicity or major infection until the patient had recovered from toxicity or infection. In the case of recovery after more than 2 weeks or no recovery, patients were withdrawn from the study.

Supportive care

During fludarabine treatment and for at least 1 year after therapy discontinuation, trimethoprim-sulphamethoxazole was given three times a week for *Pneumocystis carinii* prophylaxis. No granulocyte colony-stimulating factor (G-CSF) administration was planned in the presence of neutropenia. Patients requiring blood transfusions were given irradiated products. To prevent flu-like symptoms, paracetamol was administered 30 minutes prior to IFN-α.

Table 1. Patients' characteristics.

	No. of patients 134 (%)	*FLU+PDN 67 (%)*	*FLU+PDN +IFN-α 67 (%)*	*p value*
Gender				
Male	94 (70)	49 (73)	45 (67)	NS
Female	40 (30)	18 (27)	22 (33)	
Median age (yrs.)	54	52.3	55.7	
range	34-65	34-64	37-65	< 0.01
Median time from diagnosis (months)	12.6	12.6	13.0	NS
range	0-103	0-98	0 -103	
Rai stage				
I+II	117 (87)	58 (87)	59 (88)	NS
III+IV	18 (13)	10 (13)	8 (12)	
Median hemoglobin (g/dL)	13	13	12.9	NS
range	7.1-17.4	7.1-17.4	7.8-16.0	
Median lymphocytes ($\times 10^9$/L)	67	67	67	NS
range	2-315	2-315	9-167	
Median platelets ($\times 10^9$/L)	157	145	166	NS
range	70-410	70-410	72-318	
Bone marrow histology				
diffuse	58(43)	28(42)	30(45)	NS
non-diffuse	76(57)	39(58)	37(55)	
LDH				
normal	94(70)	45(67)	49(73)	NS
elevated	40(30)	22(33)	18(27)	

NS: not significant.

Response and toxicity evaluation

Response to induction therapy was assessed according to NCI criteria.[29] Response duration was measured from the time of achievement of the response to the time of disease progression. Disease progression was based on the presence on two monthly consecutive evaluations of one or more of the following disease-related signs: ≥ 50% increase in the absolute number of circulating lymphocytes (minimum number: at least 5000/μL); ≥ 50% increase of the size of spleen, liver and lymph-nodes (minimum diameter: at least 2 cm). Disease transformation was considered in the presence of a histologic diagnosis of lymphoma and in the case of an increase of the prolymphocyte rate ≥55% (prolymphocytoid transformation). Autoimmune hemolytic anemia was considered to have developed when there were clinical signs of hemolysis associated with a positive Coombs' test. Toxicity was evaluated according to WHO criteria. Toxicity was separately recorded and evaluated in the two phases of the study, during the induction therapy and the post-remission phase, from response to the start of a second line therapy.

Statistical analysis

Statistical analysis included an evaluation of the response rate, of the actuarial time to progression probability and of the actuarial survival probability. The corrected χ^2 test was applied to compare groups. Survival curves were calculated according to Kaplan and Meier[32] from the time of first randomization and from the time of second randomization to death, and compared with the log-rank test.[33] Response duration was calculated from the time of response to the time of disease progression or death.

The prognostic significance of the following parameters: gender (male vs female), age (≤55 vs >55 years), time from CLL diagnosis to treatment (≤12 vs >12 months), stage according to the Rai classification (I+II vs III+IV), peripheral blood lymphocyte count (≤60 vs >60×10^9/L), LDH value (normal vs elevated), rate of peripheral blood lymphocyte reduction after the second course of induction therapy (≤25 vs >25%), BM histology (non-diffuse vs diffuse), induction therapy regimen (fludarabine + prednisone vs fludarabine + prednisone + IFN-α: arm A *vs* arm B), on the probability of achieving a response was analyzed. Furthermore, the prognostic significance on response duration and survival duration of these same parameters, quality of response (CR vs PR) and type of post-remission approach (IFN-α vs no treatment) was evaluated.

In order to evaluate the relative significance of prognostic factors emerging as such from the univariate analysis, the multiple regression model of Cox was applied.[34]

Results

Clinical features of patients

The median age of the 134 CLL patients entered into the study was 54 years (range: 34-65 years). The median duration of CLL before the start of treatment was 12.6 months. More than two-thirds of patients were males and 13% had Rai stage III-IV disease. The median hemoglobin value was 13 g/dL, the median lymphocyte count 67×10^9/L and the median platelet count 157×10^9/L. The patients' characteristics are reported in Table 1.

Induction therapy

Sixty-seven patients were randomized to receive fludarabine and prednisone (arm A) and 67 were randomized to receive fludarabine, prednisone and IFN-α (arm B). The baseline clinical features of the

Table 2. Response to therapy by induction therapy arm.

	No. of patients 133 (%)	*FLU+PDN 66 (%)*	*FLU+PDN +IFN-α 67 (%)*	*p value*
Overall responses	113 (85)	57 (86)	56 (84)	NS
CR	50 (38)	30 (45)	20 (30)	NS
PR	63 (47)	27 (41)	36 (54)	

NS: not significant.

two groups of patients did not differ except for the higher median age ($p < 0.01$) of patients randomized to receive no IFN-α (Table 1).

Response to induction therapy. One patient in whom the immunologic characteristics did not fulfill the criteria for a CLL diagnosis was retrospectively excluded from the study. No protocol deviations were recorded in the induction phase. Thus, response was assessed in 133 patients. The overall response rate (CR+PR) was 85% (113 patients) with no statistically significant differences between the two arms (arm A, fludarabine, prednisone: 86% vs arm B, fludarabine, prednisone and IFN-α: 84%; $p = 0.4$).

A higher, though not significantly so, CR rate was observed in patients treated with fludarabine and prednisone compared to patients treated with these two drugs and IFN-α (45% vs 30%; $p = 0.08$) (Table 2).

Age, gender, prior CLL duration, LDH value, BM histology, PB lymphocyte count, and the introduction of IFN-α in the induction regimen, showed no significant effect on the probability of achieving a response (CR+PR) while only 2 factors showed a significant and independent effect on the probability of achieving a response (CR+PR): the baseline initial Rai stage ($p = 0.05$) and the rate of lymphocyte reduction, ≤25% or >25%, recorded after the second course of therapy ($p = 0.01$) (Table 3).

Post-induction phase

Second randomized phase of the study: *IFN-α vs no therapy*. Seventy-eight of the 113 patients who had a response (69%) were subsequently randomized to receive *maintenance* treatment with IFN-α (arm C: 41 patients) or only clinical observation (arm

Table 3. Significant and independent prognostic factors for the probability of achieving a response, response duration and survival probability (in parentheses non-significant variables).

		Independent prognostic factors	*p*	*CI 95%*
Probability of achieving a response	1st	Rai stage: I+II vs III+IV	0.05	0.99-27.99
(Age, gender, Rai stage, prior CLL duration, LDH value, BM histology, PB lymphocyte count, induction therapy regimen, rate of peripheral blood lymphocyte reduction after the second course of induction therapy)	randomized phase (133 pts)	Pb Lymph. % reduction after 2nd course: ≤25 vs >25%	0.01	1.49-29.5
Response duration probability	2nd	Response to induction: CR vs PR	< 0.01	1.31-4.06
(Age, gender, prior CLL duration, LDH value, BM histology, Rai stage, PB lymphocyte count, induction therapy regimen, rate of peripheral blood lymphocyte reduction after the second course of induction therapy and post-remission approach)	randomized phase (78 pts)			
Survival probability	1st	Age: ≤55 vs >55 years	< 0.05	0.98-3.97
(Gender, prior CLL duration, LDH value, BM histology, PB lymphocyte count, the induction therapy regimen, the quality of response, the rate of lymphocyte reduction, recorded after the second course of therapy)	randomized phase (133 pts)	Rai stage: I+II vs III+IV	0.05	0.96-4.40
(In addition to the above mentioned variables, the administration of IFN-α as maintenance therapy)	2nd randomized phase (78 pts)	Age: ≤55 vs >55 years	0.01	1.36-14.33
		Rai stage: I+II vs III+IV	0.01	1.33-14.11

Table 4. Characteristics of patients included in the 2nd randomized phase of the study.

	No. of patients 78 (%)	*IFN therapy 41 pts. (%)*	*Clinical observation 37 pts. (%)*	*p value*
Median age	55	56	54	NS
Gender				
Male	58 (74)	33 (80)	25 (68)	NS
Female	20 (26)	8 (20)	12 (32)	
Time to therapy				
≤ 1 yr	35 (48)	17 (41)	18 (49)	NS
>1 yr	43 (52)	24 (59)	19 (51)	
Rai stage				
I-II	63 (81)	36 (88)	27 (73)	NS
III-IV	15 (19)	5 (12)	10 (27)	
Induction therapy				
FLU + PDN	38 (49)	19 (46)	19 (51)	NS
FLU + PDN + IFN-α	40 (51)	22 (54)	18 (49)	
Response to induction				
CR	33 (42)	18 (44)	15 (41)	NS
PR	45 (58)	23 (56)	22 (59)	

NS: not significant.

D: 37 patients). The baseline characteristics of patients were well balanced between the two treatment groups (Table 4).

The reason for early (≤6 months) IFN-α discontinuation in 8 patients (20%) was IFN-α-related toxicity including: neurotoxicity (5 patients), persistent febrile flu-like syndrome (2 patients) and persistent thrombocytopenia (1 patient). The reasons for late (>6 months) IFN-α discontinuation were a life-threatening car accident in 2 patients, a second malignancy in 2 patients (liver: 1 patient; kidney: 1 patient) and an interstitial pneumonia of unknown origin in 1 patient. Two cases of dermatomal herpes-varicella zoster (HVZ) were observed after IFN-α discontinuation. After a median time of 27 months of IFN-α treatment (range: 6-59 months), 7 responder patients refused to continue IFN-α administration. The actuarial median response duration of the 78 randomized patients was 14 months.

While age, gender, prior CLL duration, LDH value, BM histology, Rai stage, PB lymphocyte count, the rate of lymphocyte reduction, ≤25% or >25%, recorded after the second course of therapy and the type of the induction regimen, showed no significant effect on the response duration, a significantly longer response duration was shown by 2 groups of patients: patients randomized to receive IFN-α (actuarial progression-free survival at 12 months, arm C vs arm D: 60% vs 48%; $p < 0.05$) (Figure 1) and patients who achieved a CR after induction therapy (actuarial progression-free survival at 12 months, CR vs PR: 75% vs 45%; $p = 0.001$) (Figure 2). However, in the multivariate analysis the quality of response ($p < 0.01$) emerged as the only significant and independent factor for response duration while IFN-α showed no independent prognostic significance (Table 3).

Patients not included in the second randomized phase of the study. Thirty-five patients (31%) who responded to induction therapy were not included in the second phase of the study. Sixteen patients (14%) were considered not eligible: 4 because of an infection (HBV hepatitis: 2 patients; *Listeria monocytogenes* infection: 1 patient; HVZ: 1 patient), 6 because of persistent cytopenia, 1 with autoimmune hemolytic anemia, 2 with a non-hematologic neoplasia, 2 showing a persistent liver enzyme increase, 1 with a cerebral hemorrhage.

Nineteen patients who responded (17%) were excluded from the second randomization. The reasons for the protocol deviation in the second phase of the study were: one patient with residual marked splenomegaly underwent splenectomy, 14 young patients (median age 50 years) underwent an autologous stem cell transplantation and 4 patients refused the second randomization.

Survival and factors predicting survival

Causes of death. The median follow-up was 51 months. At the time of the analysis, 41 patients (31%) had died. The main cause of death was CLL progression (51%); other causes of death were infection (15%), Richter's transformation (15%), second malignancy (12%), acute myeloid leukemia (5%) and myocardial infarction (2%). A patient included in arm A died during induction therapy because of pneumonia. The introduction of IFN-α at any time of the treatment approach was not significantly related to an increased mortality or to a specific cause of death.

Prognostic factors for survival

The overall actuarial survival probability at 6 years was 55%. The survival probability for the two induction arm groups was not significantly different (at 6 years, arm A: 57% vs arm B: 51%; $p = 0.3$). While gender, prior CLL duration, LDH value, BM histology, PB lymphocyte count, the introduction of IFN-α in the induction regimen, the quality of response, and the rate of lymphocyte reduction, (≤25% or >25% recorded after the second course of therapy) showed no significant effect on survival duration, age ($p < 0.05$) and Rai stage ($p = 0.05$) emerged as the only significant and independent parameters influencing survival probability (Table 3). When the survival probability of the 78 responsive patients included in

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