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Fludarabine treatment in B-cell chronic lymphocytic leukemia: response, toxicity and survival analysis in 47 cases

CATERINA STELITANO,* FORTUNATO MORABITO,* MARIA GRAZIA KROPP,° VINCENZO CALLEA,* FRANCESCO IULIANO,* VINCENZO ORIANA,* DOMENICO LEVATO,° FRANCESCO NOBILE,* STEFANO MOLICA,° MAURA BRUGIATELLI*

**Dipartimento di Emato-Oncologia, Azienda Ospedaliera Bianchi-Melacrino-Morelli, Reggio Calabria; °Divisione di Ematologia, Ospedale Pugliese, Catanzaro, Italy*

ABSTRACT

Background and Objective. **Fludarabine monophosphate (FAMP) is a purine analog with specific therapeutic activity in B-cell chronic lymphocytic leukemia (CLL). Its current use as front-line therapy of CLL is still a matter of debate both because of the controversial results of the clinical trials so far reported and because of the toxicity profile of the drug. In order to contribute to clarifying the possible role of FAMP, we report a retrospective analysis of the results obtained with the purine analog in CLL patients in different phases of the disease.**

Design and Methods. **Forty-seven patients affected by advanced CLL, 36% untreated, 31.9% relapsed and 31.9% resistant, were treated with FAMP 25 mg/m²/day, either for 4 days every 3 weeks in 29 cases, or for 5 days every 4 weeks in 18.The median number of FAMP cycles was 6 (range 2-11). Response was defined according to total tumor mass (TTM) score reduction and toxicity was expressed according to WHO grading criteria. The median follow-up of the series was 13 months from the beginning of FAMP therapy.**

Results. **Out of 47 evaluable patients the response rate was 74.4%, with 34% complete response (CR). The overall response rate was 94%, 80% and 46.6% in untreated, relapsed and resistant cases, respectively; a significantly higher number of responses was associated with no previous treatment and number of FAMP cycles. Fifty-three percent of all cases and 58.8% of untreated ones did not experience any toxicity. Treatment-related side effects were mainly autoimmune phenomena in untreated patients and infectious complications in treated ones. One heavily pre-treated patient died because of neurologic complications. Median time to re-treatment was 18 months (range 1-30) and was influenced by age and previous treatment. The overall median survival was 35.7 months with a significantly higher proportion of surviving cases among RAI 0-II stages, responders and patients receiving more than 5 FAMP cycles.**

Interpretation and Conclusions. **The present report confirms the high efficacy of FAMP in previously pretreated cases with acceptable toxicity and encourages its use as front-line treatment provided that the results of randomized trials demonstrate its superiority over conventional chemotherapy. The possible development of autoimmune phenomena should, however, be considered seriously.**

Key words: CLL, fludarabine, response, toxicity

Correspondence: Caterina Stelitano, M.D., Dipartimento di Emato-Oncologia, Azienda Ospedaliera Bianchi-Melacrino-Morelli, 89100 Reggio Calabria, Italy.
Phone: international + 39-0965-27191 – Fax: international + 39-0965-25082 – E-mail: morctmo@tin.it

For many years the treatment of B-cell chronic lymphocytic leukemia (CLL) did not progress significantly, although this disease is the most frequent hematologic neoplastic disease of the western world.[1,2] Perhaps because it usually develops in elderly patients and perhaps because of the frequent infectious complications due to the disease related immune impairment, chemotherapy is commonly limited to palliative rather than curative aims.[3] In an effort to improve the efficacy of treatment, combination chemotherapy, including low dose anthracyclines[4] and a high-dose daily chlorambucil regimen,[5-7] was employed with better overall results.

More recently, the introduction of fludarabine (FAMP) therapy has increased the general interest in the treatment of this disease,[8] there being the possibility of obtaining a high complete remission rate, verified at bone marrow level.[9] On the other hand, the results of the randomized trials so far reported, while indicating that FAMP produced better response than conventional chemotherapy, have not yet demonstrated a benefit in terms of survival,[10,11] leaving open the question of the role of this drug and the best timing to use it.

The toxicity profile of purine analogs is important. Differently from other chemotherapy agents, FAMP causes very few organ side-effects at conventional doses, while the main concern about its use is the prolonged and profound worsening of the immune impairment of CLL patients who are thus at risk of

severe opportunistic infections.[3,12]

In order to contribute to a better understanding of the role of FAMP in CLL, we report here a retrospective analysis of the results, in terms of efficacy and toxicity, obtained with this drug in 47 CLL patients in different phases of the disease, treated in two hematologic institutions.

Design and Methods

Patients

Forty-seven CLL patients, 30 males and 17 females, with a median age of 64.8 years (range 34-81) entered this study. The diagnosis was based on clinical features and on typical morphologic and immunologic findings.[13] Table 1 shows the main clinical and hematologic data of the patients. About half of the patients were in Rai III and IV and Binet C stages. All, but one, stage A patients displayed a high total tumor mass (TTM) score,[14] ranging from 9.4 to 19.8 (mean 13.4), and were in Rai stage I-II. The only patient with a TTM score lower than 9, which is our threshold score for starting treatment, had been resistant to a previous therapy with high dose chlorambucil. The median TTM score was 12.6 (range 6.2-32). Thirty-six percent of patients were untreated, while 32% and 32% were relapsed and resistant, respectively. Patients who required re-treatment more than one year after achievement of complete or partial response were considered to have relapsed, while patients who reached a response less than PR after the previous therapy or whose response lasted less than one year were considered to be resistant. The median number of previous treatments was 1 (range 0-3) and the median follow-up from the first FAMP cycle was 13.1 months.

Table 1. Main clinico-hematologic features at the time of FAMP therapy.

Sex, male/female	30/17
Age, median (range)	64.8 (34-81)
Rai stage	
0-I	9
II	13
III-IV	25
Binet stage	
A	12
B	12
C	23
TTM score, median (range)	12.6 (6.2-32)
Disease status	
Untreated	17
Relapse	15
Resistant	15
No. of previous lines of treatment	
0	17
1	12
2	10
≥ 3	8
No. of FAMP cycles, median (range)	6 (2-11)
Follow-up, median (range)	12.9 (2-38)

Treatment regimens and response criteria

Therapy consisted of FAMP 25 mg/m^2/day either for 4 days every 3 weeks in 29 cases, or for 5 days every 4 weeks in 18 ones. The median number of FAMP cycles was 6 (range 2-11). In addition, the vast majority of cases received prophylaxis against infections with ketoconazole and sulfomethoxazole. CR was defined as the achievement of a TTM score[14] below 2.3, partial response (PR) as more than 50% reduction of the initial TTM, progression (PD) as the increase of the TTM score while the patient was receiving treatment and no response (NR) as failure to achieve CR or PR. Response defined according to NCI-working group criteria[13] was also evaluated in our patients, but 6 out 13 of patients who reached CR according to the TTM scoring system, refused bone marrow aspirate and/or bone marrow biopsy, thus hampering this evaluation.

Statistical analysis

The non-parametric Mann-Whitney U test was used to compare two independent samples drawn from the same population. The Kruskal-Wallis analysis of variance by ranks was utilized when three or more sets of observations were made on a single sample. The chi-square test (Fisher's exact 2-tail) for 2×2 tables was used for overall comparisons of clinical responders versus non-responders. Multivariate analysis was performed using logistic regression, and covariates were added to the regression in a stepwise fashion for $p \leq 0.1$. Survival, determined from the beginning of FAMP therapy, was calculated by the method of Kaplan and Meier. Difference in survival between prognostic groups was evaluated in univariate analysis by the log-rank test, and the respective influence of the different variables, that were significant at $p \leq 0.1$ on survival was calculated in a stepwise fashion using the Cox regression method. For all analyses, the variables age, TTM score, number of previous therapies and number of FAMP cycles were transformed into binary variables by using the median as the cut-off value. All calculations were performed using the SAS/STAT software package, release 6.06 of SAS Institute Inc., 1993.

Results

Clinical response

Forty-seven patients were evaluated for clinical response by the TTM score. Thirty-four percent and 40.4% of cases achieved CR and PR, respectively. In particular, CR was obtained in 64.7%, 20% and 13.3% of patients treated at the onset, in relapse and in the resistant phase of the disease, respectively. The over-

Table 2. Response rate as function of phase of the disease. Response evaluation was defined according to TTM score reduction.

Status	*No. of cases*		*% of cases*	*p**
Untreated	17	CR	64.7	
		PR	29.7	
		Overall	94.1	
Relapsed	16	CR	20	
		PR	60	0.007
		Overall	80	
Resistant	15	CR	13.3	
		PR	33.3	
		Overall	46.6	

**Pearson chi-square.*

Table 3. Univariate and logistic multivariate regression analysis of prognostic variables of clinical response to FAMP.

Variable	*CR+PR*	*NR+PD*	**Univariate p*	*°Multivariate p*
Age, years (≤ 64.8 v > 64.8	19 v 16	5 v 7	0.5	---
Sex (male v female)	22 v 13	8 v 4	1.0	---
Rai stage (0-II v III-IV)	19 v 16	3 v 9	0.1	0.7
Binet stage (A-B v C)	20 v 15	4 v 8	0.1	0.9
TTM score (≤ 12.6 v > 12.6)	18 v 17	5 v 7	0.7	---
No. of previous therapies (0 v ≥ 1)	16 v 19	1 v 11	0.034	0.2
No. of FAMP cycles (< 6 v ≥ 6)	11 v 24	10 v 2	0.003	0.0184
FAMP schedule (4 day v 5 day)	21 v 14	8 v 4	0.7	

**Fisher's exact test; °logistic regression analysis.*

all response rate was 94.1%, 80% and 46.6% for untreated, relapsed and resistant cases, respectively (p=0.007 by Pearson Chi-Square) (Table 2). The response rate (CR+PR) was not affected by age, sex, Rai and Binet stage, or the TTM score (Table 3). A significantly higher number of responses was observed in untreated cases and in those patients treated with more than 5 cycles of FAMP. This latter variable remained significant in multivariate analysis (Table 3). Furthermore, the two different FAMP schedules used in these patients resulted to be of comparable efficacy.

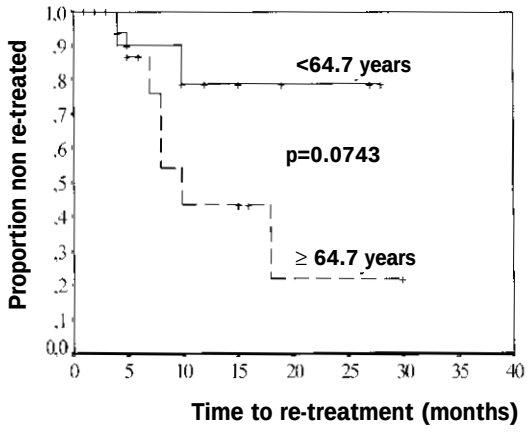
The overall median time to re-treatment was 18 months (range 1-30). The median time to re-treatment was not reached for patients i) with age > 64.7 years, as compared with 10 months (95% CI, 7 to 13) for those < 64.7 years (p= 0.0743), ii) previously untreated, as compared with pre-treated ones whose time to re-treatment was 10 months (95% CI, 7 to 13) (p=0.0163), iii) who experienced treatment-related toxicity, as compared with those who did not suffer toxic episodes (10 months; 95% CI, 2 to 18) (p=0.1) (Figure 1).

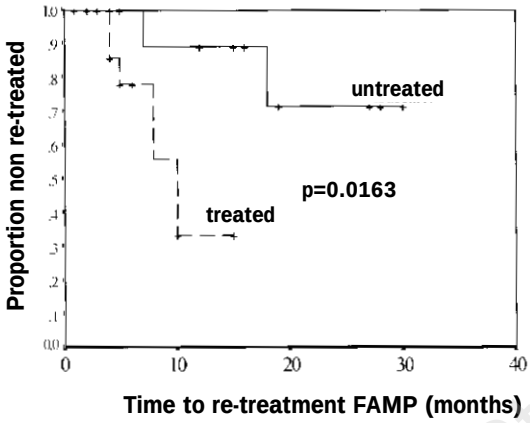
Toxicity

Toxicity evaluated after a total of 257 FAMP courses is detailed in Table 4. It is noteworthy that 53.1% of all cases and 58.8% of untreated ones did not demonstrate any toxicity. Organ toxicity was represented by a fatal neurologic complication in a heavily pre-treated patient after 9 FAMP cycles. No other organ toxicity was registered. Twenty-seven infectious episodes were reported, consisting of fever of unknown origin (8 episodes), pneumonia (4), bronchitis (4), herpes zoster (4), herpes simplex (3), interstitial pneumonia (2), fungal pneumonia (1) and infectious eczematoid dermatitis (1). The main toxi-

Table 4. Hematologic, infectious and neurologic toxicity after first line or salvage FAMP therapy.

	First-line			*Salvage*			*Total*		
	No. of episodes	*% cycles*	*% patients*	*No. of episodes*	*% cycles*	*% patients*	*No. of episodes*	*% cycles*	*% patients*
Hematologic									
Grade 1-4	2	2.0	11.7	5	3.1	16.6	7	2.7	15
Grade 4	1	1.0	5.8	4	2.5	13.3	5	1.9	11
Infectious									
Grade 1-4	5	5.0	29.4	22	13.9	73.3	27	10.5 –	57
Grade 4	–	–	–	–	–	–	– 5	1.9	– 11
Grade 5	–	–	–	5	3.1	16.6			
Neurologic									
Grade 5	–	–	–	1	0.6	3.3	1	0.4	2
Autoimmune	3	3.0	17.6	–	–	–	3	1.2	6
None			58.8			50			53.1





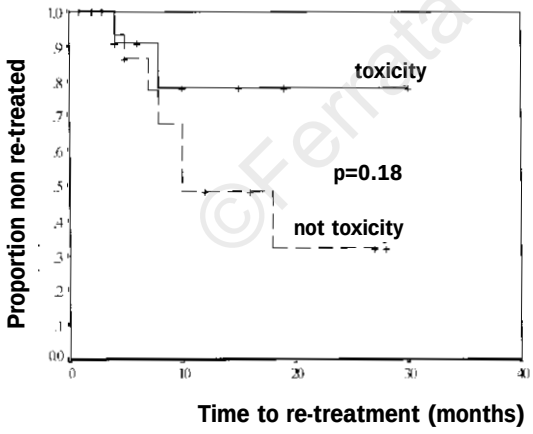


Figure 1. Kaplan-Meier curves of time to re-treatment of CLL patients stratified by age, previous therapy before fludarabine treatment and fludarabine-induced toxicity.

city for untreated patients was autoimmune complications which occurred in 3 cases: one patient developed classical antiglobulin positive autoimmune hemolytic anemia; another developed generalized

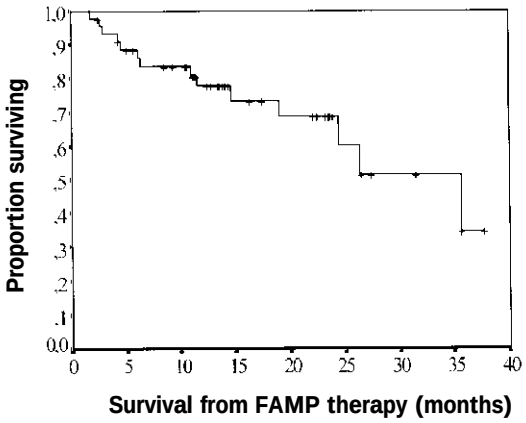


Figure 2. Kaplan-Meier survival curve calculated from fludarabine therapy. Tick marks indicate censored patients.

bullous pemphigoid and in the third, positivity for an autoantibody was demonstrated after some weeks of fever responsive to corticosteroid, while the disease was responding to FAMP therapy. Overall 15 patients died; four untreated cases died, one each of suicide, gastro-intestinal hemorrhage, lung cancer and cardiac disease. Among pre-treated patients, 2 died of their CLL, 2 of disease and infection, 3 of infection only, 2 of a second neoplasia, 1 due to neurologic toxicity and 1 of cardiac failure.

Survival

Out of the 47 patients analyzed, 33 are alive, with a median survival of 35.7 months (95% CI, 21.7 to 49.6) calculated from FAMP therapy (Figure 2). With a median follow-up of 13.1 months, the proportion of surviving cases, stratified according to prognostic parameters (Table 5), was significantly higher in patients in Rai stage 0-II (77%) as compared to those in Rai stage III-IV (62%), in responders (76%) versus non-responders (50%) and in patients treated with more than 5 FAMP cycles (88%) as compared to those receiving less than 6 cycles (45%) (Figure 3). This last variable was confirmed as statistically significant by Cox regression analysis (Table 5), while none of the other variables had a significant impact on survival.

Discussion

The use of high dose chlorambucil[5-7] and of nucleoside analogs,[3,8-11] has raised interest in an alternative non-palliative management of CLL, basically because of the quality and the high number of remissions that they can induce. Data from the present study extend and confirm prior observations on the effect of FAMP on both untreated and previously treated CLL patients. In our series of patients, treated at the onset, in relapse and in a resistant phase of disease, a response rate of 94.1%, 80% and 46.6%, respec-

Table 5. Univariate and Cox regression analysis of prognostic variables for survival calculated from the beginning of FAMP therapy.

Variable	*Probability of overall survival*	**Univariate p*	*°Multivariate p*
Age, years (≤ 64.7 v > 64.7)	.69 v .69	0.6	---
Sex (male v female)	.69 v .71	0.9	---
Rai stage (0-II v III-IV)	.77 v .62	0.0406	0.07
Binet stage (A-B v C)	.75 v .64	0.1	0.1
TTM score (≤ 12.3 v > 12.3)	.71 v .68	0.5	---
Response to FAMP (CR+PR v NR+PD)	.76 v .50	0.0078	0.6
No. of previous therapies (0 v ≥ 1)	.82 v .62	0.06	0.8
FAMP schedule (4days v 5days)	.78 v .55	0.2	
No. of FAMP cycles (< 6 v ≥ 6)	.45 v .	0.0001	0.0109

**Log-rank test; °Cox regression analysis.*

tively was obtained. As expected, a significantly higher number of responses was observed in untreated patients and in those patients treated with more than 5 FAMP cycles. This latter variable maintained its significance in multivariate analysis. It is noteworthy that the majority of resistant cases did respond, in spite of the recently emphasized poor prognosis associated with resistance to chlorambucil.[9] We can, therefore, confirm that FAMP is a very useful alternative tool for treatment of otherwise unresponsive patients.

Another possible advantage of FAMP therapy is the prolonged duration of unmaintained response. In our series, the median time to re-treatment was not reached in previously untreated patients.

In a study of long-term follow-up of CLL patients treated with FAMP,[9] response to treatment and survival were strongly correlated with the number of previous therapies, the stage of the disease and the refractoriness to alkylating agents; other parameters associated with survival were age and albumin levels. In our cohort, with a median follow-up of 13.1 months, the median survival was significantly longer in patients with early Rai stage, in responders and in patients treated with more than 5 FAMP cycles. The number of FAMP cycles resulted to be the strongest predictor of survival also in multivariate analysis; in fact, the number of FAMP courses administered may be considered a variable which surrogates other parameters such as performance status, no treatment related toxicity and absence of disease progression.

The use of FAMP as front-line therapy of CLL is still a matter of debate.[15,16] One of the reasons for refraining from the use of FAMP in untreated cases is its toxicity profile; in fact, in spite of a nearly absent organ toxicity, purine analogs seem to worsen the immune impairment typical of the natural history of CLL, consisting in frequent autoimmune[17,18] and infectious complications.[19] It is worth noting that in

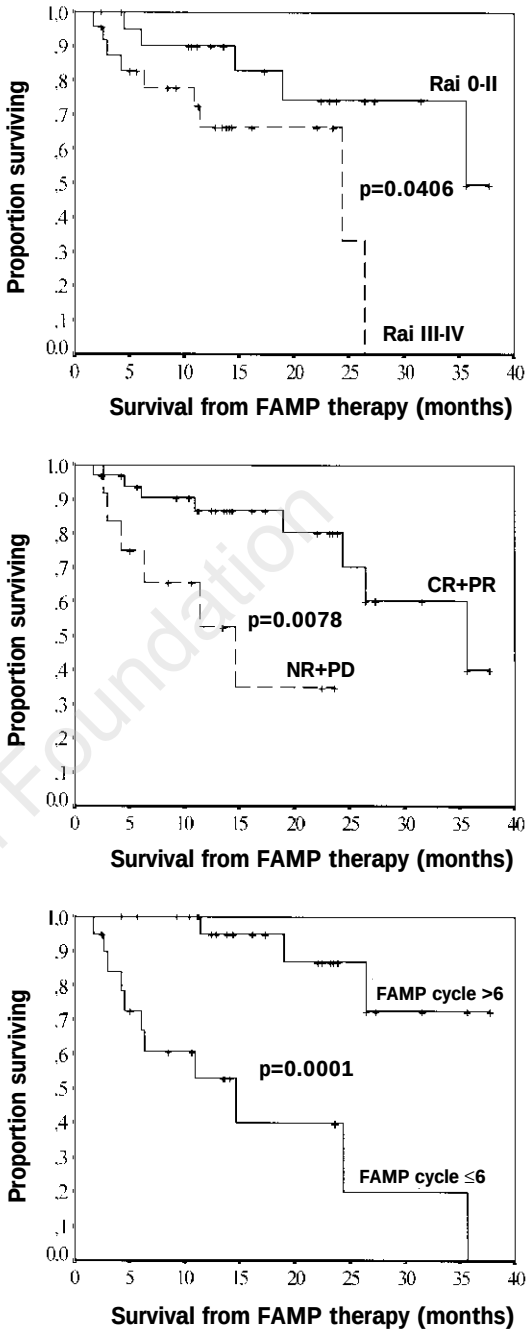


Figure 3. Kaplan-Meier survival curves calculated from fludarabine therapy of CLL patients stratified by Rai stage, response to fludarabine treatment and number of fludarabine cycles.

the present experience 53.1% of all cases and around 60% of untreated ones did not experience any toxicity, probably due to the antibiotic prophylaxis received by the vast majority of patients. We would, however, like to focus attention on the development of severe autoimmune phenomena in three of our

untreated cases. Although the role of purine analogs in determining further immune deregulation in CLL is still unclear, these complications have been repeatedly associated with purine analog treatment and should be prevented or, at least, promptly recognized. A second problem, influencing the use of FAMP as current chemotherapy is myelosuppression and infections which in CLL are also related to the inadequate bone marrow function and to the impaired humoral and cellular immunity. The present study showed an acceptable infectious toxicity with no severe episodes among untreated cases and the 3 deaths due to infection and 2 because of both disease and infection, occurring only in heavily pretreated patients. As far as the type of infection was concerned, 4 herpes zoster and 7 pneumonia (including 1 interstitial and 1 fungal) episodes were recorded. In this respect, O'Brien very recently demonstrated that the use of granulocyte colony-stimulating factor following FAMP in high risk patients with CLL significantly reduces myelosuppression and the incidence of pneumonia.[19]

In conclusion, this study confirms previous results on the efficacy of FAMP in the treatment of CLL patients and gives further information on its toxicity profile. In particular, durable responses were obtained, especially in older and untreated patients, without relevant infectious toxicity and with a very good quality of life. These results encourage its use as front-line therapy, if the conclusion of ongoing randomized trials demonstrates the superiority of this treatment over conventional chemotherapy; however, particular attention should be given to possible autoimmune complications. On the other hand, the impressive results obtained in relapsed and resistant cases would suggest its preferential use as second line therapy. The higher frequency of severe infectious complications in this setting of patients indicates the opportuneness of introducing the drug early in the course of the disease, together with prophylaxis against infections or, possibly, with concomitant use of G-CSF. It is, however, well-recognized that FAMP therapy, even as front-line treatment in young patients, cannot be considered a curative strategy. In this respect, synergistic association between the purine analog and other drugs, such as cyclophosphamide, and myeloablative treatments with peripheral blood stem cell support appear very promising in order to obtain more durable results.[20,21] Finally, better knowledge of the drug profile[22] is necessary before proposing its use with curative intent for patients in a relatively early stage of the disease, with a low tumor burden.

Contributions and Acknowledgments

CS conceived the study, was responsible for data collection and interpretation, literature analysis and wrote the manuscript. FM was responsible for data evaluation, statistical analysis and revised the paper. MGK, FI, VO and DL contributed to data collection. VC followed all the phases of the study and revised the paper. SM conceived the study, contributed with the patients and revised the paper. FN revised the final version of the paper. MB conceived the study, supervised the whole study and revised the final version of the paper.

Disclosures

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References

1. Caligaris-Cappio F. B-chronic lymphocytic leukemia: a malignancy of anti-self B cells. Blood 1996; 87: 2615-20.
2. Molica S. Prognostic value of biological variables in B-cell chronic lymphocytic leukemia. Can we improve upon clinical parameters? Haematologica 1997; 86: 705-9.
3. Rozman C, Montserrat E. Chronic lymphocytic leukemia. N Engl J Med 1995; 333:1052-7.
4. French Cooperative Group on Chronic Lymphocytic Leukemia. Is the CHOP regimen a good treatment in advanced CLL (stage B and C)? Results from two randomized trials. Leuk Lymphoma 1994; 13:449-56
5. Jaksic B, Brugiatelli M. High dose continuous chlorambucil vs intermittent chlorambucil plus prednisone for treatment of B-CLL-IGCI CLL-01 trial. Nouv Rev Fr Hematol 1988; 30:437-42.
6. Brugiatelli M, Jaksic B, Planinc-Peraica A, et al. Treatment of chronic lymphocytic leukemia in early and stable phase of the disease: long-term results of a randomized trial. Eur J Haematol 1995; 55:158-63.
7. Jaksic B, Brugiatelli M, Krç I, et al. Comparison of high-dose chlorambucil versus Binet's modified CHOP regimen in B-cell chronic lymphocytic leukemia in advanced phase: results of an international multicentric randomized trial. Cancer 1997; 79:2107-14.
8. O'Brien S, Del Giglio A, Keating MJ. Advances in the biology and treatment of B-cell chronic lymphocytic leukemia. Blood 1995; 85:307-18.
9. Keating MJ, O'Brien S, Kantarjian H, et al. Long-term follow-up of patients with chronic lymphocytic leukemia treated with fludarabine as a single agent. Blood 1993; 81:2878-84.
10. The French Cooperative Group on CLL, et al. Multicentre prospective randomised trial of fludarabine versus cyclophosphamide, doxorubicin, and prednisone (CAP) for treatment of advanced-stage chronic lymphocytic leukaemia. Lancet 1996; 347:1432-8.
11. Rai KR, Peterson B, Elias L, et al. A randomized comparison of fludarabine and chlorambucil for patients with previously untreated chronic lymphocytic leukemia. A CALGB, SWOG, CTG/NCI-C and ECOG inter-group study. Blood 1996; 88 (suppl 1):141a.
12. Juliusson G. Complications in the treatment of CLL with purine analogues. Hematol Cell Ther 1997; 39: S41-S44.
13. Cheson BD, Bennett JM, Grever M, et al. National Cancer Institute-sponsored working group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood 1996; 87:4990-7.

14. Jaksic B, Vitale B. Total tumor mass score (TTM): a new parameter in chronic lymphocytic leukemia. Br J Haematol 1981; 49:401-13.
15. Jaksic B, Delmer A, Brugiatelli M, et al. Fludarabine vs. high dose continuous chlorambucil: Interim analysis of a randomized phase II study in untreated B-cell chronic lymphocytic leukemia (B-CLL). Blood 1996; 88 (suppl 1):588a.
16. Dighiero G. Chronic lymphocytic leukemia treatment. Hematol Cell Ther 1997; 39:S31-S40
17. Mynt H, Copplestone JA, Orchard J, et al. Fludarabine-related autoimmune haemolytic anemia in patients with chronic lymphocytic leukaemia. Br J Haematol 1995; 91:341-4.
18. Weiss RB, Freiman J, Kweder SL, Diehl LF, Byrd JC. Hemolytic anemia after fludarabine therapy for chronic lymphocytic leukemia. J Clin Oncol 1998; 16: 1885-9.
19. O'Brien S, Kantarjian H, Beran M, et al. Fludarabine and granulocyte colony-stimulating factor (G-CSF) in patients with chronic lymphocytic leukemia. Leukemia 1997; 11:1631-5.
20. O'Brien S, Kantarjian H, Beran M, et al. Fludarabine (FAMP) and cyclophosphamide (CTX) therapy in chronic lymphocytic leukemia (CLL) Blood 1996; 88 (suppl 1):480 a.
21. Provan D, Bertlett-Pandite L, Zwicky C, et al. Eradication of polymerase chain reaction- detectable chronic lymphocytic leukemia cells is associated with improved outcome after bone marrow transplantation. Blood 1996; 88:2228-35.
22. Palomera L, Azaceta G, Varo MJ, Soria J, Gutierrez M. Fatal myelofibrosis following fludarabine administration in a patient with indolent lymphoma. Haematologica 1998; 83:1045-6.