Treatment of High-Risk Neuroblastoma With Triple-Tandem High-Dose Therapy and Stem-Cell Rescue: Results of the Chicago Pilot II Study

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Purpose: **To investigate whether intensive induction therapy followed by triple-tandem cycles of high-dose therapy with peripheral-blood stem-cell rescue and local irradiation will improve event-free survival for patients with high-risk neuroblastoma.**

Patients and Methods: **From August 1995 to January 2000, 25 consecutive newly diagnosed high-risk neuroblastoma patients and one child with recurrent *MYCN*-amplified disease were enrolled onto the Chicago Pilot II Protocol. After induction therapy and surgery, peripheral-blood stem cells were mobilized with three cycles of high-dose cyclophosphamide and granulocyte colony-stimulating factor. Patients then underwent triple-tandem cycles of high-dose therapy with peripheral-blood stem-cell rescue followed by radiation to the primary site.**

Results: **Twenty-two of the 26 patients successfully completed induction therapy and were eligible for the triple-tandem consolidation high-dose therapy. Sufficient numbers of peripheral-blood stem cells were collected in all but one patient. Seventeen patients were able to complete all three cycles of high-dose therapy and peripheral-blood stem-cell rescue, two patients completed two cycles, and three patients completed one cycle. There was one toxic death, and one patient died from complications of treatment for graft failure. With a median follow-up of 38 months, the 3-year event-free survival and survival rates are 57% ± 11% and 79% ± 10%, respectively.**

Conclusion: **The results of this pilot study demonstrate that it is feasible to intensify consolidation with triple-tandem high-dose chemotherapy and peripheral-blood stem-cell rescue and local irradiation, and suggest that this treatment strategy may lead to improved survival for patients with high-risk neuroblastoma.**

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DURING THE PAST 20 years, survival for children older than 1 year of age with International Neuroblastoma Staging System (INSS) stage 4[1] neuroblastoma (NB) has increased modestly, although cure rates remain low.[2-4] This improvement is thought to be because of intensification of induction chemotherapy, megatherapy consolidation, and improved supportive care.[4,5] Dose intensity correlates strongly with both response and progression-free survival,[5] and several nonrandomized studies have suggested that autologous stem-cell transplantation after myeloablative doses of chemotherapy with or without total-body irradiation results in improved overall survival.[2,3,6-10] Recently, the results of a randomized trial comparing myeloablative therapy and autologous bone marrow transplantation with chemotherapy alone conducted by the Children's Cancer Group (CCG) has been reported.[4] Three-year event-free survival (EFS) was significantly better for patients randomized to the transplant arm than for patients randomized to continuous chemotherapy (34% ± 4% *v* 22% ± 4%, respectively; $P = .034$). Although the overall EFS rate was poor, the results of this randomized study indicate that myeloablative therapy with stem-cell support improves outcome for high-risk NB patients.

To further dose-intensify therapy, some investigators have treated patients with tandem cycles of high-dose therapy in conjunction with autologous stem-cell rescue. When bone marrow was used for stem-cell support, morbidity was high and delay in hematopoietic recovery was a major problem.[11] However, recently, peripheral-blood stem cells (PBSCs) have been used for stem-cell support in a number of clinical studies involving both adult and pediatric patients, and rapid hematologic recovery has been observed.[10,12-14] Grupp et al[15] conducted a single-arm trial of PBSC-supported tandem transplantation as consolidation for high-risk NB patients. These investigators demonstrated

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that tandem transplant was feasible in this patient cohort, and that toxicity was acceptable. Furthermore, early outcome results were promising, with an estimated 3-year EFS rate of 58% (90% confidence interval, 40% to 72%).

In an effort to improve survival for high-risk NB patients, a pilot study was conducted at the Children's Memorial Hospital in Chicago using intensive induction multiagent chemotherapy followed by surgery. PBSCs were subsequently mobilized with three cycles of high-dose cyclophosphamide and granulocyte colony-stimulating factor (G-CSF). Patients then underwent triple-tandem cycles of high-dose therapy with PBSC rescue followed by local radiation to the primary site. We found that it was feasible to collect sufficient numbers of stem cells to support children through the triple-tandem high-dose therapy, and that the toxicity associated with the regimen was acceptable. Furthermore, our results suggest that this intensive, multimodality treatment strategy may improve outcome for patients with high-risk NB.

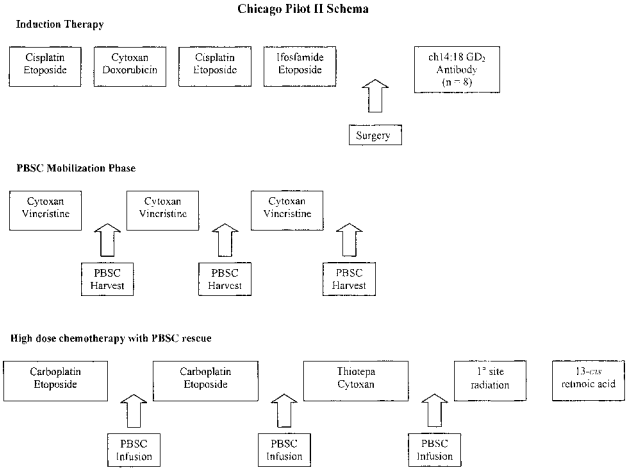

Fig 1. Summary of the Chicago Pilot II treatment schema. Therapy consisted of induction chemotherapy, surgery, PBSC mobilization, triple-tandem cycles of high-dose chemotherapy with PBSC rescue, and radiotherapy to the primary site. A subset of patients also received chimeric anti-GD_2 antibody (ch14.18) and/or 13-*cis*-retinoic acid.

PATIENTS AND METHODS

Patients

From August 1995 to January 2000, 25 consecutive newly diagnosed patients with high-risk[16] NB were enrolled onto the Chicago Pilot II Protocol. All patients were older than 1 year of age at the time of diagnosis and had either INSS stage 4 disease or *MYCN*-amplified stage 3 NB.[1] An additional child was enrolled who was initially diagnosed with a thoracic *MYCN*-amplified stage 1 NB that was treated with surgery alone. Three months after the surgical resection, the patient developed recurrent disease in the primary site. The diagnosis of NB was made on the basis of either histologic examination of tumor specimens or bone marrow infiltrated with NB tumor cells and elevated urine catecholamine levels. For all cases for which tumor tissue was obtained, the tumor was pathologically classified as favorable or unfavorable by the criteria described by Shimada et al.[17] *MYCN* copy number was determined by Southern blot analysis or by fluorescence in situ hybridization in the Pediatric Oncology Group Neuroblastoma Reference Laboratory using previously described methods.[18,19] Patients were staged according to criteria described by the INSS,[1] and extent of disease was evaluated by computed tomography of the chest and abdomen, a technetium-99 (^{99}Tc) bone scan, bilateral bone marrow aspirates and biopsy specimens, and an iodine-123–metaiodobenzylguanidine scan. The protocol was approved by the Children's Memorial Hospital Institutional Review Board, and written informed consent was obtained from the parents of each patient.

Induction Therapy

The treatment plan is summarized in Fig 1. All patients received four cycles of multiagent chemotherapy at 21-day intervals after their diagnosis was confirmed. Cisplatin and etoposide were administered during cycles 1 and 3 (Table 1). Cycle 2 consisted of cyclophosphamide and doxorubicin, and ifosfamide and etoposide were administered during cycle 4. Response to therapy was evaluated after the four cycles of induction chemotherapy with urine catecholamine levels, computed tomography scan, ^{99}Tc bone scan, and bone marrow examination, and classified according to the international criteria.[20] Surgical resection of bulky disease was then performed if (1) there was no evidence of disease progression, (2) disease was not detected by morphologic examination of the bone marrow, and (3) the surgeon considered the tumor resectable. An effort was made to surgically remove all gross disease with preservation of the kidney. Surgery was delayed in patients with persistent extensive disease that was considered unresectable by the surgeon, until after the three cycles of high-dose cyclophosphamide and stem-cell harvests were completed. A single patient underwent a third surgical procedure after completion of chemotherapy and radiotherapy in an effort to resect all residual disease. After induction therapy and surgery, the first eight patients enrolled onto the study received immunotherapy consisting of one or two cycles of chimeric antibody (ch14.18) directed against disialoganglioside (GD_2) at a dose of 50 mg/m^2/d for 4 days with GM-CSF (5 to 10 μg/kg/d for 14 days). Limited supply of antibody precluded treatment of additional patients.

PBSC Collection

Approximately 1 to 3 weeks after surgery, PBSCs were mobilized with three cycles of cyclophosphamide (2 g/m^2/d for 2 days) and G-CSF (5 to 10 μg/kg/d beginning 24 hours after completion of the cyclophosphamide) administered every 21 days. In addition, weekly vincristine (1.4 mg/m^2 1 d/wk for 3 weeks; maximum dose, 1.5 mg) was given during this phase. When the peripheral WBC count was more than 1,000 μL, PBSCs were harvested through a double-lumen cuffed central venous line (Hickman or Broviac) using a COBE Spectra pheresis machine (COBE, Denver, CO). In three patients, it was not possible to perform the pheresis though a central venous line, and a separate pheresis catheter was placed by an interventional radiologist for the harvest. The harvest was performed over 1 to 5 consecutive days after each cycle of high-dose cyclophosphamide in order to collect sufficient PBSCs to support the planned triple-tandem high-dose therapy (2.0×10^8 mononuclear cells/kg [2 to 3×10^6/kg CD34$^+$] per rescue). When mobilization after chemotherapy was not adequate to

Table 1. Induction Chemotherapy

Cycle 1	Cycle 2	Cycle 3	Cycle 4
Cisplatin 40 mg/m^2/d, days 1-5	Cyclophosphamide 1 g/m^2/d, days 1 and 2	Cisplatin 40 mg/m^2/d, days 1-5	Ifosfamide 1.8 g/m^2/d, days 1-3
Etoposide 150 mg/m^2/d, days 1-5	Doxorubicin 35 mg/m^2, day 2	Etoposide 150 mg/m^2/d, days 1-5	Etoposide 150 mg/m^2/d, days 1-3

collect the minimum number of cells, additional harvests were performed after mobilization with G-CSF alone. In one patient, it was technically not possible to collect PBSCs because of behavioral issues. This patient subsequently underwent a bone marrow harvest to obtain stem cells for rescue after a single cycle of high-dose therapy.

PBSC Processing

Samples from the individual daily harvests were obtained for total cell count, mononuclear cell count, CD34$^+$ content, hematopoietic progenitor assay, and bacterial and fungal culture. Collections from each harvest cycle were pooled and suspended in a final dimethyl sulfoxide concentration of 10% and cryopreserved in a controlled-rate freezer to −70°F. The harvest was then stored in the liquid phase of a liquid nitrogen tank until the time of PBSC rescue as previously described.[21] Cell-surface antigen expression of CD34 was identified by flow cytometry analysis using fluorescein isothiocyanate–conjugated mouse anti-CD34 monoclonal antibody (Becton Dickinson, San Jose, CA) as reported.[22]

Triple-Tandem High-Dose Therapy and PBSC Rescue

Disease response was reevaluated after the third harvest with urinary catecholamines, computed tomography scan, ^{99}Tc bone scan, iodine-123–metaiodobenzylguanidine scan, and bone marrow examination. Renal, cardiac, pulmonary, and hepatic function were also evaluated. Patients with adequate organ function, no evidence of active infection, and no evidence of disease progression proceeded to high-dose therapy. The triple-tandem cycles of high-dose therapy conditioning regimens are listed in Table 2. The first two cycles of high-dose therapy were performed in the outpatient setting if there were no medical or logistic contraindications. The indication for admission to the inpatient unit included logistical problems with outpatient management, fever and neutropenia, or any other medical condition that required close observation. The third cycle of high-dose therapy was administered in the inpatient unit. An attempt was made to administer the cycles of high-dose therapy 3 to 4 weeks apart if the patients were clinically stable, free of infection, and had a rising WBC count with a minimum absolute neutrophil count (ANC) of 250/μL off of G-CSF. Platelet recovery was not a requirement. Patients were evaluated before each high-dose treatment to reassess organ function. Patients who did not recover from infection or had significant organ dysfunction at day 35 after high-dose therapy did not proceed to the next high-dose therapy cycle.

Postrescue Therapy

After marrow and physical recovery from the final high-dose therapy cycle (ANC $>$ 1,000/μL and platelet count $>$ 30,000/μL), patients received radiation therapy at a dose of 2,400 cGy in fractions of 150 cGy/d to the primary tumor site. Disease status was reevaluated 100 days after the last high-dose therapy cycle. All patients diagnosed after September 1996 were to receive six cycles of 13-*cis*-retinoic acid (160 mg/m^2/d orally in two divided doses for 13 consecutive days in a 28-day cycle) beginning 80 to 100 days after the last high-dose therapy cycle.

Statistical Analysis

The Kaplan-Meier method was used to estimate 3-year survival and EFS rates expressed as rate ± SE. Survival and EFS functions were compared by the log-rank test.[23] The cutoff date for the analyses was June 7, 2001. The reported time for median follow-up for surviving patients was determined by calculating time from diagnosis to June 7, 2001.

RESULTS

Patients and Response to Induction Chemotherapy

The clinical characteristics of the 26 high-risk NB patients enrolled on this pilot protocol are listed in Table 3. All the patients were older than 1 year of age at the time of diagnosis, and the median age was 40 months (range, 19 to 176 months). Twenty-four patients had newly diagnosed stage 4 disease, one had *MYCN*-amplified stage 3 NB, and one child had recurrent *MYCN*-amplified NB. Twelve of the 25 tumors analyzed (48%) were *MYCN*-amplified. All 23 tumors available for pathologic analysis had unfavorable

Table 2. High-Dose Chemotherapy Phase

PBSC Rescue 1	PBSC Rescue 2	PBSC Rescue 3
Carboplatin 667 mg/m^2/d, days −3 and −2	Carboplatin 667 mg/m^2/d, days −3 and −2	Thiotepa 300 mg/m^2/d, days −6, −5, and −4
Etoposide 1,000 mg/m^2/d, continuous infusion days −3 and −2	Etoposide 1,000 mg/m^2/d, continuous infusion days −3 and −2	Cyclophosphamide 60 mg/m^2/d, days −4, −3, and −2
PBSC infusion day 0	PBSC infusion day 0	PBSC infusion day 0
G-CSF 10 μg/kg/d, beginning day +7	G-CSF 10 μg/kg/d, beginning day +7	G-CSF 10 μg/kg/d, beginning day +7

Table 3. Clinical and Biologic Characteristics of High-Risk Neuroblastoma Patients Treated With Triple-Tandem High-Dose Therapy

Characteristic	Total		Event-Free Survival ± SE	*P**
	No. of Patients	%		
Age at diagnosis				
< 12 months	0			
≥ 12 months	26		57 ± 11	
Sex				
Female	10	38	50 ± 16	.60
Male	16	62	70 ± 13	
Stage				
1	1†	4	100	.64
2	0			
3	1	4	100	
4	24	92	54 ± 11	
Primary tumor site				
Thoracic	3	12	67 ± 27	.76
Abdomen	21	81	55 ± 12	
Retroperitoneal	2	7	100	
MYCN				
Amplified	12	48	74 ± 13	.23
Not amplified	13	52	32 ± 18	
Unknown	1			
Ploidy				
Diploid	6	24	44 ± 22	.28
Hyperdiploid	19	76	57 ± 14	
Unknown	1			
Histology				
Favorable	0		0	
Unfavorable	23	100	62 ± 12	
Unknown‡	3			
13-*cis*-retinoic acid				
Yes	12		69 ± 15	.83
No	5		80 ± 18	
Chimeric 14.18 antibody				
Yes	8		75 ± 15	.44
No	16		46 ± 18	

**P* value calculated using log-rank test.

†Patient treated after localized recurrence.

‡Patients whose diagnosis was made by bone marrow examination.

histology according to the criteria described by Shimada et al.[17]

Four patients achieved a complete response after the four cycles of induction chemotherapy (Fig 1). All four patients had surgical resection of their primary tumor before the administration of induction chemotherapy. Twenty-one patients achieved a partial response (PR) after induction therapy. The bone marrow was morphologically tumor-free in all but one of these 21 patients. One patient developed progressive disease during induction. Parenteral nutrition was required during induction in 20 patients. Toxicities included 29 positive blood cultures in 19 patients (25 bacterial and four fungal). In addition, two patients had thrombotic events; one child had a pulmonary embolus, and the other developed superior vena cava syndrome. Both received anticoagulant therapy and had resolution of their symptoms. There were no toxic deaths during the induction phase of therapy. Seventeen patients underwent surgical resection of their primary tumor after completion of the induction therapy; seven were converted from PR to complete response, and two were converted from PR to very good PR after surgery. Complications of surgery included chylothorax (one patient), pleural effusion (one patient), intra-abdominal abscess (one patient), and fluid imbalance with hypotension that required admission to the intensive care unit (one patient).

Six patients received two cycles of chimeric ch14.18 anti-GD_2 antibody in combination with G-CSF after surgery. The chimeric antibody therapy was limited to a single cycle in two additional patients because of toxicity. One child developed an allergic reaction with urticaria, and the other developed transient neurologic deficits with lower extremity weakness and confusion. Patients were reevaluated for disease response after the cycles of immunotherapy, and one of the eight patients developed disease progression. Stable disease was observed in the remaining seven children.

PBSC Collection

Twenty-three patients met the protocol criteria for PBSC collection that included no evidence of disease progression and a morphologically tumor-free bone marrow. One to five phereses were completed after each cycle of cyclophosphamide, vincristine, and G-CSF in an outpatient setting, once the peripheral total WBC count was more than 1,000/μL with a monocytosis. In general, the procedure was well tolerated, and no patient developed hypotension, hypocalcemia, or citrate toxicity. Most patients were thrombocytopenic from chemotherapy and had lower platelet counts after harvest, and received platelet transfusions after the pheresis. One patient developed sepsis with *Streptococcus viridans* after the second cycle of cyclophosphamide and vincristine and died from multiorgan failure. All patients were reevaluated after completion of the three cycles of therapy, and no patient showed evidence of disease progression. Additional tumor response was seen in the three patients who did not undergo surgical resection after induction therapy.

The patients underwent leukapheresis with a median of three harvest events (range, two to five) and a median of 6.7 harvest days (range, 3 to 10 days) to collect sufficient cells for three consecutive PBSC rescues. The mean number of blood volumes pheresed per collection was 3.8. Two patients had partial collections, one (previously mentioned) was unable to cooperate, and the other died of sepsis before

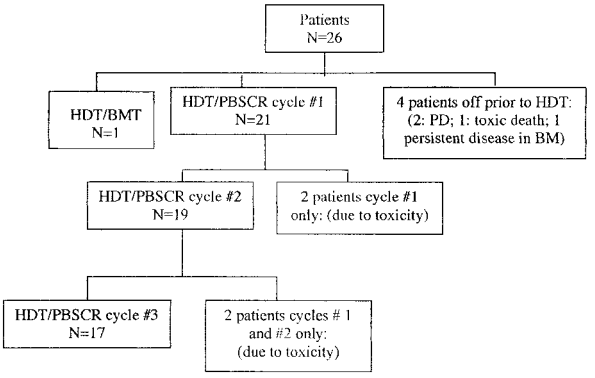

Fig 2. Patient flow through the triple-tandem cycles of high-dose therapy and PBSC rescue. HDT, high-dose therapy; BMT, bone marrow transplant; PBSCR, peripheral-blood-stem cell rescue; PD, progressive disease; BM, bone marrow.

completion of this segment of treatment. The median mononuclear cell dose per kilogram and $CD34^+$ cell dose per kilogram collected in each pheresis was 1.0×10^8 (range, 0.04 to 3.0×10^8) and 1.0×10^6 (range, 0.1 to 119×10^6). No significant difference in cell yields was observed after each cycle of chemotherapy. Nine children underwent phereses after mobilization with G-CSF because of insufficient collections after the three cycles of chemotherapy.

Triple-Tandem High-Dose Therapy and Stem-Cell Rescue

The flow of patients from diagnosis through the triple-tandem high-dose therapy and stem-cell rescues is shown in Fig 2. Four patients were off study before this phase of therapy (two with progressive disease, one with persistent disease in the bone marrow, and one patient who died from overwhelming sepsis before completion of the planned PBSC harvests). Twenty-one patients underwent at least one complete high-dose therapy cycle with PBSC rescue, and 20 underwent the procedure on an outpatient basis. One patient had to be hospitalized to receive this therapy for psychosocial reasons. The child who was unable to cooperate during the PBSC harvest underwent a single cycle of high-dose therapy and rescue with bone marrow stem cells. Two children did not proceed to the second cycle of high-dose therapy and PBSC rescue. In one case, the child developed a disseminated fungal infection and in the second case the patient suffered from severe gastrointestinal toxicity that prohibited safe administration of additional chemotherapy. Both children remain alive and free of disease 25+ and 53+ months after diagnosis. All patients had expected transplant-related toxicities including mucositis and pancytopenia. Twelve children were hospitalized for fever and neutropenia, and positive blood cultures were obtained in nine.

Nineteen patients completed the second cycle of high-dose therapy and stem-cell rescue. Seventeen patients underwent this cycle of therapy in an outpatient setting, and two received the therapy in the hospital. Fifteen children were hospitalized for fever and neutropenia, and positive blood cultures were obtained in seven. Fungal infection prevented one child from receiving the third cycle of high-dose therapy and stem-cell rescue. An additional child suffered from severe necrotizing enterocolitis that prevented additional chemotherapy. Both children are alive and free of disease with follow-up of 57+ and 63+ months from diagnosis.

Seventeen children underwent the third cycle of high-dose therapy and PBSC rescue. Fifteen children had stage 4 disease, one had stage 3 disease, and one had relapsed disease after initially being diagnosed with stage 1 *MYCN*-amplified NB. All patients received this third cycle of therapy, which consisted of thiotepa and cyclophosphamide, in the hospital rather than in the outpatient setting. The mean time from the first infusion of PBSCs to the time of the third infusion of PBSCs was 59 days (range, 47 to 83 days). Toxicities associated with this cycle of myeloablative therapy and PBSC rescue included mucositis (maximum grade 3), mild to moderate thiotepa skin toxicity, and hemorrhagic cystitis (one patient). Five patients had positive bacterial blood cultures, two had herpes zoster, and one had a localized fungal cellulitis. An additional child had prolonged pancytopenia after therapy that was believed to be consequent to graft failure. Bone marrow biopsy specimens remained aplastic 10 months after completion of the third cycle of high-dose therapy and PBSC rescue. Repeated karyotype analyses of the marrow cells were normal. This child underwent an unrelated allogeneic stem-cell transplant but unfortunately died from complications of the transplant.

Engraftment

The median times to an ANC more than 500/μL after PBSC rescues 1, 2, and 3 were 15 days (range, 10 to 27 days), 14 days (range, 8 to 65 days), and 13 days (range, 10 to 22 days), respectively. The median time to a sustained platelet count more than 20,000/μL without transfusion after PBSC rescue 3 was 26 days (range, 10 to 198 days). The platelet count in the patient noted above with the failure to engraft never recovered to over 20,000/μL, but he did not receive routine platelet transfusions after day 120 after PBSC rescue 3. Two other patients with prolonged platelet recovery appeared to have posttransplant immune thrombocytopenia, which resolved over time. The median length of hospital stay for PBSC rescue 3 was 18 days (range, 13 to 29 days).

Table 4. Treatment and Outcome of the 26 High-Risk Neuroblastoma Patients Enrolled on the Chicago Pilot II Study

Patient No.	Age (years, months)	Sex	Stage (INSS)	*MYCN*	Anti-GD_2 Antibody	Disease Status Before PBSC Rescue	No. of PBSC Rescues	13-*cis*-Reinoic Acid	Outcome (follow-up)
1	2, 9	F	1*	A	Yes	CR	3	Yes	Alive: NED (4 years, 11 months)
2	2, 9	M	3	A	No	CR	3	Yes	Alive: NED (1 year, 9 months)
3	1, 10	M	4	A	Yes	—	0	No	Relapse: (5 months) DOD (1 year, 4 months)
4	3, 5	M	4	NA	No	—	0	No	Relapse: (3 months) DOD (3 years, 3 months)
5	14, 10	F	4	A	No	—	0	No	Relapse: (3 months) Alive: (9 months)
6	3, 2	F	4	NA	No	—	0	No	Dead: Sepsis (6 months)
7	2	M	4	A	Yes	PR	1	Yes	Alive: NED (4 years, 6 months)
8	3, 11	M	4	NA	No	CR	1	No	Alive: NED (2 years, 2 months)
9	2, 7	F	4	NA	No	CR	1	No	Relapse: (1 year, 8 months) DOD (4 years, 1 month)
10	3, 11	M	4	NA	Yes	PR	2	Yes	Alive: NED (4 years, 10 months)
11	1, 7	M	4	A	Yes	CR	2	No	Alive: NED (5 years, 4 months)
12	1, 9	F	4	A	No	CR	3	Yes	Alive: NED (4 years)
13	2	F	4	A	No	CR	3	Yes	Alive: NED (3 years, 3 months)
14	2, 3	M	4	A	No	CR	3	No	Alive: NED (9 months)
15	2, 8	M	4	A	No	CR	3	Yes	Alive: NED (2 years)
16	3, 1	M	4	NA	No	CR	3	No	Alive: NED (10 months)
17	3, 5	M	4	NA	No	PR	3	No	Relapse: (12 months) DOD (1 year, 1 month)
18	3, 8	F	4	A	Yes	CR	3	No	Alive: NED (5 years, 9 months)
19	3, 9	M	4	NA	No	VGPR	3	No	Alive: NED (12 months)
20	3, 10	M	4	NA	No	CR	3	Yes	Alive: NED (2 years, 2 months)
21	5, 1	F	4	NA	No	CR	3	Yes	Relapse: (2 years, 3 months) Alive: (2 years, 10 months)
22	5, 9	M	4	A	Yes	CR	3	Yes	Dead: failure to engraft (2 years, 7 months)
23	6, 4	F	4	NA	No	CR	3	Yes	Relapse: (2 years, 5 months) Alive: (2 years, 7 months)
24	7, 2	F	4	ND	No	CR	3	No	Alive: NED (5 years, 7 months)
25	8, 1	M	4	NA	No	VGPR	3	No	Alive: NED (11 months)
26	10, 3	M	4	NA	Yes	PR	3	Yes	Alive: NED (4 years, 9 months)

Abbreviations: M, male; F, female; A, amplified; NA, nonamplified; ND, not done; VGPR, very good partial response; CR, complete response; NED, no evidence of disease; DOD, dead of disease.

*Patient with relapse after initial treatment with surgical resection alone.

Postrescue Therapy

Local radiotherapy was administered to the primary tumor site after completion of the final cycle of high-dose therapy and stem-cell rescue. To date, 19 patients have received the radiotherapy. Radiotherapy was started once the WBC count was more than 1,000/μL and the patients were clinically stable. The median time to starting radiation therapy was 70 days after the final cycle of high-dose therapy and stem-cell rescue, with a range of 45 to 106 days. One patient developed dose-limiting esophagitis and did not complete the prescribed course of radiation therapy. Twelve children received six cycles of 13-*cis*-retinoic acid administered in doses previously described after recovery from the radiation.[4] Eleven children tolerated the therapy well, with only minor problems related to dry skin. One child developed intermittent weakness and ataxia after treatment with 13-*cis*-retinoic acid.

Treatment Results

A summary of the treatment and outcome of the 26 patients is shown in Table 4. With a median follow-up of 38 months (range, 8 to 68 months) from diagnosis, the 3-year estimated EFS and survival rates for the 26 high-risk patients enrolled on this study are 57% $\pm$ 11% (Fig 3) and 79% $\pm$ 10%, respectively. To date, four patients have developed recurrent disease after consolidation with high-

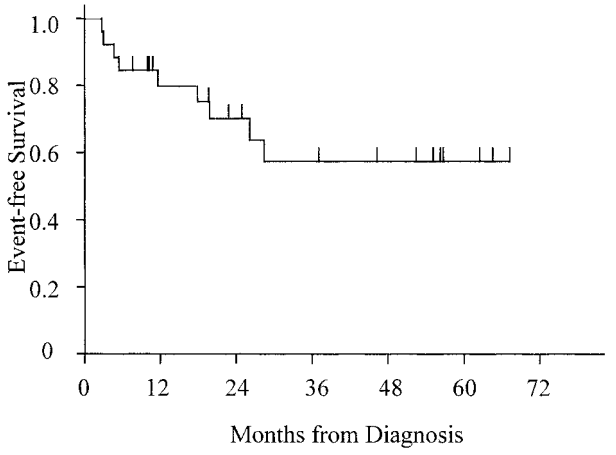

Fig 3. Kaplan-Meier analysis of EFS for the 26 patients with high-risk NB enrolled onto the Chicago Pilot II Protocol.

dose therapy plus stem-cell rescue and radiation. Two patients developed relapse in the bone marrow, one developed metastatic pulmonary disease, and one patient developed recurrence in the abdomen outside the field of radiation. For the 17 patients who have completed all three cycles of high-dose therapy and stem-cell rescue, the estimated 3-year EFS rate is 63% ± 15%. The 3-year EFS for the 15 patients with stage 4 NB who completed all three cycles of high-dose therapy and stem-cell rescue is 54% ± 12%. Both patients who completed two cycles of high-dose therapy and PBSC rescue remain alive and disease-free, and two of the three patients who underwent only one transplant are disease-free. Three-year EFS was similar for the eight patients who received chimeric ch14.18 anti-GD_2 immunotherapy compared with the 16 patients who did not receive antibody (75% ± 15% *v* 49% ± 17%, respectively; $P = .44$). Outcome was also similar for patients who did or did not receive 13-*cis*-retinoic acid (EFS, 69% ± 15% *v* 80% ± 18%, respectively; $P = .83$).

DISCUSSION

This is the first report of PBSC-supported triple-tandem transplantation in the treatment of high-risk neuroblastoma patients. We found that it was feasible to collect sufficient numbers of PBSCs to support patients through the triple-tandem courses of high-dose therapy. Although the three cycles of high-dose cyclophosphamide were primarily used to mobilize PBSCs, antitumor effects were seen. Therefore, the high-dose cyclophosphamide may also have contributed to the success of this therapy. Seventeen of the 26 children enrolled on this study were able to complete the planned triple-tandem cycles of high-dose therapy and PBSC rescue. Two patients were only able to complete two cycles of high-dose therapy and PBSC rescue, and two children underwent one cycle of high-dose therapy with PBSC rescue. With a median follow-up of 38 months, the 3-year EFS and survival rates from the time of diagnosis are 57% ± 10% and 79% ± 10%, respectively. These outcome results are significantly better than other reported series in the literature including our previous pilot study in which a single cycle of high-dose therapy and PBSC rescue was administered.[2-4,10]

Recently, the superiority of myeloablative therapy and autologous bone marrow transplant over conventional-dose chemotherapy has been definitively demonstrated in a randomized study conducted by the CCG.[4] Furthermore, small single-arm pilot studies have suggested that further dose intensification with tandem cycles of high-dose therapy and stem-cell rescue is feasible.[11,15] Grupp et al[15] reported that when PBSCs are used for rescue in lieu of bone marrow in the setting of tandem stem-cell transplantation, the rate of death because of toxicity was less than 10%, similar to that seen in the CCG study in which a single transplant was performed. Encouraging early EFS results were observed in this tandem transplant study, suggesting that this approach may improve outcome for high-risk NB patients.

Several other reports have also indicated that compared with bone marrow, the rates of transplant-related toxicities are lower when PBSCs are used to support dose-intensified therapy.[12-14,24] This is largely because of a significantly reduced cytopenic period after myeloablative therapy. Although the experience with PBSC harvest and rescue in children with high-risk malignancies is limited, the procedure does not appear to be limited by age or weight of the patient.[12,25,26] In addition, the incidence of tumor contamination has been reported to be lower in PBSC harvests than in bone marrow grafts.[10,25,27] Molecular studies using reverse transcriptase polymerase chain reaction to test for tyrosine hydroxylase expression, a marker for tumor contamination,[28] in the PBSC harvests collected in our study are ongoing and will be reported elsewhere.

In addition to dose intensification, numerous alternative therapeutic approaches have been used in an effort to achieve better cure rates for children with high-risk NB. One example is targeted immunotherapy, which exploits tumor selectivity and has minimal cross-resistance or overlapping toxicities with chemotherapy. GD_2 is suitable for targeting therapy because it is expressed at a high density in human NB tumors.[29] Several anti-GD_2 monoclonal antibodies and chimeric antibodies have been developed and tested in clinical trials.[30,31] Because antibody-dependent cell-mediated cytotoxicity is often depressed in cancer patients, in many studies cytokines have been combined with antibody

therapy to enhance effector functions.[30] Phase I and II clinical trials have demonstrated the ability of anti-GD_2 antibodies to kill tumor cells in the bone marrow, whereas the antitumor effect on bulky tumors was less clear. Encouraging results have also been reported by Cheung et al[32] when the murine anti-GD_2 antibody (3F8) was administered to high-risk patients with minimal residual disease after consolidation. To examine the clinical effect of chimeric anti-GD_2 antibody ch14.18 and GM-CSF in the setting of minimal disease, in our study antibody and cytokine were administered after four cycles of chemotherapy and surgery. Because supply of the antibody was limited, only eight patients were able to receive the immunotherapy. No survival advantage was seen in this small study for patients who received the immunotherapy.

Retinoids also appear to be effective treatment for NB. Matthay et al[4] recently reported that administration of 13-*cis*-retinoic acid after maximal tumor reduction resulted in a significant improvement in EFS. Once the results of this study were known, all newly diagnosed patients on our study were treated with 13-*cis*-retinoic acid beginning 80 to 100 days after completion of the final cycle of high-dose therapy and PBSC rescue. To date, 12 patients have completed that planned six cycles of 13-*cis*-retinoic acid therapy. In this small series, outcome was similar for those patients who received 13-*cis*-retinoic acid compared with those that did not.

The results of this pilot study demonstrate that it is feasible to treat high-risk NB patients with triple-tandem cycles of high-dose therapy and PBSC rescue after intensive induction chemotherapy and surgery, and suggest that intensification of consolidation results in improved outcome. We found that it was possible to collect a sufficient number of PBSCs to support children through triple-tandem cycles of high-dose therapy and rescue. The rate of death because of toxicity was within the range observed with other stem-cell approaches. This study supports the hypothesis that dose intensification is an important component of successful treatment of NB. However, further dose-escalation of therapy may be prohibitive. Thus, it is likely that development of targeted, tumor-specific approaches of therapy will be needed to significantly further enhance the survival for high-risk NB patients.

NOTE ADDED IN PROOF

While this article was under review, two additional patients developed prolonged pancytopenia after the third cycle of high-dose therapy and PBSC rescue. Bone marrow biopsy specimens of both patients were aplastic 6 months after completion of the high-dose therapy. Monosomy 7 was identified by karyotype analysis of the bone marrow cells from one patient, indicative of a myelodysplastic syndrome. The second patient seems to have graft failure, as no specific cytogenetic abnormality was identified in bone marrow cells. Neither patient has evidence of neuroblastoma at this time.

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