A double-blind low dose-finding phase II study of granulocyte colony-stimulating factor combined with chemotherapy for stem cell mobilization in patients with non-Hodgkin's lymphoma

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The aim of the study was to define the minimal effective dose (MED) of granulocyte colony-stimulating factor (G-CSF) among five daily doses following chemotherapy for peripheral blood stem cell (PBSC) collection. Twenty-five patients were included in this double-blind dose-finding phase II study conducted according to a two-stage Bayesian design. The estimated probabilities of success for PBSC collection for the G-CSF doses of 50, 75, 100, 125 and 150 μg/m²/day were 84%, 87.7%, 91%, 93.9 and 96.4%, respectively. Low G-CSF doses may be used with a similar probability of success as conventional doses and could allow significant savings.

Key words: stem cell mobilization, Bayesian, continual reassessment method, dose-finding, phase II, G-CSF, CD34⁺, leukapheresis.

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Autologous peripheral blood stem cell (PBSC) mobilization regimens currently include the administration of recombinant human granulocyte colony-stimulating factor (G-CSF) following chemotherapy.[1-6] Many studies have examined the impact of different doses of G-CSF to mobilize PBSC, but the standard dose (5 μg/kg/day for filgrastim, 150 μg/m²/day for lenograstim) is usually compared to higher doses.[7,8] We previously found, in a retrospective study, that a lower dose of G-CSF following chemotherapy may be sufficient to collect PBSC.[9] In order to determine the minimum effective dose of G-CSF, given after chemotherapy, for PBSC collection, we conducted a prospective double blind dose-finding phase II study in patients with non-Hodgkin's lymphoma.

Design and Methods

Patient population

Twenty-five consecutive patients between 18 and 65 years of age with non-Hodgkin's lymphoma were included in this single-center study between 2001 and 2004. The ethical committee from our institution approved the study and written informed consent was obtained from all patients. The chemotherapy regimens used to treat the patients were also used for PBSC mobilization and consisted of CHOP (day 1 doxorubicin 50 mg/m², cyclophosphamide 750 mg/m², vincristine 2 mg, plus prednisone), ACVBP (day 1: doxorubicin 75 mg/m², cyclophosphamide 1200 mg/m², vincristine 2 mg, plus prednisone) or DHAP (day 1 cisplatinium 100 mg/m², day 2 Ara-C 4 g/m², plus dexamethasone). All these regimens have a similar potential for PBSC mobilization.[10-12] Patients were excluded from this study if they had undergone previous PBSC mobilization, or had received previous radiotherapy, myeloablative therapy, interferon, fludarabine, chlorambucil or more than two different lines of chemotherapy before this mobilization attempt. Previous therapies and the characteristics of the patients are listed in Table 1.

Design and statistical analysis

The two-stage Bayesian design of this dose-finding phase II study was chosen to assess the optimal dose level of G-CSF for PBSC mobilization. The first stage focused on the dose-finding procedure until the estimated minimal effective dose (MED) was reached and the second stage focused on the reliability of the MED estimates.[13,14] The first stage used the continual reassessment method in order to determine the dose level of G-CSF associated with a 90% rate of successful PBSC mobilization. The continual reassessment method is an iterative Bayesian method based on a one-parameter model which is aimed at estimating the percentile of among k distinct dose levels d_i $(i=1,...,k)$. Each of the five dose levels tested was arbitrarily associated by the investigator (according to his personal experience) with the following initial guesses of success probability: 0.7, 0.75, 0.8, 0.85 and 0.90 for the 50, 75, 100, 125 and 150 μg/m²/day loading dose, respectively. A one-parameter logistic model (with scale parameter fixed at 3) was then used to fit the dose-response curve, with an exponential distribution (with mean=2) for the model parameter. The posterior response probability for each dose level was re-esti-

Table 1. Characteristics of the 25 patients and their chemotherapy regimens.

Patient	Age	Diagnosis	Previous therapies	Stage of disease at the time of mobilization	Chemotherapy used for stem cell mobilization
1	59	Diffuse large B cell lymphoma	3 ACVBP/2 HD-MTX/1 DHAP	PR	DHAP
2	63	Diffuse large B cell lymphoma	4 CHOP	CR	CHOP
3	29	Diffuse large B cell lymphoma	4 ACVBP/2 HD-MTX/1 DHAP	PR	DHAP
4	54	Mantle cell lymphoma	3 CHOP/1 DHAP	CR	DHAP
5	38	Diffuse large B cell lymphoma	2 ACVBP	PR	ACVBP
6	33	Diffuse large B cell lymphoma	3 CHOP	CR	Cyclophosphamide
7	64	Mantle cell lymphoma	3 CHOP/1 DHAP	CR	DHAP
8	54	Immunoblastic lymphoma	3 ACVBP/2 HD-MTX/2 DHAP	CR	DHAP
9	57	Diffuse large B cell lymphoma	3 CHOP	CR	CHOP
10	36	Diffuse large B cell lymphoma	3 ACVBP	CR	ACVBP
11	61	Diffuse large B cell lymphoma	2 CHOP	Not evaluable	CHOP
12	55	Mantle cell lymphoma	5 CHOP	PR	DHAP
13	63	Mantle cell lymphoma	3 CHOP/1 DHAP	CR	DHAP
14	36	Diffuse large B cell lymphoma	3 ACVBP	CR	ACVBP
15	41	Diffuse large B cell lymphoma	2 ACVBP	PR	ACVBP
16	55	Diffuse large B cell lymphoma	3 ACVBP	CR	ACVBP
17	51	Mantle cell lymphoma	3 CHOP/1 DHAP	CR	DHAP
18	38	Diffuse large B cell lymphoma	5 CHOP	CR	CHOP
19	50	Diffuse large B cell lymphoma	3 ACVBP	RP	CHOP
20	62	Diffuse large B cell lymphoma	4 CHOP	RP	CHOP
21	45	Mantle cell lymphoma	3 CHOP/1 DHAP	RP	DHAP
22	56	Diffuse large B cell lymphoma	3 CHOP	RP	CHOP
23	58	Diffuse large B cell lymphoma	3 CHOP	CR	CHOP
24	42	Follicular lymphoma	3 CHOP	CR	CHOP
25	56	T-cell lymphoma	3 CHOP	MR	CHOP

CR: complete remission; PR partial remission; MR: minor response; HD-MTX: high dose methotrexate.

mated after each new inclusion of patients. The dose allocated to each new patient was the dose level with the updated posterior response probability closest to the target 0.90. The first stage was stopped when either one of the following two criteria was met: (i) all doses were likely to be inefficient or (ii) the administered dose level was likely to remain unchanged until the end of the trial; if this latter criterion was fulfilled, then the first stage (dose-finding) ended and the trial moved on to the second stage. In the second stage, all patients received the dose level selected at the end of the first stage. This stage used a beta-binomial model to estimate the success rate of the estimated MED. The decision to end the second stage was based on a suitable estimation in terms of the precision of the MED: if this stopping criterion was lower than 0.05, then the trial ended. Otherwise, the trial was stopped when the fixed sample size of 25 patients was reached. The first cohort of patients received a dose of 150 μg/m^2/day with the initial guess of success probability closest to the target (0.90). The first tested dose of 150 μg/m^2/day was chosen since this is the dose currently recommended by the company producing the G-CSF for PBSC mobilization following chemotherapy. The decrease of 25 μg/m^2 between dose levels was decided arbitrarily.

G-CSF administration and PBSC collection

Chemotherapy was administered on day 1. G-CSF (lenograstim, Chugai-Rhône-Poulenc) was administered subcutaneously each day from day 7 until white blood cell (WBC) recovery and PBSC collection. We chose lenograstim because it is available in vials (103 and 263 μg) enabling it to be administered in a broad range of doses. Lenograstim was administered in a double-blind setting. WBC counts were monitored every day. Venous blood CD34$^+$ cell concentration was quantified when the WBC count recovered to 5×10^9/L. Leukapheresis was initiated if the CD34$^+$ cell concentration reached 20/μL. If three CD34 tests performed every other day did not reach this threshold despite continued administration of G-CSF, PBSC harvesting was not performed. The number of CD34$^+$ cells harvested was evaluated as previously described on each leukapheresis product obtained after two blood volumes had been processed.[6] PBSC harvesting was considered successful if at least 3×10^6 CD34 cells/kg were collected.

Assessment of engraftment

Hematologic reconstitution was assessed in 15 patients. The other patients did not receive an autograft because of death, progressive disease, decision to undergo allografting, failure to harvest PBSC or because PBSC were harvested only in the expectation of a poor evolution.

Results and Discussion

Table 2 reports the dose of G-CSF given to each patient, and the responses, with the actualized posterior estimated probability of success for each dose-level. Dose-finding was pursued until the 25th patient, due to non-fulfillment of either stopping criterion. Loading doses of 50, 75, 100, 125 and 150 μg/m^2/day were

Table 2. Sequential posterior estimated probabilities of success for the five tested doses, updated after each new patient.

			G-CSF dose finding study (μg/m²/day)				
			50	*75*	*100*	*125*	*150*
			Initial guesses of success probabilities				
			0.700	*0.750*	*0.800*	*0.850*	*0.90*
Patient number	*Allocated dose μg/m²*	*PBSC collection*	*Posterior estimated probability of success*				
1	150	Success	0.611	0.663	0.718	0.777	**0.940**
2	150	Success	0.788	0.832	0.872	0.910	0.944
3	125	Success	0.863	**0.896**	0.926	0.951	0.972
4	75	Success	**0.909**	0.934	0.955	0.971	0.985
5	50	Success	**0.937**	0.955	0.970	0.982	0.991
6	50	**Failure**	0.724	0.773	0.821	0.868	**0.914**
7*	50	Success	0.771	0.816	0.859	0.899	0.937
8	125	Success	0.794	0.832	0.876	0.913	0.947
9	125	Success	0.812	0.852	**0.890**	0.923	0.954
10	100	**Failure**	0.687	0.738	0.789	0.840	**0.892**
11	150	Success	0.704	0.754	0.803	0.853	**0.902**
12	150	Success	0.718	0.767	0.816	0.863	**0.910**
13	150	Success	0.731	0.779	0.826	0.872	**0.917**
14	150	Success	0.742	0.789	0.835	**0.880**	0.923
15	125	Success	0.755	0.801	0.846	**0.889**	0.929
16	125	Success	0.767	0.812	0.855	**0.896**	0.935
17	125	Success	0.777	0.821	0.863	**0.903**	0.939
18	125	Success	0.786	0.830	0.870	**0.908**	0.943
19	125	Success	0.795	0.837	0.877	**0.914**	0.947
20	125	Success	0.802	0.844	**0.883**	0.918	0.950
21	100	Success	0.811	0.852	**0.889**	0.923	0.954
22	100	Success	0.820	0.859	**0.895**	0.928	0.957
23	100	Success	0.827	0.866	**0.901**	0.932	0.960
24	100	Success	0.834	0.872	**0.906**	0.933	0.962
25	100	Success	0.840	0.877	**0.910**	0.939	0.964

*The dose level associated with the posterior estimated probability of success closest to the target probability of 90% is shown in bold. This was the dose that had to be administered to the subsequent patient. *The 7th patient was included before the response of the 6th patient was known. So the 7th patient was included at the same dose level as the 6th patient (50 μg/m²).*

assigned to 3, 1, 6, 9, and 6 patients, respectively. After the treatment of the planned 25 patients, the posterior estimated probabilities of success for the five doses 50, 75, 100, 125 and 150 μg/m²/day were 84.0%, 87.7%, 91.0%, 93.9% and 96.4%, respectively. The 100 μg/m²/day dose level was estimated to be the MED, as this dose level was associated with the probability closet to the target of 90% (estimated probability of success: 91.0%; and 95% credibility interval: 74.2%-98.3%). The sequential estimated probabilities of success associated with the MED are represented in Figure 1. PBSC mobilization failed in one patient treated with 50 μg/m²/day and in one other treated with 100 μg/m²/day. Neither of these patients reached a sufficient venous blood CD34⁺ cell concentration following salvage procedure using high G-CSF doses. The median time for WBC recovery, the median number of leukaphereses, the median number of G-CSF injections and the median number of CD34⁺ cells collected (6.8 to 10.7×10⁶ CD34⁺cells/kg) were similar whatever the dose of G-CSF used. No difference in hematologic reconstitution was noted between each group of patient following transplantation.

This double-blind phase II study was designed to assess the MED of G-CSF following chemotherapy in the mobilization of the CD34⁺ cells. We selected a homogeneous group of patients with non-Hodgkin's lymphoma who had not received prior heavy cumulative therapy that may compromise the mobilization.[15-17]

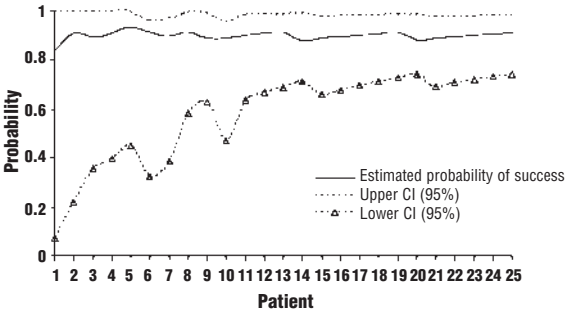

Figure 1. Sequential estimated probability of success associated with the minimal effective dose of G-CSF and 95% credibility intervals (CI).

After treatment of all patients, the posterior estimated probabilities of success for the five doses of 50, 75, 100, 125 and 150 μg/m² were 84.0%, 87.7%, 91.0%, 93.9% and 96.4%, respectively. The 100 μg/m²dose level was estimated to be the MED, as this dose level was associated with the probability closest to the target of 90%. The 8% failure rate for PBSC mobilization in this study is below the 20 to 30% usually observed.[15-17] This low incidence of failure may have been due to the selection of patients who had not received heavy previous chemotherapy. The two patients in whom PBSC mobilization failed with low doses of G-CSF also failed to

mobilize PBSC despite high doses of G-CSF administered shortly after the initial failure.[18] These failures appear to be more related to the patients' intrinsic conditions rather than to the lower G-CSF doses administered as has also been commonly observed in a similar proportion of healthy donors. It may, therefore, be speculated that the probability of success using low doses of G-CSF would be higher than that observed in this study if only patients with a good intrinsic PBSC mobilization potential are considered. In this study, the duration of a neutrophil count below 0.5×10^9/L was identical for each dose-subgroup of patients, as was already reported by others.[19,20] The number of G-CSF injections, the median number of leukaphereses and the median number of $CD34^+$ cells collected were also equivalent whatever the dose tested. So, the use of low doses of G-CSF does not increase the cost of PBSC harvesting by increasing drug administration or leukaphereses.

Further studies argue against the administration of standard or high doses of G-CSF. Martin-Mureas compared various doses of G-CSF (2.83 to 23 μg/kg) following chemotherapy and found no relationship between the dose administered and the peak level of circulating $CD34^+$ cells.[4] Bolwell compared 5, 10 and 16 μg/kg/day administered after stem cell transplantation to enhance neutrophil engraftment and concluded that there was no advantage from higher doses.[19] Toner performed a randomized study with lenograstim at a dose of 2 or 5 μg/kg/day following chemotherapy to prevent the consequences of neutropenia and did not observe differences in the measures of neutropenia, hospitalization or other clinical outcomes.[20]

In conclusion, low-dose G-CSF administration following chemotherapy for PBSC collection would allow substantial savings without toxicity.

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References

1. Chao NJ, Schriber JR, Long GD, Negrin RS, Catolico M, Brown BW, et al. Granulocyte colony-stimulating factor mobilized peripheral blood progenitor cells accelerate granulocyte and platelet recovery after high-dose chemotherapy. Blood 1993;81:2031-5.
2. Bensinger W, Appelbaum F, Rowley S, Storb R, Sanders J, Lilleby K, et al. Factors that influence collection and engraftment of autologous peripheral-blood stem cells. J Clin Oncol 1995; 13: 2547-55.
3. Gisselbrecht C, Lepage E, Molina T, Quesnel B, Fillet G, Lederlin P, et al. Shortened first-line high-dose chemotherapy for patients with poor-prognosis aggressive lymphoma. J Clin Oncol 2002;20:2472–9.
4. Martin-Murea S, Voso MT, Hohaus S, Pforsich M, Fruehauf S, Goldschmidt H, et al. The dose of granulocyte colony-stimulating factor administered following cytotoxic chemotherapy is not related to the rebound level of circulating CD34+ haemopoietic progenitor cells during marrow recovery. Br J Haematol 1998;101:582–5.
5. Lefrere F, Delmer A, Levy V, Delarue R, Varet B, Hermine O. Sequential chemotherapy regimens followed by high-dose therapy with stem cell transplantation in mantle cell lymphoma: an update of a prospective study. Haematologica 2004;89:1275-6.
6. Haas R, Mohle R, Fruhauf S, Goldschmidt H, Witt B, Flentje M, et al. Patients characteristics associated with successful mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. Blood 1994; 83: 3787-94.
7. Narabayashi M, Takeyama K, Fukutomi T, Tokuda Y, Tajima T, Okumura A, et al. A dose-finding study of lenograstim (glycosylated rHuG-CSF) for peripheral blood stem cell mobilization during postoperative adjuvant chemotherapy in patients with breast cancer. Lenograstim/Breast Cancer Study Group. Jpn J Clin Oncol 1999;29:285-90.
8. Demirer T, Ayli M, Ozcan M, Gunel N, Haznedar R, Dagli M, et al. Mobilization of peripheral blood stem cells with chemotherapy and recombinant human granulocyte colony-stimulating factor (rhG-CSF): a randomized evaluation of different doses of rhG-CSF. Br J Haematol 2002;116:468–74.
9. Lefrère F, Belanger C, Audat F, Hermine O, Cavazzana-Calvo M, Arnulf B, et al. The dose of G-CSF following chemopriming treatment does not influence stem cell apheresis yield: a retrospective study of 91 cases. Transfusion 1999; 39: 1207-11.
10. Smardova L, Engert A, Haverkamp H, Raemakers J, Baars J, Pfistner B, et al. Successful mobilization of peripheral blood stem cells with the DHAP regimen (dexamethasone, cytarabine, cisplatinum) plus granulocyte colony-stimulating factor in patients with relapsed Hodgkin's disease. Leuk Lymphoma 2005;46:1017-22.
11. Pavone V, Gaudio F, Guarini A, Perrone T, Zonno A, Curci P A, et al. Mobilization of peripheral blood stem cells with high-dose cyclophosphamide or the DHAP regimen plus G-CSF in non-Hodgkin's lymphoma. Bone Marrow Transplant 2002;29:285-90.
12. Endo T, Sato N, Mogi Y, Koizumi K, Nishio M, Fujimoto K, et al. Peripheral blood stem cell mobilization following CHOP plus rituximab therapy combined with G-CSF in patients with B-cell non-Hodgkin's lymphoma. Bone Marrow Transplant 2004;33:703-7.
13. Zohar S, Chevret S. Phase I (or phase II) dose-ranging clinical trials: proposal of a two-stage Bayesian design. J Biopharm Stat 2003;13:87-101.
14. Levy V, Zohar S, Porcher R, Chevret S. Alternate designs for conduct and analysis of phase I cancer trials. Blood 2001; 98:1275-6.
15. Dreger P, Kloss M, Petersen B, Haferlach T, Loffler H, Loeffler M, et al. Autologous progenitor cell transplantation: prior exposure to stem cell-toxic drugs determines yield and engraftment of peripheral blood progenitor cell but not of bone marrow grafts. Blood 1995; 86:3970-8.
16. Kotasek D, Shepherd KM, Sage RE, Dale BM, Norman JE, Charles P, et al. Factors affecting blood stem cell collections following high-dose cyclophosphamide mobilization in lymphoma, myeloma and solid tumors. Bone Marrow Transplant 1992;9:11-7.
17. Marit G, Thiessard F, Faberes C, Cony-Makhoul P, Boiron JM, Bernard P, et al. Factors affecting both peripheral blood progenitor cell mobilization and hematopoietic recovery following autologous blood progenitor cell transplantation in multiple myeloma patients: a monocentric study. Leukemia 1998;12: 1447-56.
18. Lefrère F, Levy V, Makke J, Audat F, Cavazzana-Calvo M, Miclea JM. Successful peripheral blood stem cell harvesting with granulocyte colony-stimulating factor alone after previous mobilization failure. Haematologica 2004;89:1532-4.
19. Bolwell B, Goormastic M, Dannley R, Andresen S, Overmoyer B, Mendez Z, et al. G-CSF post-autologous progenitor cell transplantation: a randomized study of 5, 10 and 16 μg/kg/d. Bone Marrow Transplant 1997;19:215-9.
20. Toner J, Shapiro J, Laidlaw C, Rischin, Millward M, Wolf M, et al. Low-dose versus standard-dose lenograstim prophylaxis after chemotherapy: a randomized crossover comparaison. J Clin Oncol 1998;16:3874-9.