Brief report

Impact of frontline fludarabine and cyclophosphamide combined treatment on peripheral blood stem cell mobilization in B-cell chronic lymphocytic leukemia

Olivier Tournilhac, Bruno Cazin, Stéphane Leprètre, Marine Diviné, Karim Maloum, Alain Delmer, Bernard Grosbois, Pierre Feugier, Frederic Maloisel, Florence Villard, Bruno Villemagne, Dominique Bastit, Karim Belhadj, Nabi Azar, Mauricette Michallet, Geraud Manhès, and Philippe Travade

Ongoing studies in B-cell chronic lymphocytic leukemia are evaluating autologous peripheral blood stem cell (PBSC) transplantation in first remission following fludarabine therapy. However, fludarabine could impair PBSC harvest. In 38 patients after frontline oral fludarabine and cyclophosphamide (FDR-CY) therapy, we prospectively evaluated steady state filgrastim- or lenograstim-primed PBSC mobilization to collect 2.0 × 10^6/kg or more CD34 cells. The first mobilization, performed a median of 178 days (range, 69-377 days) from the last FDR-CY course, was unsuccessful in 32 patients. This result was significantly associated with a low platelet count before mobilization but not with age, interval from last FDR-CY course, initial stage, remission status, or other blood parameters. Finally, after 1, 2, and 3 mobilizations in 27, 10, and 1 patients, 2.0 × 10^6/kg or more CD34 cells were collected in only 12. Explorations of the mechanism of poor mobilization and adaptation of PBSC harvest policies after fludarabine treatment are therefore warranted. (Blood. 2004;103:363-365)

Introduction

In B-cell chronic lymphocytic leukemia (B-CLL), treatment must be instituted for advanced (ie, B and C) stage patients.[1] Because the duration of response is correlated to the response rate,[2,3] autologous stem cell transplantation has been tested in the disease[2,4-6] with encouraging results. Therefore, ongoing cooperative studies are evaluating autologous peripheral blood stem cell (PBSC) transplantation at the time of first remission. To obtain the best response before PBSC harvest, fludarabine (FDR) is considered the most efficient drug in the treatment of B-CLL and is increasingly used as frontline therapy.[7,8] An oral formulation of this drug is available.[9] The adjunction of cyclophosphamide[10,11] (CY) or rituximab and CY[12] with FDR appears still more efficient and could lead to molecular remissions.

However, it has been suggested that occasionally in B-CLL[13-15] and in other disorders,[16-19] FDR could adversely affect PBSC mobilization. Conversely, high CD34 cell yields can be obtained in B-CLL patients treated with non-FDR–containing regimens.[20] This controversial issue has never been prospectively evaluated in a large number of B-CLL patients in first remission. The aim of this prospective study was to evaluate steady state filgrastim- or lenograstim-primed PBSC mobilization in 38 B-CLL patients in first remission after oral frontline treatment with FDR-CY.

Study design

Patients

The study involved 38 patients with B-CLL (median age, 53.5 years [range, 38-66 years]; 33 men, 5 women) in complete remission (CR) (n = 26) or partial remission (PR) (n = 12), according to the National Cancer Institute criteria,[21] after frontline treatment with FDR-CY. All gave informed consent. They had been previously enrolled in a clinical trial (ME 98123 Schering S.A.) that evaluated the efficacy and safety of 6 monthly courses of FDR (30 mg/m^2 per day) and CY (200 mg/m^2 per day) both given orally for 5 consecutive days.[11] All these patients (34 stage B and 4 stage C) had received 6 FDR-CY courses, except 4 who had received either 5 courses (n = 2), 4 courses (n = 1), or 2 courses plus 3 courses of a CHOP-like regimen (n = 1) because of toxicity. After evaluation performed 2 months after the last FDR-CY course, responding patients were considered for PBPC collection at the time of first CR or PR. According to the aforementioned trial, high-dose therapy should not be performed before relapse.

Mobilization and apheresis

Mobilization was initiated at least 2 months after the last FDR-CY course, according to published results.[13] To collect a number of CD34 cells equaling at least 2.0 × 10^6/kg body weight, all patients underwent a first steady state PBSC mobilization using either filgrastim (10 μg/kg per day) or lenograstim (7 μg/kg per day) given once a day for 4 to 6 consecutive days until adequate blood CD34 circulation was achieved. Apheresis was initiated when circulating CD34 cell levels reached 10 × 10^6/L. Two to 3 blood mass volume was processed during each apheresis performed with continuous-flow blood cell separators. If the first cell collection failed, further steady state mobilizations using the same procedure could be performed.

Statistical analysis

To avoid redundant patient-dependent variables caused by repeated mobilization courses in the same patient, we focused statistical analysis on the results of their first mobilization course only. Univariate analysis was performed using the χ^2 test to compare categorical variables and by analysis

From the Service d'Hématologie Clinique, Centre Hospitalier Universitaire, Clermont-Ferrand; Centre Hospitalier Régional Universitaire, Hôpital Claude Huriez, Lille; Centre anti-cancéreux Becquerel, Rouen; Hôpital Henri Mondor, AP-HP, Créteil; Hôpital Pitié-Salpétrière, AP-HP, Paris; Hôpital Hôtel Dieu, AP-HP, Paris; Centre Hospitalier Universitaire, Rennes; Centre Hospitalier Universitaire, Nancy-Brabois; Centre Hospitalier Régional Universitaire, Hôpital Civil, Strasbourg; Centre Hospitalier Universitaire, Hôpital Edouard Herriot, Lyon; and Département d'Information Médicale, Centre Hospitalier, Vichy; France.

Submitted May 9, 2003; accepted August 29, 2003. Prepublished online as *Blood* First Edition Paper, September 11, 2003; DOI 10.1182/blood-2003-05-1449.

Reprints: Philippe Travade, Service d'Hématologie Clinique, Hôtel Dieu, Centre Hospitalier Universitaire - 11, bd Léon Malfreyt, 63003 Clermont-Ferrand Cedex, BP 69, France; e-mail: ptravade@chu-clermontferrand.fr.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

Table 1. Steady-state filgrastim- or lenograstim-primed PBSC mobilization to collect 2.0 × 10^6/kg or more CD34 cells

	First mobilization T 178 (69-377) d		Second mobilization T 288 (243-517) d			Third mobilization T 573 d		
	No.	CD34 collected × 10^6/kg	No.	CD34 collected × 10^6/kg		No.	CD34 collected × 10^6/kg	
Patients	aphereses	1st mob	aphereses	2nd mob	1st + 2nd mob	aphereses	3rd mob	2nd + 3rd mob
1	2	3.45	—	—	—	—	—	—
2	3	2.64	—	—	—	—	—	—
3	3	2.60	—	*	—	—	—	—
4	3	2.31	—	—	—	—	—	—
5	2	2.30	—	*	—	—	—	—
6	4	2.21	—	—	—	—	—	—
7	3	1.80	—	—	—	—	—	—
8	2	1.74	—	—	—	—	—	—
9	2	1.48	2	0.92	2.40	—	—	—
10	2	1.42	2	2.20	3.62	—	—	—
11	3	1.28	3	1.84	2.67	—	—	—
12	2	1.27	1	1.76	3.03	—	—	—
13	2	0.94	—	—	—	—	—	—
14	2	0.83	—	—	—	—	—	—
15	1	0.59	—	—	—	—	—	—
16	1	0.50	—	—	—	—	—	—
17	1	0.47	—	—	—	—	—	—
18	0	—	2	2.56	—	—	—	—
19	0	—	2	1.12	—	3	1.13	2.25
20	0	—	2	0.98	—	—	—	—
21	0	—	2	0.55	—	—	—	—
22	0	—	0	—	—	—	—	—
23	0	—	0	—	—	—	—	—
24	0	—	0	—	—	—	—	—
25-38	0	—	—	—	—	—	—	—

T indicates time elapsed between the last FDR-CY course and first day of mobilization in median (range); mob, mobilization; and —, not applicable.

*Although more than 2.0 × 10^6/kg CD34 cells were stored after the first mobilization, a second mobilization was performed in patients 3 and 5 at 44 and 108 days after the first mobilization, leading to the harvest of 2.09 and 2.3 × 10^6/kg CD34 cells, respectively.

of variance to compare means. Multivariate logistic regression analysis was performed taking the final result of the first mobilization (ie, number of collected CD34 cells) as dependent variables, and the variables found significant in the univariate analysis or described in the literature as independent variables. Data analysis and statistics analysis were performed using SAS software (SAS Institute). $P < .05$ was considered statistically significant.

Results and discussion

The 38 patients underwent a total of 50 mobilizations: 1, 2, or 3 mobilizations in 27, 10, and 1 patients, respectively (Table 1). The first mobilization was performed after a median of 178 days (range, 69-377 days) from the last FDR-CY course. Apheresis was initiated in 17 (45%) patients, giving a median number of 1.48 × 10^6/kg (range, 0.47-3.45) CD34 cells. Only 6 (16%) patients achieved a CD34 cell number that equaled or exceeded 2.0 × 10^6/kg. In 21 (55%) patients, apheresis was not performed because of a poor blood CD34 level (less than 10 × 10^6/L).

Univariate analysis showed a significant association between number of CD34 cells collected and platelet count immediately before mobilization ($P = .009$), with a trend in correlation with platelet count at evaluation 2 months after the last FDR-CY ($P = .051$) but no correlation with other studied parameters (Table 2). Therefore, all patients with platelet counts lower than 150.0 × 10^9/L, either at evaluation or before mobilization, failed to mobilize adequately. In addition, achieving at least 10 × 10^6/L circulating CD34 cells was associated with platelet counts either before mobilization ($P = .009$) or at evaluation ($P = .010$) (data not shown). Multivariate analysis using age, interval time from last FDR-CY course to mobilization, initial Binet stage, disease status at evaluation, hemoglobin level, and platelet count before

Table 2. Univariate analysis to predict number of collected CD34 cells equaling or exceeding 2 × 10^6/kg after first mobilization

Variables	Results	*P*
Age, y	53.5 (38-66)	.73
Male-female ratio	33/5	.57
Initial Binet (B/C) stage before FDR-CY	34/4	.51
Blood count at presentation before FDR-CY		
Hemoglobin, g/L	135.0 (100.0-170.0)	.17
Lymphocytes, × 10^9/L	45.5 (5.0-286.0)	.99
Neutrophils, × 10^9/L	4.3 (0.0-21.0)	.77
Platelets, × 10^9/L	183.5 (57.0-279.0)	.42
No. FuCy courses (c)*	6c (n = 34); 5c (n = 2); 4c (n = 1); 2c (n = 1)	.51
Disease status at evaluation after FDR-CY: PR/CR	12/26	.63
Blood count at evaluation, 2 mo after last FDR-CY c*		
Hemoglobin, g/L	137.5 (73.0-156.0)	.64
Lymphocytes, × 10^9/L	0.7 (0.1-1.7)	.30
Neutrophils, × 10^9/L	2.9 (0.9-8.0)	.11
Platelets, × 10^9/L	178.0 (50.0-330.0)	.052
Interval from last FDR-CY c to mobilization, d	178.0 (69-377)	.19
Mobilization: filgrastim/lenograstim	25/13	.67
Blood count immediately before mobilization*		
Hemoglobin, g/L	142.0 (95.0-163.0)	.56
Leukocytes, × 10^9/L	4.3 (2.1-11.4)	.097
Neutrophils, × 10^9/L	2.8 (1.1-8.7)	.085
Lymphocytes, × 10^9/L	0.8 (0.2-5.4)	.96
Monocytes, × 10^9/L	0.3 (0.1-0.8)	.20
Platelets, × 10^9/L	177.5 (86.0-461.0)	.009

Variables were demographic, clinical, and biologic characteristics of 38 B-CLL patients. All numbers in parentheses are ranges, and all numbers preceding them are medians.
*Continuous variables.

Table 3. Combined results of first, second, and third mobilizations

Combined results	No. apheresis (n = 17), 45%	Collection of less than 2.0 × 10^6/kg (n = 9), 24%	Collection of 2.0 × 10^6/kg or more (n = 12), 31%
No. mobilization attempts	1 (n = 14)	1 (n = 7)	1 (n = 6)
	2 (n = 3)	2 (n = 2)	2 (n = 5)
	—	—	3 (n = 1)
No. aphereses	—	1 (n = 3)	2 (n = 3)
	—	2 (n = 5)	3 (n = 4)
	—	3 (n = 1)	4 (n = 3)
	—	—	5 (n = 1)
		—	6 (n = 1)
No. CD34 cells collected × 10^6/kg (median)	0	0.83	2.56
	—	(0.47-1.80)	(2.21-3.62)

— indicates not applicable.

mobilization persistently found a significant association between number of collected CD34 cells and platelet count ($P = .04$).

A second mobilization was made in 11 patients after a median of 288 days (range, 243-517 days) from the last FDR-CY course and of 140 days (range, 68-285 days) from the first mobilization. Apheresis was initiated in 8 of these patients, giving a median number of CD34 cells of 1.44 × 10^6/kg (range, 0.55-2.56). Only 5 patients achieved a CD34 cell number that equaled or was greater than 2 × 10^6/kg—2 with this second mobilization only, and 3 achieved it by cumulative results from the first and second mobilizations. In addition, though they already had 2.0 × 10^6/kg or more CD34 cells stored after the first procedure, 2 other patients had second and successful mobilizations. A third mobilization was performed in 1 patient after 573 days from the last FDR-CY course and 300 days from the second mobilization, leading to a total of 2.25 × 10^6/kg CD34 cells harvested.

The combined results of all 3 mobilizations are detailed in Table 3. These poor results were probably not caused by bone marrow involvement[16] by tumor cells at the time of mobilization because most of these patients were in CR (even when assessed by bone marrow biopsy), nor were they caused by multiple previous treatments given that FDR-CY was their first therapeutic line. As previously reported,[13-19] a negative impact of FDR on further PBSC mobilization is therefore clearly suspected, but the precise mechanism of such toxicity remains to be clarified. Jeopardized stem cell mobilization in B-CLL could depend on alterations of bone marrow stroma. The chemokine receptor CXCR4 for the stromal factor SDF-1, which is highly and functionally expressed by B-CLL cells and acts in bone marrow homing and cell survival,[22] could be one of the involved mechanisms.

Prospective studies in B-CLL are currently evaluating the impact of transplantation after frontline therapy using FDR-containing regimens. Based on this small but homogeneous series of treated B-CLL patients, these results could lead to the adaptation of PBSC harvest policies. Delaying PBSC mobilization until after FDR treatment is complete does not seem to improve the results. Limiting the number of FDR courses to the minimum needed to achieve the maximal response before mobilization and using combined mobilization with chemotherapy and G-CSF could be alternatives.

Acknowledgments

We thank Marisha Burden (University of Oklahoma College of Medicine) for technical help in manuscript preparation and Dr Odile Guibon (Laboratoire Schering, Lys-lez-Lannoy).

References

1. Dighiero G, Maloum K, Desablens B, et al. Chlorambucil in indolent chronic lymphocytic leukemia: French Cooperative Group on Chronic Lymphocytic Leukemia. N Engl J Med. 1998;338: 1506-1514.
2. Provan D, Bartlett-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;88:2228-2235.
3. Robertson LE, Huh YO, Butler JJ, et al. Response assessment in chronic lymphocytic leukemia after fludarabine plus prednisone: clinical, pathologic, immunophenotypic, and molecular analysis. Blood. 1992;80:29-36.
4. Rabinowe SN, Soiffier RJ, Gribben J, et al. Autologous and allogenic bone marrow transplantation for poor prognosis patients with B-cell chronic leukemia. Blood. 1993;82;1366-1376.
5. Khouri IF, Keating MJ, Vriesendorp HM, et al. Autologous and allogeneic bone marrow transplantation for chronic lymphocytic leukemia: preliminary results. J Clin Oncol. 1994;12:748-758.
6. Michallet M, Archimbaud E, Bandini G, et al. HLA-identical sibling bone marrow transplantation in younger patients with chronic lymphocytic leukemia: European Group for Blood and Marrow Transplantation and the International Bone Marrow Transplant Registry. Ann Intern Med. 1996; 124:311-315.
7. Leporrier M, Chevret S, Cazin B, et al. Randomized comparison of fludarabine, CAP, and CHOP in 938 previously untreated stage B and C chronic lymphocytic leukemia patients. Blood. 2001;98:2319-2325.
8. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med. 2000;343:1750-1757.
9. Boogaerts MA, Van Hoof A, Catovsky D, et al. Activity of oral fludarabine phosphate in previously treated chronic lymphocytic leukemia. J Clin Oncol. 2001;19:4252-4258.
10. O'Brien SM, Kantarjian HM, Cortes J, et al. Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. J Clin Oncol. 2001;19:1414-1420.
11. Cazin B, Maloum K, Divine M, et al. Oral fludarabine and cyclophosphamide in previously untreated CLL: preliminary data on 75 patients [abstract]. Blood. 2002;100:773.
12. Keating M, Manshouri T, O'Brien S, et al. A high proportion of molecular remission can be obtained with fludarabine, cyclophosphamide, rituximab combination (FCR) in chronic lymphocytic leukemia (CLL) [abstract]. Blood. 2002;100:205.
13. Michallet M, Thiebaut A, Dreger P, et al. Peripheral blood stem cell (PBSC) mobilization and transplantation after fludarabine therapy in chronic lymphocytic leukaemia (CLL): a report of the European Blood and Marrow Transplantation (EBMT) CLL subcommittee on behalf of the EBMT Chronic Leukaemia Working Party (CLWP). Br J Haematol. 2000;108:595-601.
14. Tedeschi A, Montillo M, Cairoli R, et al. Successful CD34$^+$ priming with intermediate dose Ara-C (ID ARA-C) in chronic lymphocytic leukemia (CLL) patients treated with sequential fludarabine (FAMP) and campath-1H [abstract]. Blood. 2002; 100:830.
15. Dreger P, Montserrat E. Autologous and allogenic stem cell transplantation for chronic lymphocytic leukemia. Leukemia. 2002;16:985-992.
16. Itala M, Pelliniemi TT, Rajamaki A, Remes K. Autologous blood cell transplantation in B-CLL: response to chemotherapy prior to mobilization predicts the stem cell yield. Bone Marrow Transplant. 1997;19:647-651.
17. Ketterer N, Salles G, Moullet I, et al. Factors associated with successful mobilization of peripheral blood progenitor cells in 200 patients with lymphoid malignancies. Br J Haematol. 1998; 103:235-242.
18. Laszlo D, Galieni P, Raspadori D, et al. Fludarabine-containing regimens may adversely affect peripheral blood stem cell collection in low-grade non Hodgkin lymphoma patients. Leuk Lymphoma. 2000;37:157-161.
19. Visani G, Lemoli RM, Tosi P, et al. Fludarabine-containing regimens severely impair peripheral blood stem cells mobilization and collection in acute myeloid leukaemia patients. Br J Haematol. 1999;105:775-779.
20. Dreger P, Viehmann K, von Neuhoff N, et al. A prospective study of positive/negative ex vivo B-cell depletion in patients with chronic lymphocytic leukemia. Exp Hematol. 2000;28:1187-1196.
21. 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;87:4990-4997.
22. Burger JA, Kipps TJ. Chemokine receptors and stromal cells in the homing and homeostasis of chronic lymphocytic leukemia B cells. Leuk Lymphoma 2002;43:461-466.