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Improved Survival of Children with Advanced Tumors by Myeloablative Chemotherapy and Autologous Peripheral Blood Stem Cell Transplantation in Complete Remission

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Sato, A., Imaizumi, M., Saisho, T., Saito, T., Yoshinari, M., Cui, Y., Suzuki, H., Koizumi, Y., Ito, T., Takai, Y., Hayashi, Y., Tamura, M. and Iinuma, K. *Improved Survival of Children with Advanced Tumors by Myeloablative Chemotherapy and Autologous Peripheral Blood Stem Cell Transplantation in Complete Remission.* Tohoku J. Exp. Med., 1998, **186** (4) 255-265 —— Five children with neuroblastoma (NB) stage IV and five children with rhabdomyosarcoma (RMS) stage III were treated with myeloablative chemotherapy and autologous peripheral blood stem cell transplantation (MCT/PBSCT) in the state of complete remission (CR) achieved by conventional therapy. PBSCs were collected in CR status using a cell separator with blood access through a double-lumen central venous catheter. PBSCs with $1.9 \pm 0.8 \times 10^5$ of CFU-GM per patient weights (kg) were infused following MCT after a period of conventional therapy for 11.1 ± 2.1 or 9.7 ± 0.9 months in NB or RMS patients, respectively. Regimen-related toxicity of MCT was tolerable and peripheral white blood cell count recovered beyond $1.0 \times 10^3/\mu l$ 10-12 days after infusion of PBSCs in all patients. All of RMS patients and three of five NB patients survived for an average of 31.6 months (ranged 10.8-58.1). The survival rate of these patients was improved as compared with our historical controls, and presumably, with that of conventional chemotherapy previously reported. Despite a limited number of patients, it appears that MCT/PBSCT may be effective in improving survival by preventing relapse which may occur thereafter if treated with conventional therapy alone. Furthermore, MCT/PBSCT reduced the duration of treatment, as compared with that of conventional chemo-

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therapy. Therefore, this study may suggest the feasibility and promise of the therapy including MCT/PBSCT for children with advanced stages of NB and RMS. ———— PBSCT; children; neuroblastoma; rhabdomyosarcoma; complete remission

Peripheral blood stem cells (PBSCs) have been utilized as a valuable source of hematopoietic stem cells to reconstitute hematopoiesis after myeloablative chemotherapy (MCT) for patients with various malignancies. As compared with bone marrow transplantation (BMT), autologous peripheral blood stem cell transplantation (PBSCT) has several advantages such as: (1) no need of anesthesia during stem cell collection; (2) a less contamination of malignant cells into collected PBSCs; (3) a rapid recovery of hematopoiesis after transplantation (Takaue et al. 1989; Hartmann et al. 1997) and (4) a benefit in reducing costs and hospitalization periods (Hartmann et al. 1997).

Although the survival rate of children with advanced solid tumors has been improved by the intensification of treatment and the development of supportive care methods, the outcome of those children remains unsatisfactory yet (Cairo 1995). For chemotherapy-sensitive malignant disorders, it has been expected that further intensification of chemotherapy with stem cell rescue would improve the outcome. Although the therapeutic effect of MCT with BMT has been investigated for advanced neuroblastoma (NB) by several groups, it seems that improved survival has not been confirmed or only limited in a certain group of patients (Ohnuma et al. 1995; Ladenstein et al. 1998). Recently, instead of BMT, MCT and PBSCT (MCT/PBSCT) has prevailed widely as a part of treatment for malignancy (Gratwohl et al. 1997). However, its clinical efficacy has not been defined thoroughly in children with advanced solid tumors, partly because most reports were studying on a relatively small number of patients in a disease-progressive status, and partly because a therapeutic strategy how to incorporate MCT/PBSCT with other therapeutic modality was diverse by investigators.

In this report, we retrospectively investigated the therapeutic effect of MCT/PBSCT in children with advanced NB and rhabdomyosarcomas (RMS) who successfully obtained complete remission (CR) with conventional chemotherapy, complete resection of primary tumor, and irradiation to the tumor bed. It is suggested that MCT/PBSCT, when performed in CR state, may be not only safe and effective in improving survival rate, but also beneficial in reducing the duration of therapy in children with advanced solid tumors.

Patients and Methods

Patients and chemo/radiotherapy

Since 1993, we have designed to perform MCT/PBSCT to children with solid tumor in the advanced stage (stage III or IV) which was defined according to the grouping systems (Evans et al. 1982; Crist et al. 1995). Until May 1998, five

children with NB and five with RMS received MCT/PBSCT in CR state achieved by conventional therapy. All NB or RMS patients were classified into stage IV or stage III, respectively, according to the clinical grouping systems. Treatment prior to MCT/PBSCT was done using the protocols as described (Hayashi et al. 1994; Crist et al. 1995). CR state was defined by complete disappearance of tumors confirmed by surgical procedure, pathological evaluation or a survey with scintigram and magnetic resonance imaging scans. Profiles of patients with MCT/PBSCT were summarized in Tables 1 and 2. Of these children, five (Cases 1, 2 and 3 in Table 1, and Cases 1 and 2 in Table 2) were children with a body weight below 20 kg. As historical controls in our hospital, four with NB stage IV and two patients with RMS stage III, who achieved CR with conventional therapy alone, were compared to patients with MCT/PBSCT.

Collection of PBSCs

PBSCs collection was principally performed in CR state after a surgical resection of primary tumor and before radiotherapy (except for an intraoperative irradiation) to tumor bed. During a BM recovery phase following conventional or the dose-intensified chemotherapy (Sawaguchi et al. 1990; Tokuda et al. 1995), PBSCs were mobilized by administration of granulocyte colony-stimulating factor (G-CSF) (2 μg/kg/day, continuous infusion). PBSCs were collected when white blood cell (WBC) count and platelet count increased to the levels of $>5\times10^3/\mu$l and $>50\times10^3/\mu$l, respectively, using a Spectra cell separator (COBE, Denver, CO, USA) with blood access through a double-lumen central venous catheter as described (Yoshinari et al. 1998). The collection procedure was terminated when a double of total blood volume of patients was processed, and PBSCs were frozen and stocked in -80°C without using a program freezer as described (Makino et al. 1991) soon after the collection was completed.

MCT and PBSCT

In MCT, all the patients received melphalan, the use of which was approved by the Institutional Review Board. Nine of ten children received High-MEC regimen consisting of carboplatin (400 mg/m^2/day continuous infusion for 24 hours on days -7 to -4), etoposide (200 mg/m^2/day infusion for 3 hours on days -7 to -4) and melphalan (40 mg/m^2/dose bolus injection three times every 12 hours on days -3 to -2). In seven of nine children, pyrarubicin (40 mg/m^2/day continuous infusion for 20 hours on days -7 to -6) was added to High-MEC regimen in order to intensify cytoreduction as described (Yoshinari et al. 1998) (Tables 1 and 2). For one NB patient who exhibited a 100-fold of N-myc amplification and bone metastasis, myeloablative therapy including melphalan and total body irradiation (TBI) with renal protection was performed (Table 1). On day 0, PBSCs were infused soon after a rapid thawing at 37°C. Hydroxyzine and hydrocortisone were given to avoid dimethylsulphoxide toxicity. G-CSF was

TABLE 1. *Characteristics of neuroblastoma*

Case	Diagnosis (Stage)	Sex	Age (yr.) at Diagnosis	Primary site	N-myc amp	Ferritin (ng/ml)	NSE (ng/ml)	Shimada class.
1.	NB (IV_B)	M	1	Adrenal	×1	N.E	153	F.H
2.	NB (IV_A)	M	2	Adrenal	×100	211	540	U.H
3.	NB (IV_A)	M	3	Adrenal	×1	N.E	120	N.E
4.	NB (IV_A)	M	6	Adrenal	×27	3360	42	U.H
5.	NB (IV_A)	F	6	Retro. p	×1	128	80	U.H

NB, neuroblastoma; F, female; M, male; retro. p, retroperitoneum; classification system; F.H, favorable histology; U.H, unfavorable histology; BM, operation; xRT, radiotherapy; in-op, intra-operative; p-op, post-operative; CR, melphalan, etoposide, and carboplatin. see Materials and Methods; TBI, total body myeloablative chemotherapy and peripheral blood stem cell transplantation.

TABLE 2. *Characteristics of rhabdomyosarcoma*

Case	Diagnosis (stage)	Sex	Age (yr.) at Diagnosis	Primary site	TNM	Histologic subtype	Meta-stasis	Surgery
1.	RMS (III)	F	0.7	Femur	$T_2N_0M_0$	Emb	—	Sec
2.	RMS (III)	M	1	Para. m	$T_2N_0M_0$	Undiff	—	—
3.	RMS (III)	F	7	Retro. p	$T_2N_0M_0$	Undiff	—	Sec
4.	RMS (III)	M	8	Retro. p	$T_2N_1M_0$	Emb	—	—
5.	RMS (III)	M	10	Orbit	$T_2N_0M_0$	Emb	—	—

RMS, rhabdomyosarcoma; F, female; M, male; Para.m, parameningeal; the retro. International Union Against Cancer (UICC); Emb, embryonal type; Undiff, operative; p-op, post-operative; CR, complete remission; High-MEC, combined mos, months; EFS, event-free survival; MCT/PBSCT, myeloablative chemotherapy

administrated from day 1 in order to promote granulocytes engraftment (Suzue et al. 1994). Children were followed up without chemotherapy after MCT/PBSCT.

RESULTS

Collection of PBSCs

Leukapheresis was performed 4.1 ± 1.8 times per patient (ranged 2 to 9), and $0.5 \pm 0.6 \times 10^5$ of CFU-GM per kg were collected in a single procedure of leukapheresis. Any serious complications of leukapheresis were not observed.

patients with MCT/PBSCT

Meta-stasis	Surgery	xRT (Gy)	Status at PBSCT	Duration (mo.) of chemotherapy	Conditioning	EFS after PBSCT (mo.)
BM	Sec	10 in-op	CR	12.0	High-MEC	3.8†
B	Sec	10 in-op	CR	9.0	TBI, VP-16, THP-ADR, CDDP, L-PAM	5.0†
B, BM	Sec	30 p-op	CR	14.3	High-MEC	58.1
Mesen	Sec	10 in-op	CR	10.4	High-MEC, THP-ADR	47.3
LN	Sec	12 in-op	CR	10.0	High-MEC, THP-ADR	14.7

amp, amplification; N.E, not evaluated; NSE, neuron-specific enolase; class., bone marrow; B, bone; Mesen, mesentrium; LN, lymph node; Sec, second look complete remission; chem, chemotherapy; High-MEC, combined regimen with irradiation; mos, months; †, dead; EFS, event-free survival; MCT/PBSCT,

patients with MCT/PBSCT

xRT (Gy)	Status at PBSCT	Duration (mo.) of chemotherapy	Conditioning	EFS after PBSCT (mo.)
(10 in-op +14 p-op)	CR	10.2	High-MEC, THP-ADR	10.8
24	CR	9.2	High-MEC, THP-ADR	17.5
12 in-op	CR	10.8	High-MEC, THP-ADR	43.4
36	CM	9.7	High-MEC, THP-ADR	37.6
48	CR	8.4	High-MEC, THP-ADR	23.4

p, retroperitoneum; TNM, a TNM classification for pediatric tumors proposed by undifferentiated type; Sec, second look operation; xRT, radiotherapy; in-op, intra-regimen with melphalan, etoposide, and carboplatin. See Materials and Methods; and peripheral blood stem cell transplantation.

Regimen-related toxicity of MCT and engraftment of PBSCs

Regimen-related toxicities (RRTs) such as severe oral mucositis, diarrhea and hemorrhagic cystitis after MCT were complicated frequently, but these toxicities were tolerable and gradually relieved as WBC counts increased in peripheral blood. PBSCs with an average of $1.9 \pm 0.8 \times 10^5$ of CFU-GM per kg were infused and WBC counts reached to $1.0 \times 10^3/\mu l$ in 10 to 12 days after the PBSCs infusion in all patients.

Survival of patients with NB or RMS

In patients with NB, three of five with MCT/PBSCT are surviving as of December 1998 without evidence of disease for 40.0 months after PBSCT (range: 14.7-58.1) (Table 1). Of those, Case 1 died of cerebral hemorrhage soon after the engraftment was achieved, and Case 2 died of recurrent NB which arised from the radio-protected region nearby the left kidney five months after MCT/PBSCT. Contrary to these patients with MCT/PBSCT, only one of four patients with NB stage IV who achieved CR with conventional therapy is surviving in our hospital (Table 3). In patients with RMS, all of five with MCT/PBSCT are surviving without evidence of disease, while one of two patients with stage III RMS who achieved CR with conventional chemotherapy is surviving without the disease (Tables 2 and 4).

Duration of treatment in patients with PBSCT at CR status

Patients with MCT/PBSCT completed whole treatments within 11.1 ± 2.1 or 9.7 ± 0.9 months for NB or RMS, respectively. By contrast, treatment periods of patients with conventional chemotherapy were much longer (20-24 months) in the protocols we used for advanced NB and RMS.

Discussion

In children with advanced solid tumors, MCT/PBSCT has been applied most frequently for NB (Takaue et al. 1989, 1992; Emminger et al. 1991; Lasky et al. 1991; Fukuda et al. 1992; Klingebiel et al. 1992; Deb et al. 1993; Caro et al. 1994; Suzue et al. 1994; Gratwohl et al. 1997; Shen et al. 1997; Urban et al. 1997; Eguchi et al. 1998). In most of these studies, MCT/PBSCT was performed in stage IV patients with residual, progressive or relapsed diseases (Takaue et al. 1989; Deb et al. 1993; Caro et al. 1994; Hayashi et al. 1994), resulting in a very poor prognosis. Unexpectedly, Ladenstein et al. (1998) reported that overall response state (i.e., CR or Partial remission [PR]) did not influence the long-term survival of patients with stage IV NB who were treated with MCT/BMT following conventional chemotherapy. However, their results (26% of actual event-free survival at 5 years) may suggest that MCT/PBSCT has little advantage over conventional therapy if it is simply incorporated into conventional chemotherapy (Berthold et al. 1990; Shuster et al. 1991).

For patients with advanced NB who achieved CR, it has been reported that MCT with autologous BMT had only a limited improvement on the outcome as compared with those treated with conventional therapy (Ohnuma et al. 1995). By contrast, Fukuda et al. (1992) reported that two of three patients with stage IV NB who received MCT/PBSCT at CR state survived for more than twenty-two months after PBSCT. Furthermore, Eguchi et al. (1998) recently reported that seven stage IV NB patients (five of thirteen who were transplanted in CR and two

TABLE 3. *Characteristics of neuroblastoma patients treated with conventional chemotherapy*

Case	Diagnosis	Sex	Age (yr) at Diagnosis	Primary site	N-myc amp	Ferritin (ng/ml)	NSE (ng/ml)	Shimada class.	Surgery	Response	Outcome Periods (mo.) after Diagnosis
1.	NB (IV_A)	M	2.4	Adrenal	×18	682	800	U.H	Prim	CR	15.7†
2.	NB (IV_B)	F	2.5	Adrenal	×26	1669	310	U.H	Sec	CR	14.5†
3.	NB (IV_B)	M	3.1	Media	×1	N.E	64	N.E	Sec	CR	98.7
4.	NB (IV_B)	M	4.2	Adrenal	N.E	117	108	N.E	Sec	CR	15.0†

NB, neuroblastoma; F, female; M, male; yr, year; media, mediastinum; amp, amplification; N.E, not examined; NSE, neuron-specific enolase; class., classification system; U.H, unfavorable histology; Sec, second-look operation; Prim, Primary operation; CR, complete femission; mos, months; †, dead.

TABLE 4. *Characteristics of rhabdomyosarcoma patients treated with conventional chemotherapy*

Case	Diagnosis (stage)	Sex	Age (yr) at Diagnosis	Primary site	TNM	Histologic subtype	Surgery	Response	Outcome Periods (mo.) after Diagnosis
1.	RMS (III)	F	0.7	Gluteus	$T_2N_0M_0$	Alveolar	Sec	CR	20.1
2.	RMS (III)	M	1.3	Gluteus	$T_2N_0M_0$	Emb	Sec	CR	17.0†

RMS, rhabdomyosarcoma; F, female; M, male; yr, year; N.E, not examined; TNM, a TNM classification for pediatric tumors proposed by the International Union Against Cancer (UICC); Emb, embryonal type; Sec, second look operation; CR, complete remission; †, dead.

of seven in PR) survived for 52–84 months after transplant. On the basis of these reports, it appears that therapeutic effects of MCT/PBSCT may be encouraging and greatly dependent on the state of disease at the time when the collection of PBSCs or MCT/PBSCT is performed. Indeed, Ladenstein et al. (1998) also indicated a significant improvement in survival rate of patients with pre-MCT/BMT conditions of CR or very good PR (VGPR) without bone lesion, as compared with that of patients with conditions of VGPR with bone lesion or PR.

In order to increase the curability of advanced tumors by treatment including MCT/PBSCT, we thought that PBSC collection should be performed at CR state to minimize the contamination of tumor cells, and that MCT/PBSCT should be completed in CR state before the risk of tumor recurrence increases. Therefore, our therapeutic strategy of this study was: (1) PBSC collection is performed soon after complete resection of primary tumor following tumor size reduction by conventional chemoradiotherapy; and (2) MCT/PBSCT is completed within one year from the beginning of therapy, beyond which the risk of relapse may gradually increase.

With this strategy, the survival rate of patients with MCT/PBSCT was improved as compared with our historical controls or, presumably, with that of patients with conventional chemotherapy previously reported (Sawaguchi et al. 1990; Crist et al. 1995; Ohnuma et al. 1995) for both NB stage IV and RMS stage III. Despite a limitted number of patients, our study suggested that MCT/PBSCT might be effective in preventing relapse which would have occurred thereafter if patients continued to be treated with conventional chemotherapy alone.

One patient (Case 2 in Table 1) relapsed after MCT/PBSCT in our study. However, his tumor exhibited extremely high risk factors and was thought to have recurred from the radio-protected region from TBI. Thus, the combination of MCT with TBI may not have failed to reduce potential risk of tumor recurrence in patients with extremely high risk factors. Furthermore, for patients with stage IV NB having high risk, intensification of induction chemotherapy with PBSC back-up may be another useful mode of PBSC utility in order to increase CR rate.

As another advantage, it is of note that MCT/PBSCT reduced therapy duration as compared with that of conventional therapy. Most patients in this study completed MCT/PBSCT, discharged within one year from diagnosis, and then were followed without chemotherapy, while conventional chemotherapy protocols for NB and RMS were continued for nearly two years. Therefore, MCT/PBSCT may be able to reduce the duration of chemotherapy without worsing the outcome and, thereby, lighten the physical and mental burden of patients and their families.

The survival rate of children with RMS in stage III or of orbit origin has been reported as 73% or 95% at five years, respectively, by conventional chemoradiotherapy and operation (Crist et al. 1995). Thus, the indication of MCT/PBSCT

for RMS stage III, especially for orbit RMS, may be controversial. However, this improved survival rate of stage III RMS was achieved by the intensive chemotherapy for approximately two years (Crist et al. 1995). Therefore, regarding MCT/PBSCT indication for RMS stage III, we made much of a benefit in reducing treatment period by MCT/PBSCT.

In this study, no serious complications were observed during leukapheresis in all of the patients, a half of whom weighed less than 20 kg, indicating that PBSCs collection was a safe procedure even for young children (Takaue et al. 1989, 1992; Lasky et al. 1991; Fukuda et al. 1992; Deb et al. 1993; Caro et al. 1994; Shen et al. 1997; Urban et al. 1997). RRT of MCT is another important factor which could influence the safety of this procedure. RRT of High-MEC plus pyrarubicin regimen we used was tolerable even in young children, and we think that this regimen can be used as MCT with PBSCT in children. However, more safe and effective regimens remain to be investigated for children with high risk factors.

In conclusion, this study suggests that MCT/PBSCT may be a safe procedure with clinical benefits for children with advanced solid tumors such as NB or RMS. Furthermore, it may be an effective strategy to perform collection of PBSCs in CR state and to complete MCT/PBSCT within one year from diagnosis for improving survival of children with advanced solid tumors. Further investigation with a larger number of patients and a longer follow-up period is needed to clarify the clinical significance and late adverse-effects of MCT/PBSCT.

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