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Original Article

Transplantation of Peripheral Blood Stem Cells Mobilized by Intensified Consolidation and Granulocyte Colony-Stimulating Factor in Acute Leukemia

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The purpose of this study was to evaluate the feasibility and efficacy of autologous transplantation of peripheral blood stem cells (PBSC) mobilized with high-dose consolidation chemotherapy and granulocyte colony-stimulating factor in patients with acute myelogenous leukemia (AML). Twenty patients received myeloablative chemotherapy or chemo-radiotherapy including total body irradiation followed by the infusion of PBSC. PBSC were collected by large-volume leukapheresis. The mean number of mononuclear cells and CD34-positive cells infused were 7.2×10^8/kg (range, 2.2- 16.6), and 6.6×10^6/kg (range, 2.1-27.7), respectively. Engraftment failure was not seen in the enrolled patients. The median time to neutrophil ($\geq 500/\mu L$) and platelet recovery ($\geq 50{,}000/\mu L$) from the transplant was 12 days (range, 8-20) and 28 days (range, 10-600), respectively. The 2-year probability of disease-free survival (DFS) and relapse were 43% and 57% for patients with AML transplanted in first complete remission (CR1). The outcome of the patients transplanted in the advanced status was significantly worse than the patients transplanted in CR1 (P=0.04). Most relapses occurred within 1 year after transplantation. Fatal hepatic veno-occlusive disease was observed in one case. Other transplantation-related toxicities were mild. Our results demonstrated that autologous transplantation of high-dose consolidation chemotherapy-mobilized peripheral blood progenitor cells is feasible in the patients with AML in CR1. To further reduce the risk of leukemia relapse, much effort should be contributed to the field of *ex vivo* purging and post-transplant immunotherapy.

Key Words: Peripheral blood stem cell, transplantation, acute leukemia

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INTRODUCTION

The use of peripheral blood stem cells (PBSC) in autologous transplantation (APBSCT) has demonstrated advantages over the use of marrow in several hematological malignancies and solid tumors.[1,2] A faster hematopoietic reconstitution in APBSCT, resulting in a lower infectious and hemorrhagic complications than autologous bone marrow transplantation (ABMT), has been observed.[3,4] In addition, a lower frequency of neoplastic cells in peripheral blood was suggested.[5]

As in ABMT, leukemic relapse remains the most frequent cause of treatment failure after APBSCT. A large retrospective study from the European Bone Marrow Transplantation Group (EBMTG) demonstrated no significant difference in disease-free survival (DFS), relapse rate, or overall survival when comparing APBSCT to ABMT.[6,7] From molecular marker studies,[8] it seems that autologous transplantation carries the risk of re-injecting occult residual leukemic cells to the patients. The number of malignant cells in APBSCT would be considerably higher than in ABMT due to the larger cell dose administered with PBSC autografts.[9] Optimizing the cell dose infused in PBSC grafts and controlling minimal residual disease by *in vitro* purging could be valid approaches to decreasing the relapse rate in this setting. However, there are currently no data correlating the cell dose infused with the risk of relapse in APBSCT. On the other hand, based on clinical and pre-clinical considerations, it has been

estimated that conditions in which leukemia will relapse solely from leukemic cells in the graft are rare.[10,11] This implies that relapse is also associated with inadquate leukemic reduction in the host. Thus, *in vivo* purging by intensified consolidation chemotherapy before transplantation could theoretically be another way to reduce the number of relapses. Some authors have used an *in vivo* purging with high-dose cytosine arabinoside (Ara-C)-based chemotherapy to try to decrease the leukemic burden in the patient and in the infused bone marrow before the collection.[12-15] In these studies, DFS was significantly better in those patients who received intensification therapy before their transplantation. Thus, it is reasonable to develop different strategies addressed at reducing minimal residual disease in the graft as well as in the host. *In vivo* puring by intensified consolidation chemotherapy before transplantation could theoretically be another way to reduce the number of relapses. The objective of our study was to evaluate the feasibility of collecting PBSC in patients with AML after intensified consolidation chemotherapy consisting of mitoxantrone, intermediate-dose cytosine arabinoside (Ara-C), and etoposide (MEC) followed by granulocyte colony-stimulating factor. And we evaluated the transplantation outcome of this strategy with adequate follow-up.

MATERIALS AND METHODS

Patients

Between January 1994 and May 1999, 20 AML patients who did not have an available HLA-identical sibling donor were offered APBSCT after myeloablative therapy. There were 13 males and 7 females with a median age of 31 years (range, 22 – 56) (Table 1). The distribution of morphologic types of AML according to the FAB classfication

Table 1. Characteristics of Patients

Patient	Age/Sex	Diagnosis	WBC ($\times 10^3/\mu L$)	CR1 (months)	Status at harvest	Status at ASCT	CR-ASCT (months)	Harvest-ASCT (months)	Conditioning regimen
1	30/M	AML(M4)	223	8	CR1	REL1	8	3	BuCy
2	22/F	AML(M4)	6	8	CR1	REL1	9	6	TBICy
3	36/M	AML(M2)	13	6	CR1	REL1	5	5	TBICyVP-16
4	25/M	AML(M4)	88	2	CR1	CR1	3	3	TBICy
5	52/M	AML(M4)	152	6	CR1	CR1	7	5	BuCy
6	31/F	AML(M4)	4	9	CR1	CR1	4	7	TBICy
7	37/M	AML(M5)	24	8	CR1	CR1	8	7	TBICy
8	30/M	AML(M1)	278	2	CR1	CR1	8	3	TBICy
9	48/M	AML(M1)	185	2	CR1	REL1	5	3	TBICy
10	31/F	AML(M1)	25	39	CR1	CR1	7	4	TBICyVP-16
11	31/F	AML(M3)	3	8	CR1	CR1	6	2	TBICy
12	29/F	AML(M3)	2	6	CR1	CR1	3	2	TBICy
13	56/M	AML(M4)	13	9	CR1	REL1	7	7	TBICyVP-16
14	20/F	AML(M5)	45	9	CR1	CR1	3	8	TBICy
15	39/M	AML(M2)	43	7	CR1	CR1	7	6	TBICy
16	24/M	AML(M1)	289	6	CR1	CR1	2	5	TBICyVP-16
17	52/M	AML(M5)	106	7	CR1	CR1	3	5	TBICy
18	19/M	AML(M2)	62	8	CR1	CR1	6	7	TBICy
19	47/M	AML(M4)	57	7	CR1	REL1	2	4	TBICy
20	32/F	AML(M2)	47	7	CR1	CR1	7	4	TBICY

AML, acute myelogenous leukemia; CR, complete remission; REL, relapse; ASCT, autologous stem cell transplantation; Bu, busulfan; Cy, cyclophosphamide; TBI, total body irradiation

was as follows: Four patients were M1, four M2, two M3, seven M4, and three M5. Informed consent was obtained from all the patients. Induction chemotherapy for all but AML/M3 patients consisted of idarubicin 12 mg/m^2 intravenously for 3 days and Ara-C 100 mg/m^2 by continuous infusion for 7 days. For AML/M3 patients, *all-trans* retinoic acid was administered for differentiation therapy.

Consolidation chemotherapy and collection of PBSC

One or two courses of high-dose consolidation chemotherapy consisting of Ara-C 1,000 mg/m^2 intravenously every 12 hours for 4 days, etoposide 100mg/m^2 intravenously for 3 days combined with mitoxantrone 10 mg/m^2 intravenously for 2 days, was begun after achieving complete remission. Human recombinant granulocyte colony-stimulating factor (Filgrastim; Kirin, Gunma, Japan) was administered at 250 μg/m^2 subcutaneously beginning one day following completion of consolidation therapy and continued until the completion of leukapheresis for harveting PBSC. Collection of PBSC was begun after WBC count of 3,000/μL was obtained during the recovery phase from high-dose consolidation chemotherapy. Large-volume leukapheresis was performed daily with a Fenwall CS3000 Plus cell separator (Baxter Healthcare Corp., Deerfield, IL, USA) according to the protocol recommended by manufacturer until a CD34$^+$ cell count $\geq$ 2.5-5.0 $\times 10^6$/kg of recipient was obtained as previously described.[16] Blood volume processed per run was at least 15 liters at a flow rate of 80 to 100mL/minute. A median of three aphereses per patient were performed. The freezing solution consisted of 20% dimethylsulfoxide (DMSO, Cryoserv, IL, USA), 20% autologous plasma, and 60% RPMI 1640 medium without phenol red (GIBCO, Grand Island, MN, USA). The leukapheresis product was mixed with an equal volume of the freezing solution, and immediately transferred to a freeze bag (Kapton-Teflon DF-700, Gambro, Schoellkrippen, Germany). Cryopreservation was performed in a controlled-rate freezer (BV-24/TRA-14, Cryoson GmbH, Schoellkrippen, Germany) at -1℃/min to -60℃/min, and then -5℃/min to -130℃. The frozen bags were stored in gas phase liquid nitrogen at -130℃ until use. Cell counts were preformed using a Coulter counter (Coulter Electronics, Hialeah, FL, USA). Samples were stained with fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated CD34 antibody (HPCA-2; Becton Dickinson, San Jose, CA, USA) and incubated for 20 minutes on the ice. After two washes with PBS containing 0.5% BSA and 0.2% sodium azid (w/v), red cells were lysed using ammonium chloride. FITC or PE isotype antibodies were used as controls. Analyses were carried out on a FACScan (Becton Dickinson). To determine the concentration of CD34$^+$ cells/mL of peripheral blood, the proportion of CD34$^+$ cells were multiplied by the WBC count.

Pretransplant regimens and APBSCT

Fouteen patients (11 AML in CR1, and 3 relapsed AML) received fractionated total body irradiation (TBI) delivered in eight fractions of 165 cGy from day -8 to -5 (total dose 13.2 Gy, lung dose limited to 9 Gy) and cyclophosphamide (CY) (60 mg/kg intravenously once daily on days -3 and -2). Four patients (2 AML in CR1, 2 relapsed AML) received VP-16 500 mg/m^2/day intravenously for 2 days in addition to TBI and CY. The pre-transplant conditioning regimen for 2 patients (1 AML in CR1, 1 relapsed AML) consisted of busulfan 4 mg/kg orally on days -8 to -5, and CY 60 mg/kg intravenously on days -3 and -2. On the day of transplantation, cryopreserved stem cell products were rapidly thawed in a 37℃ waterbath and infused through a central venous catheter without washing. 5μg/kg of G-CSF was administered intravenously from day 5 until full recovery of neutrophils in first 10 patients. The remaining patients did not received G-CSF after transplantation. Complications related to the pretransplant conditioning were classified according to the criteria proposed by Bearman et al.[17]

Supportive therapy

Patients were maintained in a laminar air flow room. They received oral ciprofloxacin and fluconazole. Anti-herpes simplex virus prophy-

laxis, acyclovir, was administered intravenously at a dose of 5 μg/kg three times a day from day −8 until day 28. Febrile neutropenic patients received broad spectrum antibiotics such as imipenem or cefoperazone/sulbactam plus aminoglycoside. Amphotericin-B was given when fever persisted for more than 5 days despite appropriate antibacterial treatment. Transfusions were given to maintain the hemoglobin level above 8 g/dL and the platelet count above 20×10^9/L. Single-donor pheresed platelets were administered if a donor was available. All blood products were irradiated with 30 Gy and leukocyte-filtered before administration except stem cell harvest products.

Statistical Analysis

Survival was estimated by the Kaplan-Meier method and compared by log-rank test.

RESULTS

PBSC collection and APBSCT

None of the patients who received intensified consolidation chemotherapy for harvesting PBSC died during therapy nor were removed from the study because of toxicity. Patients underwent PBSC collection following intensified consolidation chemotherapy supplemented with G-CSF 2 to 8 months (median 3 months) after CR. A median of 7.2×10^8 MNC/kg (range, 2.2 - 16.6), and 6.6×10^6 $CD34^+$ cells/kg (range, 2.1 - 27.7) were collected in a median of three aphereses (range, 1 - 5). Six patients relapsed before APBSCT. Median interval time from CR to transplantation was 6 months (range, 2 - 9) for patients at CR, and 6 months for patients at relapsed status.

Hematopoietic resonstitution

All patients reached a complete neutrophil recovery. The median number of days needed to reach an absolute neutrophil count (ANC) greater than 500/μL and 1,500/μL were 12 (range, 8 - 20) and 14 (range, 8 - 28) after transplantation, respectively (Table 2). The administration of recombinant human G-CSF did not result in the expedited ANC recovery (data not shown). The median number of days needed to reach platelet count greater than 50,000/μL without transfusion support was 28 (range, 10 - 600). Five patients continued to be dependent on the platelet transfusion for more than 3 months, and three of these patients relapsed within 12 months after transplantation. Among the remaining 2 patients, one patient who received 5.6×10^6/kg $CD34^+$ cells, never reached platelets ≥ 50,000/μL. The patient remained platelet and red blood cell transfusion-dependent for 9 months after transplantation without evidence of recurrent leukemia. The other patient who received 8.5×10^6/kg $CD34^+$ cells, were dependent continuously on platelet transfusion up to 22 months after transplantation, and ultimately recovered platelet count above 50,000/μL without transfusion support. There was no significant correlation between platelet or neutrophil recovery and the number of $CD34^+$ cells infused (data not shown).

Table 2. Stem Cell Data and Hematologic Recovery after APBSCT

MNC $\times 10^8$/kg	7.2	(2.2-16.6)
$CD34^+ \times 10^6$/kg	6.6	(2.1-27.7)
No of Apheresis	3	(1-5)
Days to reach ANC ≥ 500/μL	12	(8-20)
≥ 1,500/μL	14	(8-28)
Days to reach platelet ≥ 50,000/μL	28	(10-600)
No. of RBC transfusion	4	(2-20)
No. of platelet transfusion	23	(4-48)
Number of febrile days	5	(0-20)
Days of systemic antibiotic treatment	22	(0-38)
Days of hospitalization	38	(32-71)

MNC, mononuclear cells; ANC, absolute neutrophil count; RBC, red blood cell; Data represent median values (range).

APBSCT-related complications

Transplantation-related toxicity is detailed in Table 3. Severe toxicity associated with DMSO administration was not observed. The busulfan-associated seizure activity was not seen. Oral mucositis and gastrointestinal symptoms such as nausea, vomiting, and diarrhea, were commonly observed. Four (20%) of the patients were found to have grade 3 or more mucositis, and all of them

Table 3. Transplant-related Toxicity

Toxicity	No. of patients	%
Febrile episode	18	90
Documented infection	4	20
Mild to moderat e(grade I/II)	4	20
Severe (grade III/IV)	0	0
Hemorrhage	4	20
Mild to moderate (grade I/II)	4	20
Severe (grade III/IV)	0	0
Nausea and vomiting	17	85
Mild to moderate (grade I/II)	12	60
Severe (grade III/IV)	5	25
Mucositis	16	80
Mild to moderate (grade I/II)	12	60
Severe (grade III/IV)	4	20
Diarrhea	10	50
Mild to moderate (grade I/II)	9	45
Severe (grade III/IV)	1	5
VOD	2	10
Mild to moderate	1	5
Severe	1	5
TRM	1	5

VOD, veno-occlusive disease; TRM, transplantation-related mortality

required parenteral nutritional support. Bladder toxicity including hemorrhagic cystitis was not observed. Febrile episodes were documented in 18 patients. Clinically or microbiologically-documented infection was demonstrated in four patients (20%); One with septicemia, two with pneumonia, and one with catheter infection. Bleeding episode due to severe thrombocytopenia was found in four patients, none of them had severe hemorrhage. Two patients receiving busulfan as a conditioning regimen developed hepatic veno-occlusive disease (VOD), one with moderate and the other with severe form. One patient transplanted in the first relapse succumbed to VOD, and multi-organ failure on day 137.

Disease-free survival (DFS) and clinical outcome after APBSCT

The actuarial probabilities of DFS is shown in Fig. 1. The 2-year probability of DFS for patients with AML transplanted in CR1 was 43%. With a median follow-up of 17 months, seven patients are alive and in CR1 with a DFS of 12.5 months (range 2-58). Among patients transplanted in CR1, eight transplanted after 6 months or more from the documentation of CR1 showed high DFS rate (62%) compared to that of the six patients (30%) transplanted within 6 months of entering the CR1, although statistical significance was not obtained probably due to the small number of patients (Fig. 1). The 2-year probability of relapse for AML patients transplanted in CR1 was 57%. Five patients transplanted in the advanced status relapsed again within 12 months of transplantation with a median leukemia-free interval of 8 months, significantly worse than the patients transplanted in their CR1 (P= 0.04, Fig. 1). A

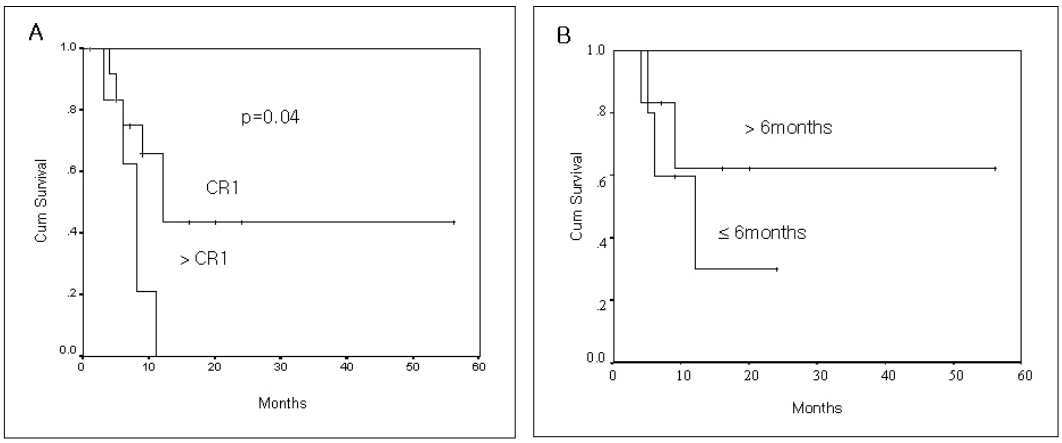

Fig. 1. Disease-free survival(DFS) of the patients with AML. A. DFS according to the disease-status at the time of transplantation: CR1 vs >CR1 B. DFS of the patients transplanted in CR1 according to the interval from CR to transplantation: ≤6 months vs >6 months

patient transplanted in the relapsed status died of severe hepatic VOD on days 137.

DISCUSSION

This study confirmed rapid neutrophil recovery and low-transplant related mortality in patients with AML after myeloablative therapy and APBSCT. Recent reports demonstrated that both the neutrophil and platelet recovery were significantly more rapid in patients with AML when autografted with PBSC compared with bone marrow; neutrophil recovery around days 12 - 13, and platelet recovery around days 25 - 42,[18,19] similar to the result of this study. In transplantation with bone marrow in patients in CR1, recovery of neutrophils occur around days 30 through 45 and recovery of platelets around days 70 through 100, with very wide ranges and extremes up to 1 years for neutrophils and up to 2 years for platelets.[20] These delayed engraftment might become more prominent in chemotherapy purged marrow autograft.[21,22] Fast hematologic recovery in APBSCT seems to be associated with administration of large number of committed hematopoietic stem cells. However, even with PBSC, delays in platelet recovery can occur and extreme values have ranged up to 2 years.[18,19] Engraftment profiles of a few cases in this study also showed similar features. Five patients (3 patients in CR1, and 2 relapsed patients) continued to be dependent on the platelet transfusion for more than 3 months. Three of them relapsed within 12 months of transplantation, suggesting a delayed platelet recovery as an important prognostic factor. Because they received more than 5 $\times 10^6$/kg $CD34^+$ cells, delayed platelet recovery in these patients may not be due to the insufficient number of stem cells infused. An inadequate bone marrow hematopoietic microenvironment could contribute to delayed hemopoietic reconstitution.[23]

Although transplant-related mortality of autologous transplantation is known to be significantly lower than that of allogeneic transplantation, most studies have reported the incidence of transplant-related mortality to be as high as 5 - 10%.[24,25] In this study there was no transplant-related mortality in early post-transplant period (within 100 days), altough one relapsed patient at the time of transplantation suffered from severe hepatic VOD. He died of multi-organ failure associated with VOD 137 days after transplantation. Fatal VOD in this patient may have been related to repeated intensive chemotherapy prior to transplantation and possibly use of busulfan as a conditioning regimen. An increased incidence of hepatic VOD has been linked to pre-transplant conditioning with busulfan in allogeneic marrow transplantation.[26,27] Selection of busulfan as a conditioning regimen for heavily pretreated patients with advanced disease needs more careful consideration about hepatic VOD. Transplantation-related toxicity, other than hepatic VOD, was not great, thus demonstrating the feasibility of APBSCT even in the patients over age 40.

Leukemic relapse has been the most frequent cause of treatment failures in APBSCT. Thus, it is reasonable to develop different strategies addressed at reducing minimal residual disease (MRD) in the graft as well as in the host. In vivo purging by intensified consolidation chemotherapy before transplantation could be an effective way to that purpose. Some authors have used an *in vivo* purging with high-dose Ara-C to try to decrease the leukemic burden in the patient and in the infused bone marrow before collection.[12-15] Stein et al.[12] reported a series of 60 AML patients in CR1, 44 of whom underwent ABMT with a probability of relapse of 33% and a DFS of 61% at 2 years. Schiller et al.[15] has shown the feasibility of performing APBSCT after high- dose Ara-C ($2\,g/m^2/12\,h \times 8$ doses) in 43 AML patients in CR1, with an actuarial DFS at 12 months 47%. Likewise, Martin et al.[15] reported the results of APBSCT in 32 patients with AML in CR1. Twenty-two of the patients received one or two intensification cycles with high-dose Ara-C ($1.5\,g/m^2/12h \times 6$ doses) before transplantation. The authors reported an actuarial probability of relapse and DFS at four years of 42.6% and 57.4%, respectively. In that study, DFS was significantly better in those patients who received intensification therapy before their transplant. Reichle et al.[14] reported similar results concerning 16 patients with AML in CR1 who underwent PBSC collections after high-dose Ara-C. In this series, the actuarial relapse-free survival at 26 months

was 57%. Recently, Gondo et al.[19] reported a five-year DFS of 70.7% in 42 AML patients who underwent APBSCT in CR1 with PBSC collected after high-dose Ara-C. According to these results, it seems reasonable to perform PBSC collections after administration of high-dose Ara-C in an attempt to reduce the MRD further. Our group performed PBSC collection after high-dose combined consolidation therapy (MEC) with Ara-C (1 g/m^2/12 h × 8 doses), mitoxantrone (12 mg/m^2/day × 3 days), and VP-16 (100 mg/m^2/day × 2 days) to reduce the tumor burden both in the patients and in the PBSC collected. This MEC regimen has been shown to be effective for post-remission therapy and salvage chemotherapy.[28] Our intensification regimen was well tolerated in comparison to the 3 g/m^2 Ara-C used by Stein et al.[12] prior to transplantation, who report 8% of significant toxicity following high-dose Ara-C. In addition, there is no significant negative effect of intensification therapy on the number of CD34$^+$ cells collected in our patients. Some authors have expressed concern about the potential risk of increasing the cytotoxic treatment before auto-transplantation in an attempt to prevent recurrence.[11] This strategy could damage the repopulating capacity of the grafts, leading to delay in the hematopoietic recovery, higher transplantation-related morbidity and mortality. However, despite the greater amount of chemotherapy administered before transplantation, we did not observe the difficulty in collecting PBSC and the significant delay in the hematologic recovery. Actually, the high number of PBSC collected in our group could be due to the synergistic effect of high-dose chemotherapy and G-CSF. G-CSF supplementation after high-dose chemotherapy was found to be correlated with enhanced stem cell mobilization.[29] Our results are comparable to those results obtained in APBSCT after high-dose intensification therapy by several groups. The 2-year probability of DFS for patients with AML transplanted in CR1 was 43%.

Patient selection and prognostic variables may have affected the outcome of APBSCT. There are few reports describing the prognostic factors for DFS and relapse after APBSCT for AML. Körbling et al.[25] have suggested that monocytic type of AML is a poor prognostic factor. A fact that half of the patients included in this study were monocytic leukemia (M4, or M5) may explain the high relapse rate after APBSCT. Age, disease status at transplantation, and the cytogenetic findings have been shown to be significantly associated with clinical outcomes.[18,19,24] As expected, the patients underwent APBSCT in CR1 was better than patients receiving APBSCT in advanced status in our study. It has been recently shown that the karyotype at diagnosis is the most important prognostic factor to be considered in AML patients grafted in CR1.[19,30] Unfortunately, we were not able to study the impact of chromosome abnormalities on the results of APBSCT. The timing of APBSCT may also be an important factor. It was shown that the transplantation outcome was significantly better in patients autografted late, when in CR.[31] This has been mainly explained by a selection bias, with patients transplanted late having a higher probability of being cured spontaneously. Another explanation about this point is that late autografts may have taken advantage of more consolidation chemotherapy administered before marrow collection, leading to a better *in vivo* purging of the graft.[23,31] This study showed similar results that AML patients autografted within 6 months of entering a CR showed somewhat poorer survival rate compared with patients autografted after 6 months or more, although not supported by statistical power due to small number of patients.

In conclusion, transplantation of autologous peripheral blood hematopoietic stem cells procured after dose-intensive consolidation chemotherapy supplemented with granulocyte colony-stimulating factor provides a reliable source of hematopoietic support for patients receiving dose-intensive myeloablative chemoradiotherapy. The stem cell product led to rapid hematopoietic recovery with a low transplantation-related morbidity. However, APBSCT was still associated with high relapse rate. To further reduce the risk of leukemia relapse, much effort should be contributed to the field of *ex vivo* purging, post-transplant immunotherapy including dendritic cell-based leukemia vaccination.

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