A Dose-finding Study of Glycosylated G-CSF (Lenograstim) Combined with CHOP Therapy for Stem Cell Mobilization in Patients with Non-Hodgkin’s Lymphoma

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Background: Peripheral blood stem cell (PBSC) reinfusion has been widely used for hematopoietic reconstitution after high-dose chemotherapy. However, the optimal dose of granulocyte colony-stimulating factor (G-CSF) for PBSC mobilization in combination with chemotherapy for autograft remains unknown.

Methods: To find the optimal dose of glycosylated G-CSF (lenograstim) for PBSC mobilization in combination with chemotherapy for aggressive non-Hodgkin’s lymphoma (NHL), we conducted a dose-finding study on 43 newly diagnosed patients who had unfavorable prognostic factors. They received four to six courses of cyclophosphamide, doxorubicin, vincristine and prednisolone combined with lenograstim every 2 weeks (biweekly CHOP therapy). PBSC apheresis was started after the third course of biweekly CHOP therapy. Lenograstim was given daily from day 3 until the day of the last apheresis. The optimum dose of lenograstim was assessed based on mobilization efficacy and safety profiles at a daily single dose of 2, 5 and 10 μg/kg for each level.

Results: The collected number of CD34+ cells in the first apheresis products was higher in the 5 μg/kg group than in the 2 μg/kg group (median, 4.22 x 10⁶ vs 2.49 x 10⁶ CD34+ cells/kg, P = 0.051). The highest dose of 10 μg/kg (median, 2.99 x 10⁶ CD34+ cells/kg) failed to show a dose dependence in PBSC mobilization. The efficacy and safety of the 5 μg/kg dose were further confirmed in an additional 19 patients.

Conclusions: The present study suggests that the recommended dose of lenograstim for PBSC mobilization with CHOP therapy in untreated NHL is 5 μg/kg.

Key words: peripheral blood stem cell – G-CSF – lenograstim – mobilization – non-Hodgkin’s lymphoma – CHOP therapy

INTRODUCTION

Peripheral blood stem cell (PBSC) reinfusion has been widely used for hematopoietic reconstitution after high-dose chemotherapy (HDC). For the purpose of collecting a sufficient number of PBSCs, numerous studies have shown that the addition of hematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) to myelosuppressive chemotherapies enhances the mobilization of PBSCs while reducing hematologic toxicities (1–3). However, the dose of G-CSF combined with chemotherapy for PBSC mobilization is usually on the basis of one ampoule/vial per day (estimated range 3–6 μg/kg daily), which means that the design of mobilization protocols remains empirical. In the meantime, dose-
escalating studies of G-CSF for PBSC mobilization in healthy donors for allogeneic stem cell transplantation have been performed (4–6). The use of G-CSF alone for healthy donors showed a dose dependence in PBSC mobilization and also adverse effects such as bone pain, fever and elevation of serum alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and glutamic oxaloacetic transaminase (GOT). However, the optimal dose of G-CSF for PBSC mobilization in combination with chemotherapy for autograft has not yet been extensively studied.

The role of HDC with autologous hematopoietic stem cell (AHSC) reinfusion in the treatment of aggressive non-Hodgkin’s lymphoma (NHL) has been analyzed in many studies. The role of HDC for patients with relapsed NHL responding to salvage chemotherapy has already been confirmed (7); however, whether this treatment has a role as consolidation or front-line therapy in high-risk NHL is still controversial (8).

In the consolidation or up-front setting, primary induction chemotherapy is very important for purposes of both tumor reduction and PBSC mobilization. Many kinds of chemotherapy regimens have been used in conjunction with G-CSF for mobilization. Until now, high-dose use of single cytotoxic agents such as cyclophosphamide, cytosine arabinoside or etoposide has appeared to be an effective method for mobilization (9,10). Furthermore, a number of studies have revealed the efficacy of combination chemotherapy regimens for PBSC mobilization particularly in NHL (11,12). For patients with aggressive NHL, cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) therapy remains the best available combination chemotherapy (13). Therefore, HDC followed by AHSC reinfusion after CHOP therapy should be assessed as one of the suitable treatment strategies to improve the poor prognosis of aggressive NHL patients.

Here we report the results of a dose-finding study of glycosylated G-CSF (lenograstim) for PBSC mobilization in newly diagnosed patients with aggressive NHL of unfavorable risk groups who were scheduled to undergo biweekly CHOP therapy before HDC.

**PATIENTS AND METHODS**

**Patients**

Forty-three patients with aggressive NHL were enrolled in this study between July 1995 and December 1997; 24 patients were entered into a dose-escalating study of lenograstim and the remaining 19 patients were entered into a confirmatory study using the proposed dose of lenograstim.

The eligibility criteria for this study were as follows: patients aged 15–59 years, with newly diagnosed intermediate- or high-grade NHL according to the Working Formulation (14), high- or high-intermediate risk groups according to the age-adjusted international prognostic index (15), clinical stage II–IV according to the Ann Arbor staging classification (16), Eastern Cooperative Oncology Group performance status of 0–3 and adequate bone marrow and organ functions.

This study was approved by the institutional review board of each participating institution. All patients gave written informed consent.

**TREATMENT PLAN**

The treatment was made up of three parts as follows: (a) pre-mobilization phase, the first two courses of biweekly CHOP therapy to reduce tumor load and to avoid the contamination of lymphoma cells in PBSCs; (b) mobilization phase, additional courses of biweekly CHOP to collect PBSCs; and (c) transplant phase, HDC followed by PBSC reinfusion.

**PRE-MOBILIZATION PHASE**

All patients were treated with two courses of biweekly CHOP therapy with a fixed dosage of lenograstim [cyclophosphamide 750 mg/m² i.v. on day 1, doxorubicin 50 mg/m² i.v. on day 1, vincristine 1.4 mg/m² i.v. on day 1 and prednisolone 100 mg p.o. on days 1–5, every two weeks, and glycosylated G-CSF (lenograstim; Chugai Pharmaceutical, Tokyo, Japan) 2 μg/kg subcutaneously (s.c.) daily from day 3 to day 13 or to the day of absolute neutrophil count (ANC) >5000/μl, whichever came first].

**MOBILIZATION PHASE**

All patients were scheduled to receive an additional two to four courses of biweekly CHOP therapy to collect PBSCs. Lenograstim was administered subcutaneously daily from day 3 until the day of the last apheresis. Twenty-four patients enrolled in the dose-escalating study were sequentially allocated to one of the three lenograstim dosage groups (2, 5 or 10 μg/kg body weight). The dose of lenograstim was started from the lowest dose, 2 μg/kg, to higher doses, 5 and 10 μg/kg, enrolling eight patients in each dosage group after confirming the safety at each dose level. Adverse drug reactions (ADRs) of lenograstim were graded according to the toxicity criteria of the Japan Clinical Oncology Group (17), which is an expanded version of the National Cancer Institute Common Toxicity Criteria version 1.0. Apheresis was performed once or twice when the leukocyte count increased to >10 000/μl after leukocyte nadir as described previously (18). The minimum required number of PBSC for autologous transplant was defined in this study as >1×10⁶ CD34+ cells/kg.

**TRANSPLANT PHASE**

Patients underwent HDC consisting of carboplatin 200 mg/m², etoposide 250 mg/m², cyclophosphamide 1200 mg/m² and dexamethazone 25 mg/m² i.v. daily from day −7 to −3, followed by PBSC reinfusion on day 0. After PBSC reinfusion, lenograstim was administered intravenously at a fixed dosage of 5 μg/kg from day 1 until the day when ANC recovered to >5000/μl.
IN VITRO ASSAYS

CD34+ cells and colony-forming unit of granulocyte macrophage (CFU-GM) were measured three times per week for peripheral blood samples and for all the products of apheresis as described previously (18,19). These assays were conducted centrally, CD34+ cells at the laboratories of BML, Kawagoe, Japan and CFU-GM assay at the National Cancer Center Hospital, Tokyo, Japan.

In brief, flow cytometric determination of CD34+ subset was accomplished with a Cytoron Absolute (Ortho, Raritan, NJ) by two-color direct immunofluorescence that used fluorescein isothiocyanate-labeled anti-CD34 antibody (HPCA-2; Becton-Dickinson, San Jose, CA) and phycoerythrin-labeled anti-CD33 antibody (Anti-CD33-PE; Pharmingen, San Diego, CA, USA). The total percentage of CD34+ events was calculated as the sum of the CD33+ and CD33– subset percentages. To evaluate CD34+ cells/µl of peripheral blood sample, the percentage of CD34+ cells was multiplied by the mononuclear cell count.

CFU-GMs were assessed in a semi-solid 0.3% agar culture system consisting of 20% fetal calf serum (GIBCO BRL Life Technologies, Rockville, MD), 20 ng/ml of recombinant GM-CSF (Kirin Brewery, Tokyo, Japan) and 20 ng/ml of recombinant interleukin-3 (Kirin Brewery). Cultures were plated in triplicate at 2 × 10^6 cells and incubated at 37°C in a humidified environment with 5% CO₂. The number of colonies was counted after 14 days of incubation.

STATISTICAL ANALYSIS

Differences among the dose groups regarding patients’ characteristics were tested using a one-way analysis of variance for age and the chi-squared test for other parameters. Statistical comparisons were carried out between the dose groups. The non-parametric Dunnett test (20) was used for multiple comparisons regarding the mobilization of CD34+ cells and CFU-GMs in peripheral blood samples and apheresis products. The trend in days to peak level of CD34+ cell in peripheral blood was analyzed using the one-sided Jonckeere–Terpstra test.

Hematological recovery of ANC and platelet count after PBSC reinfusion were calculated using the Kaplan–Meier method.

RESULTS

STUDY COMPLIANCE

All 24 patients in the dose-escalation section were evaluable for efficacy and safety in the mobilization phase and 20 out of 24 were subsequently treated with HDC followed by PBSC reinfusion. Additionally, 19 patients were enrolled in the study to confirm the efficacy of 5 µg/kg of lenograstim, the dose recommended from the results of the preceding dose-escalation study. Seventeen out of the 19 patients who were enrolled in the confirmatory study were evaluable, but two were excluded owing to the ineligible histological diagnosis, although these cases were evaluated for safety of lenograstim. Thirteen out of
the 19 patients were treated with HDC thereafter. A total of 137 aphereses were performed (median four aphereses, range 1–7 per patient).

Clinical characteristics of the patients according to each dosage group of lenograstim are listed in Table 1. The mean age was significantly older for the 10 μg/kg group and the imbalances in gender and body weight were observed in which more female and lighter patients were entered into the 5 μg/kg group.

PBSC MOBILIZATION

**PEAK VALUE OF CD34+ CELLS AND CFU-GMS IN PERIPHERAL BLOOD SAMPLES**

The dose effects of lenograstim on PBSC mobilization were assessed from the peak value of CD34+ cells and CFU-GMs (Table 2). No significant correlation was found between the lenograstim dose and the peak values of CD34+ cells and CFU-GM in peripheral blood. There was no significant correlation of age, gender, body weight and bone marrow involvement with PBSC mobilization (data not shown).

**CD34+ CELLS IN THE FIRST APERHESIS PRODUCTS**

Table 3 shows the results of the first apheresis. There were no clear dose-dependent relationships in the amount of PBSC harvested in terms of CD34+ cells, except for the borderline significance between 2 and 5 μg/kg ($P = 0.051$). Table 3 also shows the number of patients who achieved an adequate number of PBSCs for autologous transplantation by the first apheresis. The minimum amount of PBSC, $1 \times 10^6$ CD34+ cells/kg, could be obtained in all patients in the three dose groups but one in the 2 μg/kg and one in 10 μg/kg group.

**TIME COURSES OF LEUKOCYTE COUNTS, CD34+ CELLS AND CFU-GMS IN PERIPHERAL BLOOD**

The time courses of mean leukocyte counts (Fig. 1), CD34+ cells and CFU-GMs (Fig. 2) in peripheral blood are shown for 24 patients who received 5 μg/kg of lenograstim during the third course of biweekly CHOP therapy, including both the dose-escalation and confirmatory sections. The peak values for CD34+ cells and CFU-GMs were observed around the first apheresis.

![Figure 1](image-url)
G-CSF dose for PBSC mobilization in NHL

day 12 (Fig. 2). A rapid leukocyte count recovery occurred after the nadir and a high leukocyte count level continued during the peak period for CD34+ cells and CFU-GMs (Figs 1 and 2). The days to peak CD34+ values were shortened with the dose increment of G-CSF: 13.1 ± 0.4 in the 2 μg/kg, 11.1 ± 0.5 in the 5 μg/kg and 11.9 ± 0.7 in the 10 μg/kg group, respectively (mean ± SE, P = 0.023 by Jonckeere–Terpstra test). For comparison, the time course of mean CD34+ cells and CFU-GMs in peripheral blood from eight patients who received 2 μg/kg of lenograstim in the dose-escalation section and those from eight patients who received 10 μg/kg of lenograstim in the dose-escalation section are shown in Figs 3 and 4, respectively.

**EFFECT OF REPEATED CHOP THERAPY ON PBSC MOBILIZATION**

The mobilization effect of lenograstim on CD34+ cells in peripheral blood was higher in the third course of biweekly CHOP therapy compared with the later courses of biweekly CHOP therapy, as shown in Fig. 5. Accordingly, the number of CD34+ cells and CFU-GMs harvested decreased in the later courses (data not shown).

**HEMATOLOGICAL RECOVERY AFTER HDC FOLLOWED BY PBSC REINFUSION**

All 33 patients who received HDC followed by PBSC reinfusion showed rapid ANC recovery (median; 10 days >500/μl and 10 days >1000/μl after reinfusion) and platelet count recovery (median; 10 days >20 000/μl and 13 days >50 000/μl).

**ADVERSE DRUG REACTIONS (ADRS) OF LENOGRASTIM ADMINISTRATION**

**MOBILIZATION PHASE**

The safety profile of lenograstim for PBSC mobilization was similar among the three dose groups and all of the observed ADRs were already known and manageable. In a total of 24 patients in the dose-escalating section, grade 2 ADRs were observed in four patients [elevation of LDH in one, elevation of C-reactive protein (CRP) in one and fever in two] in the 2 μg/kg group, none in the 5 μg/kg group and four [elevation of ALP in two, fever in one, back pain in one] in the 10 μg/kg group. In the 19 patients in the confirmatory section, grade 4 neutropenia after apheresis and grade 4 interstitial pneumonitis were observed in one patient each. Both ADRs were reversible.

**AFTER PBSC REINFUSION**

Lenograstim was well tolerated in the transplant phase. The main observed ADRs (>grade 2) were elevation of ALP in two, LDH in seven, GPT (grade 2 in one, grade 3 in one) and fever (grade 2 in three, grade 3 in one). Grade 3 elevation of CRP together with a rash and fever during neutropenic period occurred in one patient and grade 3 elevation of GPT occurring just after the completion of lenograstim administration in one patient. Both patients recovered shortly thereafter.

**DISCUSSION**

Several randomized phase III trials suggested that consolidation or up-front HDCs followed by AHSC support might be beneficial in high-risk patients with aggressive NHL, younger...
than 60 years (21–23). However, other randomized studies of HDC after abbreviated induction therapy showed negative results (24,25). The international consensus conference concluded that the role of HDC with AHSC support as a front-line therapy for the treatment of aggressive NHL is still controversial (8).

There is now a consensus that the addition of hematopoietic growth factor to chemotherapy increases the number of circulating PBSCs in comparison with chemotherapy or growth factor alone. Our study attempted to investigate an optimal dose of lenograstim for efficient PBSC mobilization and also to standardize the harvesting methods during biweekly CHOP therapy before HDC in patients with poor-prognostic aggressive NHL.

The present study showed that 5 μg/kg of lenograstim is recommended for combined use with biweekly CHOP therapy. This recommendation is based on the following two findings: a higher dose of 10 μg/kg failed to show an increase in the number of mobilized PBSCs and a lower dose of 2 μg/kg showed that a two-week interval of CHOP therapy is too short for maximum efficiency of mobilization.

The relatively lower mobilization effect observed in the 10 μg/kg dose in the present study might be partly explained by older patients being allocated to the dose group. Weaver et al. reported that patients over 50 years old were less likely than younger patients to harvest optimal CD34+ cells (26). However, there was no correlation between age and CD34+ cell mobilization in this study (data not shown). Fairly large inter-patient variation in mobilized progenitor cells was observed in the present study. Martin-Murea et al. also reported no relationship between the dose of G-CSF and the circulating peak level of CD34+ cells or the CD34+ cells collected (27). Their study, including 120 patients with multiple myeloma with G-CSF

Figure 4. Time courses of mean CD34+ cells and CFU-GMs with their standard error (SE) values in peripheral blood from eight patients in the dose-escalation section who received 10 μg/kg of lenograstim during the third course of biweekly CHOP therapy. Day 0 is the first day of the third course of biweekly CHOP therapy.

Figure 5. Serial changes of CD34+ cells with their standard error (SE) values in peripheral blood samples from 21 patients who received 5 μg/kg of lenograstim during the third to fifth course of biweekly CHOP therapy.
dosage from 300 up to 1200 µg/m², failed to show a difference between 300 and 600–1200 µg/m² by matched-pair analysis of 30 cases each. Recently, Demirer et al. reported the results of a randomized evaluation of different doses of G-CSF (8 vs 16 µg/kg) following mobilization chemotherapy in previously treated patients with various malignancies (28). Although they found a dose–response effect on the collected cell yields, there was no difference between the two groups regarding the parameters of peritransplant morbidity. They suggested that 8 µg/kg may be used in view of cost effectiveness. Compared with a dose-dependent efficiency in G-CSF-induced PBSC mobilization in healthy donors for allograft, PBSC mobilization with chemotherapy and G-CSF for autograft is multifactorial. The lack of a clear dose–response relationship for G-CSF in this study might imply that higher doses of G-CSF are not necessary for previously untreated patients in combination with chemotherapy.

The present study showed that 5 µg/kg of lenograstim can be recommended for PBSC mobilization from the viewpoints of efficiency and safety in the dose-escalating study and in the later confirmatory section. This conclusion corresponds well with our similar dose-finding study of lenograstim for PBSC mobilization in patients with postoperative breast cancer treated with adjuvant cyclophosphamide, doxorubicin and 5-fluorouracil therapy (19).

In the present study, the timing of apheresis was decided according to the recovery of the leukocyte count to >10 000/µl during treatment after the nadir. Mobilized levels of CD34+ cells in peripheral blood show that days between days 11 and 13 are the best timing for sufficient PBSC collection by an apheresis at a dose of 5 µg/kg. The day of the CD34+ peak shifted earlier as the dose of lenograstim was increased. When the interval of CHOP therapy is shortened from the standard 3 weeks to 2 weeks, for the purpose of intensification of chemotherapy, it might not be long enough to keep the chemotherapy scheduling using 2 µg/kg of G-CSF for PBSC mobilization. Van Os et al. (29) and Charrier et al. (30) reported that G-CSF administered after repeated exposure to cytotoxic agents seemed to damage the hematopoietic stem cell. Martinez et al. showed that the administration of G-CSF for 5 days in a normal donor may reduce the number of progenitor cells in bone marrow by increasing their mobilization into peripheral blood (31). The most likely mechanism for G-CSF-induced damage to hematopoietic stem cells after multiple application of cytotoxic agents is the increased proliferation of stem cells in response to G-CSF at the expense of self-renewal. Some progenitor cells, which are not harvested in the apheresis product, appear to home in on bone marrow and others are destined to be matured in peripheral blood. When the next course of chemotherapy is initiated prior to homing of the remainder of mobilized PBSCs after harvest, the progenitor cells that are still circulating in peripheral blood could be damaged by chemotherapeutic agents. The present study also showed that the peripheral blood CD34+ levels around day 14 on which the next CHOP course was to be administered were still high and a clear tendency for decreased mobilization effects of lenograstim was observed as the CHOP cycle proceeded.

Almost all patients actually achieved sufficient PBSCs (>1 × 10^6 CD34+ cells/kg) collection with one or two aphereses. It has been reported that the threshold number of PBSCs needed for successful durable engraftment varies widely from a minimum of 1 × 10^6 to 5 × 10^6 CD34+ cell/kg (32, 33). Mitterer et al. showed a good correlation between the number of CD34+ cells in peripheral blood and in the apheresis products (34). They indicated that more than 40 CD34+ cells/µl in peripheral blood was highly predictive of sufficient PBSC collection of >2.5 × 10^6 CD34+ cells/kg with a single apheresis. The ratio of patients in whom >40 CD34+ cells/µl were mobilized in peripheral blood in this study was 63% (5/8), 86% (6/7) and 100% (7/7) with lenograstim dosages of 2, 5 and 10 µg/kg, respectively.

Thirty-three patients went on to the HDC followed by PBSC reinfusion. They all received the reinfusion of sufficient amounts of PBSCs and received lenograstim from the day after PBSC. Rapid hematological recovery in terms of numbers of neutrophils and platelets was observed in all patients.

In conclusion, 5 µg/kg/day of lenograstim is the recommended dose for PBSC mobilization following CHOP therapy in untreated patients with NHL.

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