

Autologous Stem Cell Transplantation for High-Risk Neuroblastoma

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ABSTRACT

Despite initial responses, long-term survival for children with high-risk neuroblastoma using conventional chemoradiotherapy is only 15%. The recently completed phase 3 national randomized trial (Children's Cancer Group [CCG] 3891) demonstrated that myeloablative consolidation using carboplatin, etoposide, melphalan, and total body irradiation (TBI) with purged autologous bone marrow infusion (autoBMT) significantly improved 3-year event-free survival (EFS) compared with nonmyeloablative chemotherapy consolidation ($34\% \pm 4\%$ vs. $22\% \pm 4\%$; $P=.034$). Therapy with 13-*cis* retinoic acid (13-cRA) postconsolidation also significantly improved EFS ($46\% \pm 6\%$ vs. $29\% \pm 5\%$; $P=.027$). The best outcome was achieved with the combination of myeloablative chemotherapy and autoBMT plus 13-cRA, with an estimated EFS of $38\% \pm 6\%$ from the time of diagnosis. This approach is now the standard for therapy of high-risk neuroblastoma. However, relapses indicate the need for more effective primary site control, as well as agents active against resistant minimal residual disease (MRD).

Our subsequent CEM-LI (carboplatin, etoposide, melphalan, and local irradiation) pilot study eliminated TBI from the transplant regimen. This allowed significant dose escalation of carboplatin and etoposide. Stem cell infusion was either purged autologous marrow or purged peripheral blood stem cells (PBSCs). Local radiation was given to all primary sites, as well as residual metastatic sites. Posttransplant therapy was allowed and may have included 13-cRA and/or anti-G_{D2} antibody and granulocyte-macrophage colony-stimulating factor (GM-CSF). One hundred six children were transplanted in first ($n = 77$) or second ($n = 29$) remission. The 3-year EFS for all patients transplanted in first remission was $64\% \pm 9\%$; that for stage 4 patients >1 year of age in first remission was $61\% \pm 12\%$. Relapses occurred at primary and distant ($n = 3$), primary ($n = 2$), and distant ($n = 18$) sites; only 3 of 20 reviewed were within the irradiation field. This strategy appears to have decreased primary site relapse.

The next phase 3 Children's Oncology Group (COG) study (A3973) will use intensive induction chemotherapy, followed by consolidation with CEM-LI and posttransplant therapy with 13-cRA. Patients will be randomized to receive either purged or unpurged PBSCs to determine the effect of purging on EFS and MRD. A second randomization adding anti-G_{D2} antibody (ch14.18) and GM-CSF and interleukin (IL)-2 (COG-P9842) after autoSCT vs. 13-cRA alone will determine if EFS can be improved by another therapy directed against MRD.

Future therapies in development include chemotherapy resistance modifiers (such as glutathione depletion with buthionine sulfoximine [BSO]), targeted radiotherapy with ¹³¹I-metaiodobenzylguanidine (¹³¹I-MIBG), and novel retinoids (fenretinide). Integration of these strategies should further improve the outcome of children with high-risk neuroblastoma.

INTRODUCTION

Neuroblastoma, the second most common solid tumor in children, can be stratified into risk groups at diagnosis using age, tumor stage, histopathology, and the presence of amplification of the *MYCN* oncogene.¹⁻³ Conventional-dose chemoradiotherapy can achieve a complete response in the majority of high-risk patients; however, the long-term survival is only 15%.¹ Pilot studies suggested that high-dose myeloablative chemotherapy may improve the outcome.⁴⁻¹² This provided the rationale for a national randomized prospective trial, CCG 3891,¹³ to determine if myeloablative chemotherapy and transplantation with purged autologous bone marrow could achieve a better EFS than nonmyeloablative chemotherapy. The observation of a high incidence of relapse in patients who had no measurable tumor posttransplant⁸ led to another pilot study to determine the toxicity of 13-cRA in the posttransplant setting.¹⁴ It had been shown in the laboratory that 13-cRA could induce differentiation, decrease proliferation, and downregulate *MYCN* expression in neuroblastoma tumor cell lines, including some established from refractory tumors after autoBMT.¹⁵⁻¹⁸ The effect of 13-cRA on EFS when given on a randomized basis posttransplant was also examined in the CCG 3891 trial. This article summarizes the results of the CCG 3891 study, as well as preliminary results of a successor pilot transplant study (CEM-LI) that eliminated total body irradiation from the transplant regimen, escalated doses of the chemotherapy, and intensified the local radiation therapy. Trials currently in progress that are piloting novel transplant regimens and/or posttransplant therapies for resistant minimal residual disease in high-risk neuroblastoma are also summarized.

MATERIALS AND METHODS

For the clinical studies described, written informed consent was obtained from the parent and/or guardian, and studies were approved by the participating

institutions' investigational review boards. Eligible patients were 1–18 years of age for CCG 3891 and 9 months to 25 years of age for the CEM-LI pilot. High-risk neuroblastoma was defined as all patients with Evans' syndrome (ES) stage IV¹⁹ >1 year of age at diagnosis; ES stage IV <1 year of age at diagnosis with *MYCN* amplification; ES stage III with *MYCN* amplification, unfavorable histopathology, and/or serum ferritin >143 ng/mL; ES stage II with *MYCN* amplification; or ES stage I/II with development of bone metastases after surgical resection alone. Patients transplanted on both CCG 3891 and the CEM-LI pilot received autologous stem cells purged at the Neuroblastoma Purging Center of the CCG. Bone marrow was purged using the previously published method,^{7,20,21} with immunomagnetic beads. PBSCs were purged with a modification of this method, using an additional initial step with carbonyl iron²² to remove monocytes and neutrophils. All infused stem cell products had no evidence of neuroblastoma tumor cells by immunocytology,²³ as performed by the Neuroblastoma Reference Lab of the CCG, with a sensitivity of 1 tumor cell per 10⁵ nucleated bone marrow cells.

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Methods for this study have been published previously.¹³ The patient population consisted of children ages 1–18 years with newly diagnosed high-risk neuroblastoma entered on a prospective randomized national study from 1991 to 1996, including 434 with ES stage IV >1 year of age, 72 with ES stage III with high-risk features, 1 with ES stage II with *MYCN* amplification, 13 with ES stage I or II who developed bone metastases after surgical resection only, and 19 <1 year of age with ES stage IV and *MYCN* amplification. There were no significant differences in the prognostic features of the randomized groups for either the first randomization (at 8 weeks from diagnosis) to transplant vs. continuation chemotherapy or the second randomization (at completion of the assigned chemoradiotherapy arm) to 13-cRA vs. no further therapy. Doses for the transplantation regimen from CCG 3891 are summarized in Table 1. Carboplatin and etoposide were given on days –7 through –4 as a continuous intravenous (IV) infusion. Melphalan was given as 140 mg/m² on day –7 and 70 mg/m² on day –6. The TBI was given as 333 cGy/day on days –3 through –1. The 13-cRA was given every 28 days as 160 mg/m²/day for 14 days, followed by a 14-day rest, beginning at day 84 after completion of chemoradiotherapy. Local radiotherapy (1000 cGy over 5 days) was given at the end of the induction chemotherapy to persistent metastatic sites and to the primary site only if there was residual tumor. Further details of the CCG 3891 study and the statistical analyses used have been published previously.¹³

Table 1. Comparison of Total Doses of Chemotherapy Drugs and Radiation for CCG 3891 and CEM-LI Regimens*

	CCG 3891	CEM-LI (GFR >100)	CEM-LI (GFR <100)
Carboplatin, mg/m ²	1000	1700	AUC 16.4
Etoposide, mg/m ²	800	1350	800
Melphalan, mg/m ²	210	210	180
Local radiation, cGy	1000†	2100‡	2100‡
Total body irradiation, cGy	1000	None	None

*AUC, area under the curve; GFR, glomerular filtration rate in cc/min per 1.73 m². †To residual measurable tumor sites pretransplant. ‡To all primary sites and any residual metastatic sites.

CEM-LI Pilot Study

Analyses reported here include patient accrual from 1991 to 1999. This study is still open and pending final analysis. Eligible patients included those with high-risk neuroblastoma who had been treated with any accepted induction therapy (including CCG 3891,¹³ N6,²⁴ or other) in first remission and patients with stage III/IV who were in at least a partial remission after any reinduction therapy for relapse. One hundred six children were transplanted in first ($n = 77$) or second ($n = 29$) response. The chemotherapy regimen consisted of continuous-infusion carboplatin (escalated from 250–425 mg/m² per day), etoposide (escalated from 200–375 mg/m² per day) given IV days –7 through –3, and melphalan (fixed dose of 70 mg/m² per day) by bolus IV infusion days –7 through –4. Purged autologous bone marrow or peripheral blood stem cells were infused on day 0, followed by either GM-CSF 250 µg/m² per day or G-CSF 10 µg/kg per day until neutrophil engraftment occurred. The carboplatin dosage was calculated based on the pretransplant glomerular filtration rate (GFR) using the pediatric Calvert formula²⁵ for those patients with a GFR <100 cc/min per 1.73 m², and this group of patients were dose-escalated separately from those patients with a GFR >100 cc/min per 1.73 m². Local irradiation was given as two 150-cGy fractions per day, at least 4 hours apart, for a total dose of 1500–2100 cGy to the primary site regardless of extent of residual tumor and to any residual metastatic sites before transplant. Posttransplant therapy was allowed at the discretion of the treating physician, and included cRA, anti-G_{D2} antibody plus GM-CSF, fenretinide, and/or gene therapy. Life-table estimates were calculated according to the Kaplan-Meier procedure.²⁶ The standard errors of the life-table estimates of event-free survival were calculated according to the method described by Peto et al.²⁷ Events considered were disease progression, death from any cause, and/or a second neoplasm, whichever occurred first.

RESULTS

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Myeloablative chemoradiotherapy and purged ABMT significantly improved the 3-year EFS from the time of the first randomization (8 weeks from diagnosis) compared with nonmyeloablative chemotherapy consolidation ($34\% \pm 4\%$ vs. $22\% \pm 4\%$; $P=.034$).¹³ Those patients randomized after completion of either arm of chemoradiotherapy to receive 13-cRA also had a significantly improved EFS at 3 years from the time of randomization ($46\% \pm 6\%$ vs. $29\% \pm 5\%$; $P=.027$). The estimated EFS 3 years from the second randomization was $55\% \pm 10\%$ for those patients assigned to transplantation followed by 13-cRA. The estimated 3.7-year EFS from the time of diagnosis was $38\% \pm 6\%$ for patients receiving transplantation and 13-cRA, compared with only $17\% \pm 4\%$ in the group treated with conventional-dose chemotherapy alone. For patients undergoing the first randomization treatment, there were 7% (9 of 129) treatment-related deaths vs. 1% (1 of 150) in those randomized to continuation chemotherapy ($P=.013$).

CEM-LI Regimen

The maximal tolerated doses of this regimen determined for patients with a GFR >100 cc/min per 1.73 m² were carboplatin 1700 mg/m² and etoposide 1350 mg/m² given with melphalan 210 mg/m². There were no toxic deaths in 58 patients transplanted in first remission with a GFR >100 cc/min per 1.73 m². For patients with a GFR <100 cc/min per 1.73 m², the maximal tolerated dosage was determined initially as a carboplatin area under the curve (AUC) of 16.4 with etoposide 1000 mg/m², based on 6 patients treated at these doses. After further accrual, the etoposide dose was decreased to 800 mg/m² and the melphalan dose was decreased to 180 mg/m², based on observation of additional toxicity. Accrual of patients to evaluate this dose-reduction level is continuing. Among 18 first-remission patients with a low GFR, there were 2 toxic deaths (11%). Overall, there were 2 of 76 (3%) toxic deaths in first-remission patients.

The EFS at 3 years from time of transplantation was $64\% \pm 9\%$ for all patients transplanted in first remission. The 3-year EFS was $61\% \pm 12\%$ for stage 4 patients >1 year of age ($n = 56$) in first response, of whom 70% received 13-cRA, 20% anti-G_{D2} antibody, and 4% fenretinide posttransplantation. Relapses in the 58 patients transplanted in first response occurred at primary and distant ($n = 3$), primary ($n = 2$), and distant ($n = 18$) sites. Three of the 20 relapse sites reviewed were within the irradiation field.

DISCUSSION

The results of the randomized prospective CCG 3891 trial¹³ have established myeloablative chemoradiotherapy with autologous transplantation followed by 6 months of 13-cRA as the standard of therapy for high-risk neuroblastoma. However, with an estimated survival from diagnosis of only 38% for patients treated with this standard, novel approaches are clearly needed to further improve outcome. The primary site is a common site of relapse posttransplantation,⁸ indicating that local control needs to be addressed. In addition, CCG 3891 used total body irradiation, which is associated with increased acute toxicity in the early posttransplant period, as well as late effects including abnormalities in growth, thyroid dysfunction, abnormal dental development, and cataracts.²⁸⁻³²

We hypothesized that eliminating TBI would allow dose escalation of the chemotherapy used in the CCG 3891 transplant regimen and more intensive local radiation to the primary site, which would improve the EFS. As shown in Table 1, the doses of carboplatin and etoposide were successfully escalated in patients with a GFR >100 cc/min per 1.73 m²; however, this was not possible in the low GFR cohort due to toxicity. Current neuroblastoma regimens all contain nephrotoxic agents and, since nephrectomy is not uncommon with primary tumor resection, tailoring dose escalations based on renal function is an important issue to give most effective yet tolerable doses to all patients. The CEM-LI regimen had 3% (2 of 76) toxic deaths in first-remission patients vs. 7% (9 of 129) of patients transplanted with the CCG 3891 regimen. There were no toxic deaths on the CEM-LI regimen among patients with a normal GFR. Primary site relapses were observed in only 5 of 23 patients (22%) with the CEM-LI regimen. This suggests an improvement over previous studies in which radiation was not given consistently to the primary site, and local relapse occurred in ~50% of patients.⁸ This observation needs to be confirmed in a larger number of patients. Distant relapses remain a significant issue, indicating the need for more effective therapy for minimal residual tumor following transplantation. The outcome with CEM-LI compares favorably with the CCG 3891 TBI regimen; however, there are differences in the prognostic features of the 2 patient populations that affect retrospective comparisons. These include induction therapy before transplant, response status before transplant, time from diagnosis to transplantation, and posttransplant therapy. We conclude from the CEM-LI pilot results that this regimen is well tolerated, the elimination of TBI does not adversely affect outcome, and more consistent intensive local radiation may decrease relapse at the primary site.

The next cooperative group study, A3973, will open this year in the COG. This study will use a more aggressive induction than CCG 3891 based on the N6 regimen,²⁴ which has reported the highest response rates to date. All patients will then be transplanted with the CEM-LI regimen, followed by 6 months of 13-cRA.

This study will provide a larger patient population to establish the EFS of the CEM-LI regimen and the incidence of primary site relapse. The A3973 study will use peripheral blood stem cells instead of bone marrow, based on data showing more rapid engraftment with PBSCs^{33–36} and preliminary reports of a lower content of neuroblastoma tumor cells than in marrow.^{37–39} Patients will be randomized at diagnosis to receive either purged or unpurged PBSCs, with the study end point to determine differences in EFS and overall survival between these 2 groups in patients with stage IV tumors who are >1 year old at diagnosis. The pilot of purged PBSC is near completion in the final cohort of patients on the CEM-LI study. Preliminary data in children with neuroblastoma who have no detectable tumor by immunocytology have shown the feasibility of collecting sufficient numbers of PBSCs after 2–3 cycles of induction chemotherapy and normal engraftment of the purged PBSCs.

The issue of purging stem cells for transplantation has never been examined in a randomized study. Previous studies for neuroblastoma have used either purged or unpurged stem cells. Some data suggest an advantage for purging, but no definitive conclusion can be made.^{4–7,10,12,13,39–45} Gene marking studies in neuroblastoma⁴⁶ have demonstrated that tumor cells infused in marrow grafts can be found at sites of relapse. The minimum number of neuroblastoma tumor cells required to initiate tumor regrowth is not known and is likely to vary with the biologic characteristics of the individual tumor. The A3973 study will determine in a prospective randomized manner whether purging of PBSCs is associated with a significant difference in event-free survival in high-risk neuroblastoma.

The clinical standard for using stem cell products is the absence of neuroblastoma tumor cells detectable by immunocytologic assay, with a sensitivity of 1 tumor cell in 10^5 mononuclear cells.²³ Recently, more sensitive assays using reverse transcription–polymerase chain reaction (RT-PCR) methodology with markers of tyrosine hydroxylase and protein gene product (PGP) 9.5⁴⁷ can detect as few as 1 tumor cell in 10^6 mononuclear cells. Approximately 25% of purged bone marrow samples from the CCG 3891 study that were negative for tumor by immunocytology had detectable tumor by RT-PCR analysis (R.C.S., personal communication). The prognostic significance of these RT-PCR findings is not known, and will require a larger sample of patients. The A3973 study will perform RT-PCR analysis on all stem cell products to determine if there are differences in purged vs. unpurged PBSCs and whether tumor detected by RT-PCR has prognostic significance. Minimal residual tumor will also be assessed by RT-PCR and MIBG scans at various points during and after completion of therapy to determine if these assessments can predict outcome.

Other posttransplant therapies for neuroblastoma may further improve EFS when used in combination with 13-cRA. The ganglioside G_{D2} is expressed on the surface of almost all neuroblastoma tumor cells and is involved in the attachment of tumor

cells to the extracellular matrix. Antibodies against G_{D2} have antitumor activity that can be augmented by GM-CSF and/or IL-2, which activate monocytes and lymphocyte-activated killer cells and enhance their ability to kill neuroblastoma tumor cells in combination with antibody.⁴⁸⁻⁵² Both murine and chimeric antibodies to G_{D2} have demonstrated clinical responses in patients with recurrent neuroblastoma, especially in marrow metastases.^{49,53-58} The CCG 0935A phase 1 study, which is a pilot of the chimeric anti-G_{D2} antibody ch14.18 given with GM-CSF alternated with IL-2 and concurrent with 13-cRA, will be completed this year. The successor P9842 phase 3 cooperative group study will randomize patients from the A3973 COG study described above to 13-cRA alone vs. 13-cRA plus the CCG 0935A regimen, following transplantation with CEM-LI. The study end point will be differences in EFS and survival between the 2 groups. Differences in minimal residual tumor in patients treated on either study arm, as measured by RT-PCR of blood/marrow and MIBG scans, will also be examined as a secondary aim.

Another synthetic retinoid, *N*-(4-hydroxyphenyl)retinamide or fenretinide,^{59,60} has potential as a future posttransplant therapy for minimal residual tumor. Fenretinide has demonstrated activity against neuroblastoma cell lines,⁶¹⁻⁶⁴ including those resistant to 13-cRA. Unlike 13-cRA, fenretinide induces apoptosis rather than differentiation.⁶³ Its mechanisms of action include an increase in ceramide levels,⁶⁴ increase in oxidative radicals,⁶⁵ and antiangiogenesis.⁶⁶ Its major toxicity is nyctalopia, and no significant hematopoietic toxicity has been reported^{60,67-69}; therefore, it should be well tolerated after intensive chemoradiotherapy. A pediatric phase 1 study of oral fenretinide on an intermittent high-dose schedule (given tid for 7 days, followed by a 2-week rest) is currently in progress in the CCG (study 09709). Toxicity to date has been minimal at doses up to 1395 mg/m² per day, with preliminary analysis demonstrating that plasma levels can be achieved in patients which are comparable to those required in vitro for activity against neuroblastoma tumor cell lines (J.G.V., personal communication). Response data are blinded until completion of the study. A phase 2 study of fenretinide in neuroblastoma is planned in COG, pending determination of the maximal tolerated dosage. A future phase 3 study of fenretinide in the posttransplant setting is possible, pending results of these preliminary studies.

Agents that can reverse resistance to chemotherapy in neuroblastoma relapsing after myeloablative therapy should provide another avenue to improve outcome. BSO is a selective inhibitor of γ -glutamylcystein synthetase, the rate-limiting enzyme in glutathione synthesis.⁷⁰ BSO causes depletion of intracellular glutathione levels, which can enhance alkylator activity.⁷⁰⁻⁷² BSO exhibits cytotoxic activity as a single agent in in vitro neuroblastoma. The combination of BSO and the alkylator melphalan is synergistic in vitro against neuroblastoma cell lines, including some derived from patients transplanted with high-dose melphalan (210 mg/m²) regimens.^{73,74} This synergy is most striking at levels of melphalan that

can be achieved in patients with myeloablative doses. A phase 1 study of BSO and melphalan⁷⁵ given at 15 mg/m² has demonstrated 27% responses in 20 evaluable patients with recurrent neuroblastoma, including 7 partial responses, 1 minor response, and 9 stable disease. The major toxicity was hematopoietic. A successor phase 1 study of BSO with escalating doses of melphalan (40–170 mg/m²) given with autologous stem cell support is planned to open this year in the New Approaches to Neuroblastoma Therapy (NANT) Consortium. Pending the toxicity and response data from the NANT study, the BSO/melphalan regimen may be incorporated into frontline studies.

Metaiodobenzylguanidine

MIBG is a guanethidine derivative that structurally resembles norepinephrine and is concentrated in adrenergic tissue.⁷⁶ MIBG can be labeled with radioactive isotopes of iodine and used for diagnostic imaging (¹²³I) or for both imaging and therapeutic treatment (¹³¹I) of neuroblastoma, which is a tumor of neuroectodermal origin.^{77–80} Multiple studies using ¹³¹I-MIBG in children with neuroblastoma have reported response rates from 10% to 50%, and none have observed nonhematologic dose-limiting toxicity.^{77,79,81–84} A recently completed phase 1 study of ¹³¹I-MIBG for recurrent neuroblastoma at the University of California–San Francisco⁸⁵ defined the maximal dose of ¹³¹I-MIBG for hematopoietic toxicity as 12 mCi/kg, with no significant nonhematologic toxicity at doses up to 18 mCi/kg. The response rate among the 30 patients treated was 37%, including 1 complete, 10 partial, 3 minor, and 10 stable disease. Based on the response data and the hematopoietic dose-limiting toxicity that can be abrogated by stem cell support, a phase 1 study will open this year in the NANT Consortium using ¹³¹I-MIBG in combination with the CEM-LI regimen and autologous stem cell support. Eligible patients will include those with poorly responding neuroblastoma after induction chemotherapy and those who develop progressive disease. ¹³¹I-MIBG administration will be followed 2 weeks later by CEM-LI, with dose escalation of both ¹³¹I-MIBG and CEM beginning at levels below the maximally defined doses. This study may provide a more effective myeloablative regimen that uses an agent specifically targeted to neuroblastoma.

CONCLUSION

Significant progress has been made in the last decade in the therapy of high-risk neuroblastoma, with improvement in survival from 15% to 40% through the implementation of more intensive induction regimens, myeloablative consolidation with autologous transplantation, posttransplant therapy directed against minimal residual disease, and improvements in supportive care. Future studies are needed to

design more effective induction and myeloablative regimens with agents that can reverse chemoradiotherapy resistance and/or have novel mechanisms of action that are non-cross-resistant with chemoradiotherapy, for maximal reduction of tumor burden. Novel therapies directed against minimal residual disease will also be critical. The role of stem cell purging also needs to be defined. Finally, the long-term effects of these aggressive therapies must be monitored carefully to provide quality of life for the expected increased number of survivors.

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