

# Stable Hematopoietic Recovery after Peripheral Blood Stem Cell Transplantation in Patients Receiving High-dose Chemotherapy for Advanced Germ Cell Tumors

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**Abstract.** *Aim:* The objective of this study was to evaluate the utility of CD34-positive peripheral blood stem cell transplantation (PBSCT) in the hematopoietic recovery, in patients receiving high-dose chemotherapy (HDCT), for advanced germ cell tumor (GCT). *Materials and Methods:* This study included 41 patients with advanced GCT, who were treated with HTCT combined with PBSCT. PBSCs were harvested after conditioning chemotherapy followed by the administration of granulocyte colony-stimulating factor. A retrospective analysis of a total of 86 PBSCTs was carried out focusing on the effects of several factors, including age (<35 years versus ≥35 years), CD34-positive cell dose (<5.0x10<sup>6</sup>/kg versus ≥5.0x10<sup>6</sup>/kg), indication of HDCT (first-line versus salvage) and previous history of HDCT before PBSCT (with versus without), on hematopoietic recovery after PBSCT. *Results:* The median number of CD34-positive PBSCs collected during a single apheresis and the median cumulative number of CD34-positive PBSCs from each patient were 8.3x10<sup>6</sup>/kg and 23.2x10<sup>6</sup>/kg, respectively. The median number of PBSCT performed in each patient was two and the median number of CD34-positive cells transplanted during a single course was 5.7x10<sup>6</sup>/kg. The median recovery times to white blood cells (WBC) greater than 500/ $\mu$ l, 1000/ $\mu$ l and 2000/ $\mu$ l were 8, 9 and 10 days, respectively, following PBSCT, while that to neutrophils greater than 500/ $\mu$ l and that to platelets greater than 50000/ $\mu$ l were 9 and 13 days, respectively, following PBSCT. Only the recovery time to platelet count greater than 50000/ $\mu$ l was significantly affected by age; however, there were no significant differences in the recovery

of WBC, neutrophils and platelets in relation to several parameters examined. *Conclusion:* These findings suggest that CD34-positive PBSCT may facilitate stable hematopoietic recovery after HDCT in patients with advanced GCT, and that HDCT, if combined with PBSCT, could be performed with comparative safety in such patients, irrespective of their individual characteristics.

Approximately 70-80% of patients with advanced germ cell tumors (GCTs) achieve a durable complete response to cisplatin-based combination chemotherapy with surgical resection of residual masses. However, the prognosis of the remaining 20-30% of patients with refractory or relapsed diseases after first line chemotherapy remains poor, despite the introduction of several kinds of salvage chemotherapy (1). Recently, high-dose chemotherapy (HDCT), combined with autologous peripheral blood stem cell transplantation (PBSCT) has been increasingly used for several kinds of malignant disease, including advanced GCT (2). Although the prognostic benefit of HDCT in patients with advanced GCT has not been clearly demonstrated in randomized trials, several investigators reported the favorable outcomes of HDCT as first-line, as well as salvage regimens, compared with those of conventional-dose chemotherapy (3-5).

Autologous stem-cell rescue is necessary for patients who receive HDCT, in order to promote hematopoietic engraftment, shorten the period of neutropenia and thrombocytopenia, and decrease the risk of infection and bleeding (2). Stem cells collected from peripheral blood by leukapheresis after the administration of HDCT have largely replaced bone marrow as the source of stem cells, because it is a less invasive procedure and allows a more rapid hematological recovery (6). Considering these findings, administration of an HDCT regimen would be dependent on effective hematopoietic support, particularly PBSCT. To date, factors regulating hematopoietic recovery after HDCT followed by PBSCT have been investigated in several kinds of malignant disease (2, 7-11); however, there has been no study analyzing such factors in patients with

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**Table I.** Characteristics of 41 patients with advanced germ cell tumors.

Median age (years, range)	32 (17-62)
Site of primary tumor (%)	
Testis	34 (82.9)
Retroperitoneum	4 (9.8)
Mediastinum	2 (4.9)
Intrapерitoneum	1 (2.4)
IGCCCG classification (%)	
Good	11 (26.8)
Intermediate	13 (31.7)
Poor	17 (41.5)
Indication of HDCT (%)	
First-line	30 (73.2)
Salvage	11 (26.8)
Regimen of HDCT (%)	
ICE	33 (80.5)
T-ICE	8 (19.5)
Median cycles of HDCT (range)	2 (1-6)

IGCCCG: International Germ Cell Cancer Collaborative Group; HDCT: high-dose chemotherapy; ICE: ifosfamide, carboplatin, etoposide; T-ICE: ICE plus paclitaxel.

advanced GCT. In this study, therefore, we retrospectively reviewed the clinical data from 41 patients with advanced GCT, who were treated with HDCT combined with PBSCT, in order to characterize the features of hematopoietic recovery following PBSCT in these patients.

## Materials and Methods

This study included a total of 41 male patients who were diagnosed as having advanced GCTs, including 7 cases of extragonadal origin, between November 1994 and December 2005, and were subsequently treated with HDCT combined with PBSCT. The characteristics of these 41 patients are summarized in Table I.

In this series, after conditioned cisplatin-based combination chemotherapy, daily administration of subcutaneous recombinant human granulocyte colony-stimulating factor (rhG-CSF) at a dosage of 5 µg/kg/day was started on the day the leukocyte count had decreased to <1000/µl and continued until completion of apheresis. As a rule, leukapheresis was performed over 2 consecutive days with the Cobe Spectra continuous flow cell separator (Cobe Laboratories, Lakewood, CO, USA). The leukapheresis product was centrifuged, and platelet-rich plasma was reinfused into the patient. The remaining peripheral mononuclear cells containing PBSCs were mixed with Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, Grand Island, NY, USA) containing 5% dimethyl sulfoxide (Kyokuto Pharmaceutical, Tokyo, Japan) and 4% human serum albumin. The mixture was transferred into freezer bags and frozen to -85°C with a cryopreservation device. A small sample removed before cryopreservation was used for a CD34-positive cell assay by flow cytometry (Becton Dickinson, Sunnyvale, CA, USA). On the day of transplantation, the bags were thawed in a water bath at 37°C.

As HDCT regimens, ICE (ifosfamide, 7.5 g/m<sup>2</sup>; carboplatin, 1250 mg/m<sup>2</sup>; etoposide, 1500 mg/m<sup>2</sup>) or T-ICE (ICE plus paclitaxel, 175 mg/m<sup>2</sup>) therapy was administered in 33 or 8 patients, respectively.

**Table II** Results of peripheral blood stem cell harvest.

No. of patients following regimen of conditioning chemotherapy (%)	
BEP	35 (85.4)
VIP	5 (12.2)
VAB-6	1 (2.4)
Total no. of apheresis sessions	111
Median no. of apheresis sessions per patient (range)	2 (1-8)
Median no. of collected CD34-positive cells at a single apheresis session x10 <sup>6</sup> /kg (range)	8.3 (0.2-114.2)
Median cumulative no. of collected CD34-positive cells per patient x10 <sup>6</sup> /kg (range)	23.2 (5.4-196.2)

BEP: bleomycin, etoposide, cisplatin; VIP: etoposide, ifosfamide, cisplatin; VAB-6: cisplatin, vinblastine, cyclophosphamide, actinomycin, bleomycin.

**Table III.** Results of PBSCT.

Total no. of transplantations	86
Median no. of transplantations per patient (range)	2 (1-6)
Median no. of CD34-positive cells transplanted at a single course x10 <sup>6</sup> /kg (range)	5.7 (1.5-18.5)
Median time to recovery (days, range)	
Elevation of WBC from nadir	7 (4-10)
WBC >500/µl	8 (5-12)
WBC >1000/µl	9 (6-13)
WBC >2000/µl	10 (8-14)
Neutrophils > 500/µl	9 (7-13)
Platelets > 50000/µl	13 (4-28)

PBSCT: peripheral blood stem cell transplantation.

At 72 h after the last administration of chemotherapy, the PBSCs were intravenously reinfused. The day of PBSCT was set as day 0, all patients received rhG-CSF at a dosage of 5 µg/kg/day, starting on day 1 and continuing until the leucocyte count was greater than 1500/µl. Red blood cell and platelet transfusions were occasionally performed to maintain hemoglobin levels greater than 8.0 g/dl and platelet levels greater than 20000/µl, respectively.

The log-rank test was used to compare hematopoietic recovery following PBSCT according to several factors, including age (<35 years versus ≥35 years), CD34-positive cell dose (<5.0x10<sup>6</sup>/kg versus ≥5.0x10<sup>6</sup>/kg), indication of HDCT (first-line versus salvage) and previous history of HDCT, before apheresis (with versus without). Statistical calculations were performed using Statview 5.0 software (Abacus Concepts, Inc., Berkely, CA, USA), and *p* values <0.05 were considered significant.

Table IV. Association between several factors and hematopoietic recovery after PBSCT.

Variable	Median time to recovery (days, range)											
	Elevation of WBC from nadir	p-value	WBC >500/ $\mu$ l	p-value	WBC >1000/ $\mu$ l	p-value	WBC >2000/ $\mu$ l	p-value	Neutrophils >500/ $\mu$ l	p-value	Platelets >50000/ $\mu$ l	p-value
Age (years)		0.55		0.22		0.66		0.098		0.079		0.006
Younger than 35	7 (4-10)		8 (5-12)		9 (8-13)		10 (8-14)		9 (8-15)		13 (7-17)	
35 or older	7 (5-9)		8 (5-10)		9 (6-12)		10 (8-11)		9 (7-10)		14 (4-28)	
CD34-positive cell dose		0.16		0.28		0.061		0.26		0.11		0.59
Less than $5.0 \times 10^6/\text{kg}$	7 (5-10)		9 (6-12)		9 (8-12)		10 (8-14)		9 (8-13)		13 (9-17)	
$5.0 \times 10^6/\text{kg}$ or greater	7 (4-10)		8 (5-11)		9 (6-13)		9 (8-14)		9 (7-13)		13 (4-28)	
Indication of HDCT		0.13		0.20		0.45		0.48		0.36		0.39
First-line	7 (4-10)		8 (5-12)		9 (8-13)		9 (8-14)		9 (8-13)		13 (7-28)	
Salvage	8 (5-10)		9 (5-10)		9 (6-11)		10 (8-12)		9 (7-10)		13 (4-17)	
Previous history of HDCT before PBSCT		0.94		0.34		0.091		0.79		0.96		0.78
With	7 (5-10)		8 (6-11)		9 (7-13)		10 (8-14)		9 (8-13)		13 (7-28)	
Without	7 (4-10)		9 (5-12)		9 (6-12)		10 (8-14)		9 (7-12)		13 (4-24)	

PBSCT: peripheral blood stem cell transplantation; WBC: white blood cell; HDCT: high-dose chemotherapy.

## Results

The outcomes of PBSC harvest are presented in Table II. In this series, a total of 111 leukapheresis sessions were performed in 41 patients. The median number of CD34-positive PBSCs collected during a single apheresis session and the median cumulative number of CD34-positive PBSCs from each patient was  $8.3 \times 10^6/\text{kg}$  and  $23.2 \times 10^6/\text{kg}$ , respectively.

Table III summarizes the features of a hematopoietic recovery following 86 PBSCTs performed in 41 patients. The association between several factors and hematopoietic recovery after PBSCT was then investigated. As shown in Table IV, recovery time to a platelet number greater than  $50000/\mu\text{l}$  was significantly affected by age; that is, significantly earlier recovery of platelet count was observed in patients younger than 35 years. However, there were no significant differences in the recovery of WBC, neutrophils and platelets, in relation to the other parameters examined.

## Discussion

HDCT is currently regarded as one of the useful therapeutic options for patients with advanced GCT (3-5). Furthermore, combining PBSCT with HDCT has been shown to produce rapid hematopoietic engraftment, reducing the duration of neutropenia and thrombocytopenia, and decreasing the risk of life-threatening infection and bleeding (2). However, the significance of PBSCT in patients with advanced GCT has not been well investigated; accordingly, in this study, we retrospectively reviewed our experience of 86 PBSCTs

performed in 41 patients with advanced GCTs, who were treated with HDCT.

The number of CD34-positive stem cells required for prompt hematopoietic recovery remains controversial. Although approximately  $5 \times 10^6/\text{kg}$  CD34-positive cells has been regarded as the standard dose for PBSC rescue following HDCT (2), some recent studies suggested that a lower dose of CD34-positive stem cells could achieve a similar hematopoietic recovery (12). In this series, the median cumulative number of CD34-positive PBSCs in each patient was  $23.2 \times 10^6/\text{kg}$ , ranging from  $5.4$  to  $196.2 \times 10^6/\text{kg}$ . These findings showed that a target CD34-positive cell dose of approximately  $5.0 \times 10^6/\text{kg}$  is easily achievable in the majority of patients with advanced GCT. Furthermore, patterns of hematopoietic recovery after PBSCT observed in this series were similar to previous studies (2, 7, 8, 10), and there were no late engraftment failures or toxic deaths.

Although factors that affect hematopoietic recovery following HDCT and PBSCT in advanced GCT have not been well characterized, several studies have investigated such factors in various kinds of other malignant diseases (2, 7-11). For example, a close association between the administered CD34-positive cell dose and the rate of hematopoietic engraftment after HDCT for hematological malignancies has been reported (7, 8). It has also been suggested that there are factors other than CD34-positive cell dose involved in hematopoietic recovery after HDCT, such as the gender, kind of malignant disease (*i.e.*, hematological *versus* non-hematological) and duration of previous chemotherapy (9-11). In this series, only recovery time to platelet count greater than  $50000/\mu\text{l}$  was significantly affected

by age, while there were no significant differences in the recovery of WBC, neutrophils and platelets in relation to the parameters examined. These findings suggest that the majority of patients with advanced GCT may achieve stable hematopoietic recovery after HDCT, by combining CD34-positive PBSC rescue. These stable features of hematopoietic recovery in GCT patients may be due to the younger patient age, compared with that in other malignant diseases.

In conclusion, despite the small number of patients analyzed in this study, the present outcomes suggest that CD34-positive PBSCT may facilitate the prompt and stable hematopoietic recovery following HDCT in patients with advanced GCT, and would allow HDCT to be performed with comparative safety in such patients, irrespective of their individual characteristics.

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