Tandem High-Dose Therapy in Rapid Sequence for Children With High-Risk Neuroblastoma

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Purpose: Advances in chemotherapy and supportive care have slowly improved survival rates for patients with high-risk neuroblastoma. The focus of many of these chemotherapeutic advances has been dose intensification. In this phase II trial involving children with advanced neuroblastoma, we used a program of induction chemotherapy followed by tandem high-dose, myeloablative treatments (high-dose therapy) with stem-cell rescue (HDT/SCR) in rapid sequence.

Patients and Methods: Patients underwent induction chemotherapy during which peripheral-blood stem and progenitor cells were collected and local control measures undertaken. Patients then received tandem courses of HDT/SCR, 4 to 6 weeks apart. Thirty-nine patients (age 1 to 12 years) were assessable, and 70 cycles of HDT/SCR were completed.

Results: Pheresis was possible in the case of all patients, despite their young ages, with an average of 7.2×10^6 CD34$^+$ cells/kg available to support each cycle. Engraftment was rapid; median time to neutrophil engraftment was 11 days. Four patients who completed the first HDT course did not complete the second, and there were three deaths due to toxicity. With a median follow-up of 22 months (from diagnosis), 26 of 39 patients remained event-free. The 3-year event-free survival rate for these patients was 58%.

Conclusion: A tandem HDT/SCR regimen for high-risk neuroblastoma is a feasible treatment strategy for children and may improve disease-free survival.

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$\mathbf{M}$ULTIAGENT CHEMOTHERAPY and combined-modality treatment strategies have improved outcome for pediatric patients with leukemias and localized solid tumors. These strategies have also improved rates of remission induction for patients with high-risk solid tumors, but the impact on long-term disease-free survival is less clear. The hypothesis that further dose escalation may cure more children with malignancies such as high-risk neuroblastoma[1] has led investigators to explore the use of myeloablative regimens for remission consolidation. Experience with autologous bone marrow transplantation (ABMT) has been variable, with early studies suggesting that ABMT may improve overall survival or lengthen time to progression.[2] Recently, the Children's Cancer Group (CCG) published the results of study 3891, which found statistically significant improvement in event-free survival (EFS) among patients randomized to consolidation with ABMT compared with those randomized to continuation chemotherapy.[3] The goal in designing ABMT regimens is to use effective drug combinations whose doses can be safely escalated, while ensuring rapid bone marrow reconstitution. Another goal has been to determine which stem-cell product and which tumor-purging strategy minimize tumor cell contamination.

Tandem AMBT was tried in children with neuroblastoma, but toxicity was a problem, especially delay in hematopoietic recovery when bone marrow was used as stem-cell support.[4] To lessen the toxicity of ABMT, many oncologists have moved to using peripheral-blood progenitor cells (PBPCs) as a stem-cell source (stem-cell rescue [SCR]). Clinical trials involving adult patients with solid tumors have demonstrated rapid hematopoietic recovery with the use of PBPCs. In adult patients, collection of enough stem or progenitor cells for support through tandem transplantation courses has been shown to be a valid approach.[5,6] PBPC support through single transplantation has also been explored in children[7,8] and has resulted in

rapid engraftment,[9] but there are no published pediatric trials of tandem transplantation using PBPCs.

We conducted a single-arm trial of PBPC-supported tandem transplantation at a limited number of institutions. We studied the feasibility of stem-cell collection in small patients, the manipulation of stem cells with CD34^{+} selection, and the toxicity and feasibility of using two sequential myeloablative regimens. After completion of induction and local control measures, patients on this study underwent tandem HDT with two important design features: separate non–cross-reactive regimens for each HDT and rapid progression from the first to the second HDT/SCR. This study demonstrates that tandem transplantation is feasible with acceptable toxicity and adequate recovery of hematopoiesis in patients supported with unprocessed PBPC. Hematopoietic recovery was similar to the overall group in the subset of patients who received CD34^{+}-selected PBPC in an effort to further minimize the burden of infused tumor.[10]

PATIENTS AND METHODS

Eligibility Criteria

Previously untreated patients with neuroblastoma who were more than 1 year old were eligible for enrollment if they had Evans stage IV disease or Evans stage III disease with *MYCN* amplification. Evans staging was used during the study; however, all four patients with stage III disease also had International Neuroblastoma Staging System stage 3 disease. Thus, the latter staging is reported.[11] Diagnosis was confirmed pathologically in each case by biopsy or resection of the primary tumor or of metastatic tumor from an accessible site. Diagnosis of neuroblastoma could also be made from a bone marrow aspirate or biopsy specimen revealing tumor cells accompanied by increased urinary catecholamine levels. Each patient's staging evaluation included computed tomography of both the chest and the primary tumor site, a ^{99}Tc bone scan, bilateral bone marrow aspirates and biopsies, and, when possible, an [^{125}I]metaiodobenzylguanidine scan. Additionally, urinary catecholamine levels were measured and *MYCN* amplification was determined by fluorescence in situ hybridization or Southern blotting. *MYCN* amplification was determined on bone marrow samples (if adequate tumor existed for analysis) or tissue obtained from the primary or metastatic site.

Protocols and consent forms were approved by each participating hospital's institutional review board, and informed consent was obtained from the parents of each child after the diagnosis was confirmed. Patients were enrolled at the Dana-Farber Cancer Institute (DFCI) and Children's Hospital in Boston, MA, Children's Hospital of Philadelphia (PA), Emory University in Atlanta, GA, and Primary Children's Hospital in Salt Lake City, UT. All consecutive eligible patients presenting to the participating institutions were offered enrollment onto the protocol between 1994 and 1998 (DFCI) or between 1995 to 1996 and 1998 (other institutions).

Treatments

Patients underwent induction chemotherapy after confirmation of their diagnosis, although entry onto the protocol could be delayed by one cycle in the case of patients with stage 3 neuroblastoma in whom

MYCN amplification was subsequently demonstrated. Samples were obtained after the second course of chemotherapy, to confirm remission of disease in the bone marrow. Determination of such remission was based both on morphologic examination (Wright-Giemsa stain) and on neuroblastoma immunocytochemistry (ICC) (performed by BIS Laboratories, Reseda, CA; referred to here as standard ICC). Patients were eligible for pheresis if ICC revealed less than 5% neuroblasts. Eligible patients underwent PBPC pheresis after recovery from neutropenia after chemotherapy cycle 3 and, if more PBPCs were needed, after recovery after cycle 4. The first pheresis was delayed one cycle if adequate marrow clearing was not achieved after cycle 2. After the fourth or fifth cycle of induction chemotherapy, each patient underwent resection of the primary mass. Determination of the existence of residual disease, both gross and microscopic, was made in conjunction with the surgeon, pathologist, and radiotherapist.

The treatment plan is summarized in Fig 1, and details of drug doses and days of administration are listed in Tables 1 and 2. Each course of chemotherapy was followed by administration of daily recombinant human granulocyte colony-stimulating factor (rhuG-CSF) (5 μg/kg during nonpheresis cycles), starting 24 hours after completion of drug administration and continuing until the absolute neutrophil count (ANC) was greater than 10,000/μL after the nadir. Chemotherapy courses were scheduled 21 days apart, but delays were allowed until the ANC exceeded 750/μL (48 hours after stopping rhuG-CSF therapy) and the platelet count was greater than 75,000/μL. In the case of patients with gross or microscopic residual disease after surgery, the presurgical extent of primary tumor underwent local irradiation (10.8 to 24 Gy) before the first HDT/SCR.

Stem-Cell Collections

Patients underwent their first PBPC collection after the third cycle of induction chemotherapy or after gross residual bone marrow disease had been cleared. Collections were started 1 to 4 days after the ANC reached 1,000/μL after the nadir. Administration of rhuG-CSF (5 to 10 μg/kg/d) was continued until pheresis was complete. Products intended for use were collected, with a goal of 4 × 10^{6} CD34^{+} cells/kg (minimum, 1 × 10^{6} cells/kg) available for each cycle of HDT/SCR. A backup product was collected, with the goal of 2 × 10^{6} CD34^{+} cells/kg (minimum, 1 × 10^{6} cells/kg) for cryopreserving separately.

Initially, PBPCs were cryopreserved without further processing. After an initial group of patients had undergone tandem transplantation without unacceptable toxicity, subsequent patients at DFCI/Boston Children's Hospital and the Children's Hospital of Philadelphia received CD34^{+}-selected PBPCs. In the case of these patients, after cryopreservation of an aliquot of unselected PBPCs, the first day's collection was held overnight and pooled with the second day's collection.[12] Then both pheresis products underwent CD34^{+} selection using the CellPro Ceprate SC device (CellPro, Bothell, WA). This device selects for and concentrates CD34^{+} cells, yielding two- to three-log depletion of CD34^{−} cells, including possible contaminating tumor cells.[13] Samples obtained from both the input and the selected populations for assessing for neuroblastoma contamination were cryopreserved. Some patients, especially in the early phase of the study, also had bone marrow harvested and cryopreserved as a stem-cell backup, although investigators were later given the option of using unselected PBPCs as a backup instead of bone marrow.

HDT and SCR

HDT/SCR conditioning regimens are summarized in Table 2. Toxicities (Cancer and Leukemia Group B toxicity criteria) for all patients
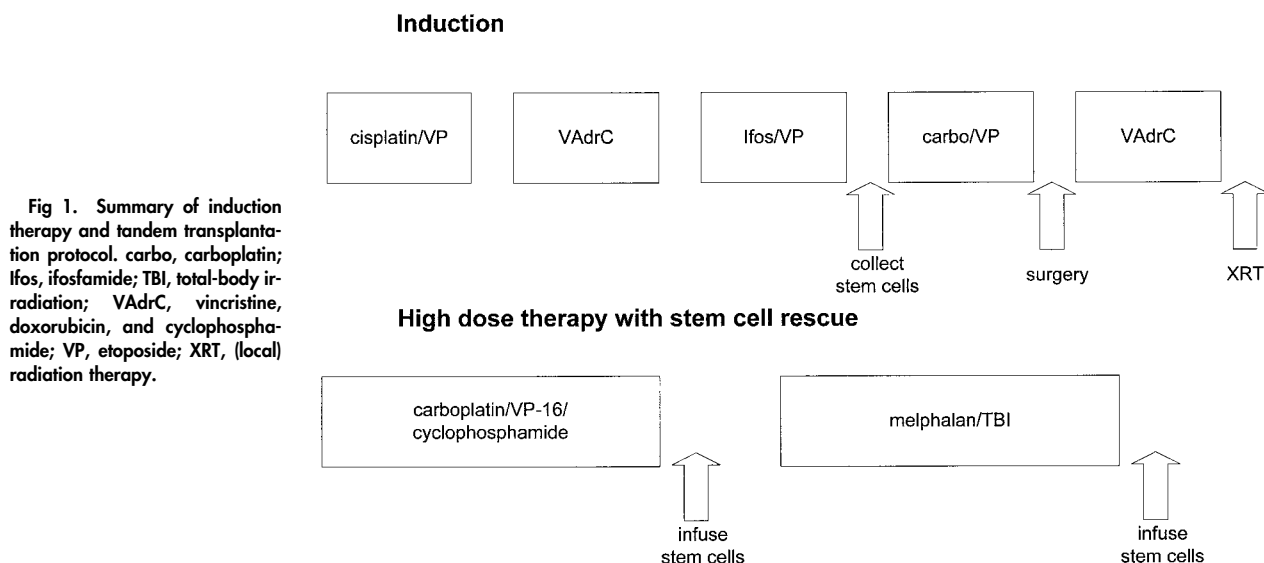
Induction

Fig 1. Summary of induction therapy and tandem transplantation protocol. carbo, carboplatin; Ifos, ifosfamide; TBI, total-body irradiation; VAdrC, vincristine, doxorubicin, and cyclophosphamide; VP, etoposide; XRT, (local) radiation therapy.

during HDT/SCR were recorded. When the ANC was greater than $500/\mu L$ for 3 consecutive days, neutrophil engraftment was considered to have occurred, with the first of the 3 days recorded as the date. The first day on which platelet transfusions were not required for the subsequent week was considered the date of platelet transfusion independence. The diagnosis of veno-occlusive disease (VOD) was made using standard clinical criteria.[14]

Patients who completed induction therapy were considered eligible for the first HDT/SCR if enough stem cells had been collected after clearing of bone marrow disease, there was no subsequent evidence of disease progression, and the patients had fully recovered from the last course of chemotherapy. Patients were evaluated for adequate renal, cardiac, pulmonary, and liver function before HDT/SCR, and there could be no evidence of active infection. There were further requirements to be met before for the second HDT/SCR was begun. Patients had to have tolerated the first cycle well without evidence of life-threatening lung or cardiac toxicity and without evidence of other, irreversible organ toxicity or severe VOD (considered present when the total bilirubin level was > 10 mg/dL). The backup stem-cell product had to be available to be considered for use in the second HDT/SCR (ie, it could not have been infused after the first HDT/SCR). Restaging of disease took place only if clinically indicated; if patients had evidence of recurrent or progressive disease, they were removed from the study. The protocol required that patients be ready for the second HDT/SCR on day 42 after the first HDT/SCR, although the actual day of stem-cell

reinfusion was occasionally later for scheduling reasons or to permit completion of antibiotic therapy. The intent was to proceed with the second HDT/SCR rapidly. The relatively short interval between transplantations was chosen to maximize treatment intensity and was predicated on the belief that any patient not ready for the second HDT/SCR because of severe organ toxicity or poor hematopoietic reconstitution within the 42-day interval would not be a good candidate for a second procedure.

Statistical Considerations

EFS was measured from the time of diagnosis to the day of relapse or progression, the day of death in remission, or the last date on which the patient in remission was known to be alive. The Kaplan-Meier method[15] for incomplete data was used to estimate the EFS curves; confidence intervals are based on normal theory, and Greenwood's formula was used to obtain SEs. Descriptive statistics were used in the analysis of platelet and neutrophil engraftment.

Immunocytochemical Detection of Neuroblastoma

Clinical samples of blood and bone marrow were analyzed at BIS Laboratories using standard ICC, with a cocktail of three antibodies specific for neuroblastoma, as previously described.[16] Patient samples were analyzed at the time of diagnosis; before chemotherapy cycle 3 to determine eligibility for pheresis; and 1 month after the second

Table 1. Induction Chemotherapy*

Cisplatin/VP	Carboplatin/VP	VAdrC	Ifos/VP
Cisplatin 40 mg/m²/d days 1-5	Carboplatin 500 mg/m²/d days 1-2	Vincristine 1.5 mg/m² day 1	Ifosfamide 2,000 mg/m²/d days 1-5
Etoposide 150 mg/m²/d days 2-4	Etoposide 150 mg/m²/d days 1-3	Doxorubicin 75 mg/m² day 1-2, 48-hour continuous infusion	Etoposide 150 mg/m²/d days 1-5
		Cyclophosphamide 1,000 mg/m²/d days 1-2	

Abbreviations: VP, etoposide; VAdrC, vincristine, doxorubicin, and cyclophosphamide; Ifos, ifosamide.
*Delivered at 21-day intervals and delayed until the ANC was greater than $750/\mu L$ and the platelet count was greater than $75,000/\mu L$.

Table 2. High-Dose Treatments

First HDT/SCR	Second HDT/SCR
Etoposide 2,400 mg/m² days −6 through −4, 72-hour continuous infusion	Melphalan 60 mg/m²/d days −5 through −3
Carboplatin 667 mg/m²/d days −6 through −4	TBI (12 Gy)
Cyclophosphamide 1,800 mg/m²/d days −3 through −2	PBPC infusion day 0
PBPC infusion day 0	

Abbreviation: TBI, total-body irradiation.

HDT/SCR. Additionally, aliquots from daily PBPC collections underwent additional immunocytochemical analysis (referred to as investigational ICC), the goal being to quantify the degree of depletion of contaminating neuroblastoma obtained by CD34 selection. For these studies, an aliquot of pheresate (10^8 cells) was $CD34^+$-selected using the Ceprate LC laboratory $CD34^+$ selection column (CellPro). The Ceprate LC uses the same monoclonal antibody for $CD34^+$ selection (12.8), as well as the same biotin-avidin affinity system, as the Ceprate SC clinical $CD34^+$-selection device but is scaled for smaller numbers of cells (maximum, 3×10^8). These pheresis samples were incubated with anti-CD34 antibody 12.8 at 20 μg/mL and then processed on the Ceprate LC system. Both pre- and post-CD34 fractions were analyzed for the presence of neuroblastoma using ICC in a slide-based assay using a cocktail of four antibodies that recognize neuroblastoma cells (181.4, ERIC-1, UJ13A, and 5.1H11)[17] and antibody-labeled cells detected in an immunoalkaline phosphatase assay (Vector Laboratories, Inc, Burlingame, CA). Cell-seeding experiments with the neuroblastoma cell line CCL-127 have shown that this investigational ICC assay has a sensitivity of 10^{-5} to 10^{-6} (data not shown). The positive control CCL-127 line and the negative control (patient specimen with nonimmune mouse serum), as well as the pre- and post–$CD34^+$-selection specimens, were independently interpreted by two investigators.

RESULTS

Thirty-nine patients (21 boys and 18 girls) were enrolled and were assessable for response (Table 3). Median age was 3 years.

Feasibility of PBPC Collection

All but one patient were eligible for pheresis after two or three cycles of chemotherapy. The ineligible patient went off study because of persistent marrow disease after cycle 3

Table 3. Patient and Disease Characteristics at the Time of Diagnosis

	No. of Patients
INSS stage and *MYCN* amplification status	
Stage 4 and amplification present	19
Stage 4 and no amplification	14
Stage 4 and unknown status	2
Stage 3 and amplification present	4
Primary site of disease	
Abdomen/adrenal glands	37
Paraspinal	1
Unknown	1
Metastasis to bone	28

NOTE. Median age of the 39 patients was 3 years (range, 1 to 12 years).
Abbreviation: INSS, International Neuroblastoma Staging System.

of chemotherapy. The ages of patients at the time of pheresis ranged from 14 months to 12 years, and PBPC collection was successfully completed in all eligible patients. Enough $CD34^+$ cells for infusion as well as backup were collected from most patients in a single cycle (3 days of pheresis). The lowest acceptable number of $CD34^+$ cells available for each HDT/SCR was 10^6/kg, and the goal of 4×10^6 was met in the majority of cases (64% of infused products contained at least 4×10^6 $CD34^+$ cells/kg). There were no significant complications during pheresis. Adequate venous access is a potential problem in smaller patients who require pheresis.[18] In all patients more than 3 years of age and most patients younger than 3 years, pheresis was accomplished with an 8-French double-lumen tunneled pheresis catheter (Medcomp, Harleysville, PA). In some of the smallest patients (those weighing < 12 kg), placement of a permanent pheresis catheter was deemed infeasible, and a single-lumen catheter or temporary pheresis catheter was placed on the opposite side of the patient's double-lumen catheter and used as a draw line, with return through the double-lumen catheter. Femoral line placement was not necessary in any patient. Targets for collection included an inlet rate of 2 mL/kg/min and a duration of 4 hours. This resulted in the processing of an average of six to eight blood volumes during each collection. Prior studies documented diminishing returns when collections exceeding this volume were attempted.[19] Collections in younger children yielded sufficient progenitor cells. Overall, a mean of 7.2×10^6 $CD34^+$ cells/kg was infused during each SCR, with similar numbers infused in patients older than 3 years (7.4×10^6/kg) and patients younger than 3 years (7.0×10^6/kg).

Feasibility and Toxicity of PBPC-Supported Tandem HDT/SCR

Thirty-seven patients underwent at least one complete HDT/SCR and were assessable for transplantation toxicity. One patient was taken off study because of failure to clear the bone marrow adequately before pheresis. A second patient withdrew from the study at parental request after five cycles of chemotherapy and local control. She subsequently underwent single transplantation at another institution and
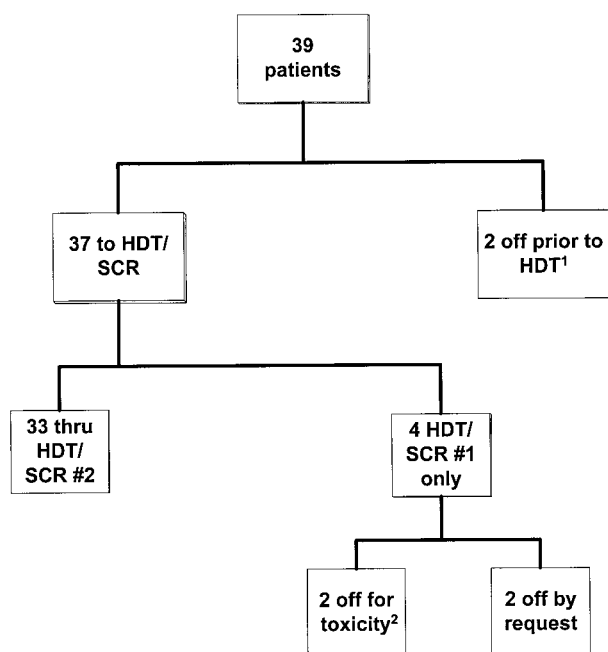
Fig 2. Patient flow through the tandem transplantation protocol. [1]One patient did not have adequate clearing of marrow disease. The second was taken off study at parental request. [2]One patient developed clinical sepsis during conditioning, resulting in multiorgan system dysfunction. The second developed viral hepatitis during HDT/SCR.

then received anti-GD2 antibody therapy. She remained in remission but was censored from the analysis at the time of withdrawal from our study. Of the 37 patients completing the first HDT, 33 (89%) also completed the second HDT/SCR. Two did not proceed after the first HDT/SCR, at parental request; and two patients experienced toxicity during the first HDT that made them ineligible for the second HDT/SCR (Fig 2).

Toxicity of tandem transplantation. All patients had expected transplant-related toxicities including mucositis and pancytopenia. One patient developed reversible congestive heart failure associated with fluid overload during the second HDT/SCR. Another patient developed a clinical sepsis syndrome during conditioning for the first HDT and did not proceed to the second HDT/SCR.

Because of the potential hepatotoxicity of tandem courses of high-dose chemotherapy followed by total-body irradiation (TBI), we monitored patients closely for development of VOD and other evidence of hepatic injury. Three patients developed VOD during HDT/SCR. All episodes occurred during the second procedure, and patients were treated supportively, with two receiving investigational defibrotide therapy.[20] One patient developed hepatomegaly and fluid retention without hyperbilirubinemia or evidence of cardiac

dysfunction during the first HDT/SCR. This did not recur during the second course. One patient developed persistently increased transaminase levels without hyperbilirubinemia. Viral cultures suggested that the hepatitis might be related to adenovirus infection, and the transaminase levels normalized without further sequelae, although the patient did not meet criteria needed to proceed to the second HDT/SCR.

Treatment-related mortality occurred in three patients. All deaths occurred after the second HDT/SCR, after engraftment of neutrophils. One patient had an episode of *Enterobacter* sepsis with fever and hypotension progressing to acute respiratory distress syndrome and multiorgan system failure. Parainfluenza was also isolated from a tracheal aspirate obtained at admission. A second patient developed significant VOD on day 14 after the second HDT/SCR and, while VOD was improving, developed fever and fatal respiratory compromise. Cytomegalovirus infection was noted at autopsy, with diffuse involvement of the lungs, liver, and adrenal glands. The third death was that of a patient with presumed viral sepsis, again after engraftment after the second HDT/SCR. Cultures obtained at the time of flexible sigmoidoscopy grew adenovirus, and despite broad spectrum antibiotic therapy (bacterial, fungal, and viral), the patient developed pneumonia and severe myocardial dysfunction.

Recovery of hematopoiesis. Hematopoietic recovery supported by PBPCs was rapid. The ANC reached $500/\mu L$ in a median of 11 to 12 days. Platelet transfusion independence after the second HDT/SCR occurred in a median of 29 days (the recommended transfusion trigger point for platelets was $20,000/\mu L$ or less). The subset of patients infused with CD34^{+}-selected PBPCs had engraftment kinetics similar to those of the overall group (Table 4). Each patient experienced engraftment between the first and second HDT. One patient, who had experienced prompt engraftment after the first HDT, had no signs of neutrophil recovery on day 28 following the second HDT. This patient received the backup stem-cell product on day +31, after which engraftment occurred on day +55 (24 days after the second infusion).

Treatment Results

Patients were eligible to proceed to the HDT/SCR portion of the protocol if they showed no progressive disease and experienced acceptable clearing of bone marrow disease. The flow of patients through the protocol stages is summarized in Fig 2. Results of Kaplan-Meier analysis of EFS among these patients are shown in Fig 3A. EFS among patients infused with unselected PBPCs compared with EFS among patients who received CD34^{+}-selected stem-cell

Table 4. Engraftment Data

	n	No. of Days to ANC > 500/mm³		No. of Days to PTI	
		Median	Range	Median	Range
First HDT/SCR					
All patients	37	11	9-28	—	—
Patients with CD34-unselected PBPCs	19	11	9-28	—	—
Patients with CD34+-selected PBPCs	18	11	10-17	—	—
Second HDT/SCR					
All patients	33	12	9-55	29	10 -103
Patients with CD34-unselected PBPCs	17	12	9-55	31	12 -103
Patients with CD34+-selected PBPCs	16	11	10-41	28	10 -100

Abbreviation: PTI, platelet transfusion independence.

products is shown in Fig 3B. With a median follow-up of 22 months (from diagnosis), 26 of 39 neuroblastoma patients remained event-free. The 3-year point estimate of EFS for these patients is 58% (90% confidence interval, 40% to 72%).

Tumor Cell Contamination of PBPC Products

Assessment of blood, bone marrow, and PBPC collections for tumor was possible using standard ICC. Of 29

Fig 3. (A) Kaplan-Meier analysis of EFS among all 39 patients with high-risk neuroblastoma. (B) Kaplan-Meier analysis of EFS among patients who received unselected PBPCs or CD34+-selected PBPCs.

pooled PBPC collections analyzed, only one PBPC product was found to be minimally positive (one neuroblastoma cell per 10^5 cells counted) by standard ICC. Because of the large number of cells required for this analysis (10^7, or a substantial fraction of a graft), only five samples obtained after CD34+ selection were assayed for tumor cell contamination, and none of these were positive. Pre- and postselection samples from all patients were available for quantitative radiotherapy–polymerase chain reaction analysis, and this analysis is under way.

For determination of the degree of neuroblastoma purging in clinical specimens, aliquots of PBPC products were analyzed using a separate, investigational immunocytochemical assay. These aliquots were then CD34+-selected using the laboratory-scale Ceprate LC column (see Patients and Methods). The CD34+-selected cells were next analyzed for remaining neuroblastoma contamination using investigational ICC. As summarized in Table 5, nine of 30 pheresis specimens tested were found to have detectable tumor by this assay, and CD34+ selection provided more than 0.4 to more than 2.6 logs of tumor cell purging. No specimen free of detectable tumor before CD34+ selection subsequently became positive after CD34+ selection, and all specimens with detectable tumor decreased in tumor burden after CD34+ selection (six of nine had no detectable tumor after CD34+ selection). These data support the use of CD34+ selection to reduce the amount of contaminating neuroblastoma in pheresis products.

DISCUSSION

The role of dose intensification using stem-cell support has been explored extensively in the treatment of metastatic solid tumors in adults. In this report, we have described the first experience with PBPC-supported tandem transplantation in children. We found this treatment to be feasible, with enough PBPCs collected during induction chemotherapy for support through tandem courses of HDT/SCR. The transplantation regimens were generally well tolerated, with
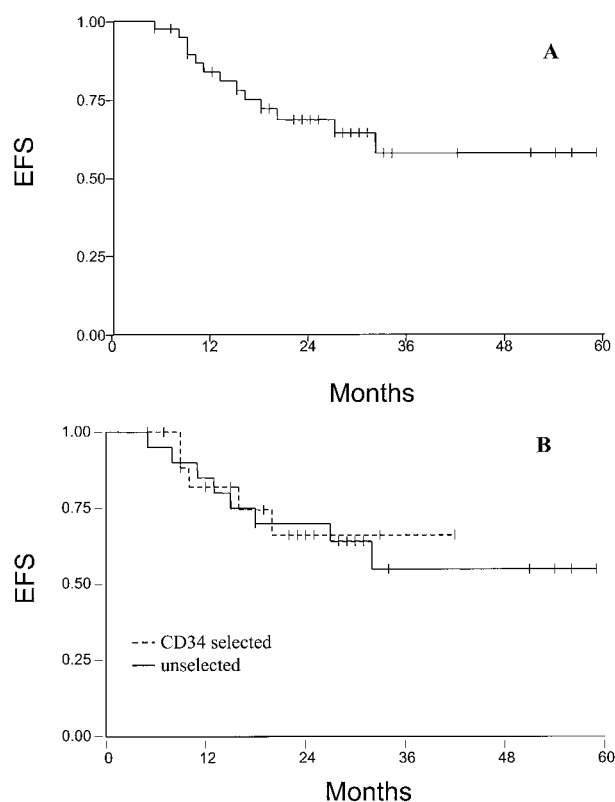
Table 5. Detection of Neuroblastoma by ICC

| | | Standard ICC | Investigational ICC | | | | |
| | | NB Cells Before Selection* | NB Cells Before Selection† | | NB Cells After Selection‡ | | |
ID No.	Induction Therapy Cycle/Day of Pheresis	No. per 10^5 PBPCs Counted	Total No.	No. per 10^6 PBPCs Counted	Total No.	No. per 10^6 PBPCs Counted	Log Depletion
D10	3/2	1	13	4.3	1	0.8	0.8
	3/3		65	21.7	2	1.5	1.6
D11	3/4	0	11	2.9	0	0	> 0.5
	4/1		57	25	4	4	0.8
	4/4		6	2.7	0	0	> 0.4
D12	3/4	0	1,100	367	0	0	> 2.6
D13	3/1	0	750	200	0	0	> 2.2
	3/2		150	50	0	0	> 1.7
	3/3		45	12	0	0	> 1.1

Abbreviation: NB, neuroblastoma.

*Thirty individual pheresis aliquots were analyzed; only those with tumor detected by standard or investigational ICC are included.

†A minimum of 2.25×10^6 cells were analyzed from each specimen.

‡A total of 1 to 2×10^6 CD34$^+$-selected cells were analyzed for each aliquot.

rapid recovery of hematopoiesis and acceptable rates of toxicity and transplant-related mortality.

Use of ABMT or allogeneic bone marrow transplantation as part of treatment for pediatric solid tumors has been previously studied in patients with neuroblastoma. CCG used ABMT with purged bone marrow support in patients with advanced-stage neuroblastoma, and survival among transplant recipients (38%) was somewhat better than expected from historical controls.[21-23] Use of allogeneic bone marrow provided no survival advantage. CCG recently reported data from study 3891, a randomized study comparing continuation chemotherapy with transplantation of purged autologous bone marrow after induction therapy.[3] These data point to the superiority of ABMT over continuation chemotherapy. The overall EFS rate in this phase III study was 30%, with intent-to-treat analysis showing a significantly improved EFS rate (measured from 8 weeks after diagnosis) in the ABMT group (34%) compared with the chemotherapy group (22%). Overall survival was not affected at the time of the analysis; however, patients whose high-risk neuroblastoma recurs rarely respond to salvage treatment. CCG-3891 used TBI as part of the preparative regimen. Avoiding using TBI in these young patients, the Pediatric Oncology Group studied the use of "megadose" chemotherapy for consolidation of complete or partial remissions, using purged marrow support. In the concurrent CCG and POG trials, relapse rates 3 years after diagnosis were comparable but unacceptably high (60% to 75%).[3,24]

The suggestion that very high doses of chemotherapy and chemoradiotherapy might improve overall survival in pediatric patients with high-risk solid tumors led us to explore the use of tandem HDT. The theoretic advantages of tandem transplantation include exposure to potentially non–cross-resistant therapies in rapid sequence, exposure of tumor to multiple effective agents at maximum-tolerated doses, and possible modulation of the tumor microenvironment during the first transplantation, potentiating cell kill with the second course. Tandem transplantation has been studied in adults with breast cancer, multiple myeloma, germ cell tumors, and lymphomas, and some promising early results have been reported.[25-28] The median interval between cycles in these studies ranged from 25 days to more than 6 months.

There are few published studies of tandem transplant therapy in children. Kajiume et al[29] reported on treatment of six pediatric patients with a regimen consisting of one submyeloablative cycle of chemotherapy supported by the CD34$^-$ fraction of CD34$^+$-selected PBPCs, followed by further chemotherapy and HDT/SCR supported by the CD34$^+$ fraction of the same PBPC collection. Philip et al[4] reported on treatment of recurrent or refractory disease using a tandem regimen with bone marrow as a source of stem cells. This regimen required a significant delay between transplantations and was associated with slow engraftment and a high rate of death due to toxicity (24%), but the 5-year survival rate in this high-risk group of patients was 32%. Our early results compare favorably with these findings, and our study indicates that rates of transplant-related toxicities are lower when PBPCs are used to support dose-intensified therapies. Ladenstein et al,[30] analyzing data from the European Bone Marrow Transplant Registry, reported a 10% rate of transplant-related mortality in a heterogeneously treated group of patients who underwent tandem AMBT with marrow support. The rate of death due to toxicity that we report here (8%) is similar to the mortality rate of 6% in CCG-3891 and in a recent study of

single-cycle, PBPC-supported HDT/SCR in children with high-risk malignancies.[3,8]

An important question relating to autotransplantation is whether tumor cells reinfused with the stem-cell product contribute to the likelihood of relapse. Gene-marking studies in patients with neuroblastoma who underwent transplantation showed marked tumor cells at sites of relapse, suggesting that infused clonogenic tumor increases the potential for relapse.[31] However, no clinical study has shown that tumor cell purging improves patient outcome. Many of the strategies used to purge tumor cells from harvested marrow, such as immunomagnetic purging, may significantly delay engraftment (already delayed in patients in whom bone marrow was used as support compared with patients receiving PBPCs).

An alternative, pursued in this study, is to use CD34$^+$ selection of PBPCs. This strategy has three advantages. The first is that PBPCs may have less tumor cell contamination than marrow may have, leaving less tumor to purge. The patients on this study had minimal contamination of the PBPC product even before CD34$^+$ selection. The second advantage of CD34$^+$ selection is that there is no resulting delay in engraftment kinetics, despite a decrease in the number of stem cells infused.[13] The third advantage is that positive selection of stem cells is not dependent on tumor type for depletion, as long as the tumor cells do not express CD34 or an epitope recognized by the anti-CD34 monoclonal antibody used for selection. In this regard, Voigt et al[32] recently reported the labeling of some neuroblastoma cell lines by some anti-CD34 monoclonal antibodies. However, these investigators stained fresh neuroblastoma specimens with the 12.8 anti-CD34 monoclonal antibody used in the Ceprate system and saw no labeling. Other studies have not supported recognition of neuroblastoma by anti-CD34 antibodies or enrichment of neuroblastoma by CD34$^+$ selec-

tion. Greenfield et al[33] attempted to label tumor specimens with anti-CD34 antibodies and found no labeling of 29 neuroblastoma specimens. We have found that CD34$^+$ selection depletes neuroblastoma cells from PBPCs, as has been shown by others.[34-37] Determination of the clinical relevance of CD34$^+$ selection and the impact of purging on EFS will require a larger clinical trial.

In summary, we have shown that it is feasible to treat newly diagnosed high-risk neuroblastoma in pediatric patients using intensive induction chemotherapy followed by tandem HDT/SCR in rapid sequence. The rate of death due to toxicity (8%) is within the range observed with other stem-cell transplantation approaches. Additionally, we have shown that collecting PBPCs is practical in this population, which included children as young as 14 months and weighing as little as 9 kg, and that use of PBPCs results in rapid engraftment, shortening the period of neutropenia and thus lessening the risk of HDT. In a subset of our patients, CD34$^+$ selection was used and resulted in evidence of tumor cell depletion and having no effect on engraftment, demonstrating the feasibility of this approach for potentially decreasing tumor contamination. Although further follow-up is needed to determine whether tandem transplantation with PBPC support has an impact on long-term EFS, early results are encouraging. In part because of these findings, the Children's Oncology Group is planning a randomized trial comparing single and tandem HDT/SCR in patients with high-risk neuroblastoma.

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