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Role of Myeloablative Therapy in Improved Outcome for High Risk Neuroblastoma: Review of Recent Children's Cancer Group Results

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The use of new strategies for dose intensification using peripheral blood stem cell or autologous purged bone marrow rescue has raised expectations for cure in advanced neuroblastoma, although conflicting reports exist regarding the efficacy of these approaches. Using risk groups based on both biological and clinical staging, the Children's Cancer Group (CCG) has conducted a series of pilot studies to test new induction, consolidation and myeloablative regimens to attempt to improve outcome. We summarise below the outcome and prognostic factor analysis for the pilot chemotherapy trial, CCG-(CCG-321P2), and the use of high dose myeloablative chemoradiotherapy with allogeneic (CCG-321P1) or autologous purged bone marrow rescue (CCG-321P3) for high risk neuroblastoma patients who were progression-free at the end of induction chemotherapy. After autologous bone marrow transplantation (ABMT), progression-free survival (PFS) at 4 years was 38% (median follow-up 4 years). Prognostic factors for relapse after ABMT included pre-BMT disease status, bone marrow tumour content at harvest, extent of primary resection at diagnosis, and time to ABMT. *MYCN* amplification, age, stage, and pre-BMT myeloablative regimen were not significant. Allogeneic BMT did not have a better outcome than ABMT. In a retrospective, non-randomised comparison of ABMT and chemotherapy, there was a significant difference in PFS for stage IV patients. High risk subgroups possibly benefiting from ABMT could be identified, including those with tumour *MYCN* amplification, over 2 years at diagnosis, and those not in complete remission at the end of induction. A randomised prospective trial comparing myeloablative therapy with ABMT to continuous infusion consolidation chemotherapy is currently underway in CCG to determine the relative benefit.

Keywords: neuroblastoma, bone marrow transplantation, prognostic factors, allogeneic, autologous
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INTRODUCTION

MYELOABLATIVE therapy followed by bone marrow or peripheral blood stem cell rescue is under extensive investigation for treatment of childhood high risk neuroblastoma, because of the persistently dismal outcome with conventional chemotherapy [1]. Some previous reports of such treatment, using either allogeneic or autologous bone marrow transplantation (BMT), have suggested an improved outcome compared with historical patients treated with standard chemotherapy [2–7].

However, no prospective randomised trial has yet been completed to control for bias in patient selection. In addition, the necessity for bone marrow purging to remove autologous tumour cells and the relative benefits of autologous compared with allogeneic BMT have not been elucidated. These issues will be examined below in the context of three recent concomitant Children's Cancer Group (CCG) pilot studies for high risk neuroblastoma: CCG-321P2 using combination chemotherapy for 1 year, CCG-321P1 using myeloablative chemoradiotherapy and allogeneic BMT, and CCG-321P3, using myeloablative chemotherapy and autologous purged BMT.

PATIENTS AND METHODS

Three pilot studies were open to high risk neuroblastoma from CCG institutions, including CCG-321P2, open to accrual from 18 September 1986 to 1 February 1991; CCG-321P1, open to accrual from 10 February 1986 to 3 October 1991, and CCG-

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321P3, open to accrual from 18 October 1985 to 7 February 1994. Eligibility requirements included age 1–18 years at diagnosis, and stage IV neuroblastoma or stage III with either unfavourable histopathology by the Shimada classification [8, 9], serum ferritin >142 ng/ml [10], or amplification of tumour *MYCN* [11]. Diagnosis was based on unequivocal pathological appearance of the tumour or presence of bone marrow metastases together with elevated urinary catecholamines. Tumour evaluations included computerised tomography or magnetic resonance imaging of the primary tumour, bone scan, skeletal survey, and bilateral bone marrow aspirates, biopsies, and immunocytology [12]. Appropriate informed consents for the initial chemotherapy induction protocol and the subsequent allogeneic or autologous BMT were obtained according to the requirement of local institutional review boards.

Induction therapy in most cases conformed to the treatment prescribed by CCG-321P2 (Table 1), with 5 monthly courses of cisplatin, cyclophosphamide, doxorubicin and etoposide [13, 14]. In addition, delayed primary tumour resection was accomplished, when possible, with local radiation given for residual primary and metastatic disease. Patients, for whom autologous BMT was planned, had bone marrow harvest with tumour depletion by immunomagnetic beads [15] after the fourth cycle of chemotherapy. Some patients received a different induction therapy on CCG-321P3 using similar drugs in a different schedule, but these were not included in the comparative analyses of outcome between chemotherapy and BMT. Evaluation of tumour status was performed at the end of the induction period.

Eligibility for allogeneic or autologous BMT was dependent on the absence of disease progression during induction, normal organ function, and time of less than 36 weeks from diagnosis at the time of BMT. BMT was not mandatory, and the decision whether to continue chemotherapy or to proceed to BMT was by investigator and parental choice. Any patient who had a HLA-compatible sibling was eligible for allogeneic BMT on CCG-321P1, while those high risk neuroblastoma patients who had no donor were eligible for autologous BMT on CCG-321P3.

The conditioning regimens for the BMT patients combined myeloablative chemotherapy with cisplatin or carboplatin, melphalan, teniposide or etoposide, with or without doxorubicin, along with 10 Gy of total body irradiation (Table 2) [2, 16]. The first regimen utilised cisplatin, teniposide, doxorubicin, melphalan and total body irradiation (TBI). The second change to continuous infusion cisplatin over 4 days, continuous infusion of etoposide over 4 days, eliminated the doxorubicin owing to excessive toxicity, and kept the melphalan and total body irradiation the same. The third regimen substituted carboplatin for cisplatin, and tested escalating doses of carboplatin and etoposide.

Life table methods were used to estimate the progression-free survival (PFS) from time of BMT or chemotherapy [17]. The log rank statistic was used to compare the PFS probabilities between patient subgroups. Relative risk analysis was performed using the regression method of Cox. For comparison of BMT to chemotherapy, the time from diagnosis to transplant was treated as a time-varying covariate in order to allow for the varying times that ABMT took place. The need for this type of analysis to assess the effect of ABMT on outcome is imposed by the requirement that patients going to ABMT on CCG-321P3 had to be progression-free at the time of ABMT. Therefore, a simple comparison of Kaplan–Meier curves from the date of chemotherapy study would give a positively biased appraisal for the effect of ABMT, since the chemotherapy group includes all patients with early disease progression. For the Kaplan–Meier curves, the PFS of the subset of chemotherapy patients that had remained event-free and not transplanted for 8 months, was compared from that time to the PFS from the data of transplant for the patients who underwent BMT. This time was chosen since it was approximately equal to the median time to BMT.

RESULTS

Chemotherapy (CCG-321P2)

A total of 241 eligible patients with a median follow-up of 41 months (range 20–80 months) were treated on CCG-321P2. There were 36 high risk stage III patients and 205 stage IV patients. Patients who subsequently went off study for autologous or allogeneic BMT are censored at the time of discontinuing protocol therapy. The overall 3 years PFS for the group is 25%. Several clinical and biological prognostic factors were examined: age, histology, sites of metastases at dignosis, induction response, tumour *MYCN* amplification, and surgical resection. Significant favourable factors with a P-value <0.05 included age 1–2 years compared with >2 years at diagnosis, single copy tumour *MYCN*, complete response to induction therapy, and gross complete surgical resection [14].

Autologous BMT

A total of 147 children received myeloablative chemoradiotherapy and autologous purged BMT after completion of induction therapy. 73 of these had received induction therapy on CCG-321P2, while the rest had received induction with similar drugs on other schedules. Three conditioning regimens were used (Table 2): VAMP-TBI (n = 45), PEM-TBI (n = 54), and CEM-TBI (n = 48). The overall 3 year and 4 year PFS from time of BMT was 45% and 38%, respectively, with a median follow-up of 34 months. No difference in outcome was seen for the three conditioning regimens, although the toxic death rate decreased successively from 22 to 6%. Interestingly, unlike the chemotherapy regimen, tumour *MYCN* amplification was not a significant unfavourable factor for relapse after BMT. Tumour burden at the time of BMT was important, as shown by the

Table 1. Induction therapy

Week									
0	4	8	12	13	17	18	21	22	26
CDEC*	CDEC	CDEC	BM Harvest†	CDEC	Surgery	CDEC	Local radiation	CDEC‡	BMT

*CDEC, cisplatin, 60 mg/m^2/6 h, day 0; doxorubicin, 30 mg/m^2, day 2; etoposide 100 mg/m$_2$/1 h, days 2 and 6; cyclophosphamide, 30 mg/kg, days 4 and 5; †For autologous BMT; ‡Optional if delay necessary for timing of BMT.

Table 2. Pre-BMT chemotherapy protocols

	VAMP-TBI (1985–1988)	PEM-TBI (1988–1990)	CEM-TBI (1990–1994)
Cisplatin	90/m² d−9*	120/m² d−8 to −5†	
Carboplatin			640–1200/m² d−8 to −5†
Doxorubicin	45/m² d−7		
Etoposide		400/m² d−8 to −5†	500–800/m² d−8 to −5†
Teniposide	150/m² d−7, −4		
Melphalan	140 mg/m² d−6	140 mg/m² d−6	140 mg/m² d−6
	70 mg/m² d−5	70 mg/m² d−5	70 mg/m² d−5
TBI	333 cGy d−3 to −1	333 cGy d−3–−1	333 cGy d−3 to −1

*8 h infusion; †Continuous 24 h infusion; TBI, Total body irradiation. The CEM regimen included a dose escalation every 4–9 patients as shown.

result that incomplete surgical resection at diagnosis, incomplete tumour response to induction therapy, and residual tumour by bone marrow immunocytology at harvest were all significant ($P < 0.05$) for relapse. In addition, time from diagnosis to BMT was also significant, with patients transplanted before 8 months doing better than those transplanted later [2, 14].

Allogeneic BMT compared with autologous BMT

Although a total of 44 patients received allogeneic BMT, for the purposes of valid comparison, only the 20 patients who were treated with the CCG-321P2 induction described above and received the PEM-TBI (Table 2) conditioning were compared with the 36 patients who received the same induction and conditioning regimens on CCG-321P3. The overall PFS rates were not significantly different for these two groups, 25% for the allogeneic and 45% for the autologous BMT patients ($P = 0.051$), even though their disease response status at the time of BMT were equivalent. The toxic death rate for the allogeneic group was 20%, compared with 8% for the autologous group. The relapse rates were also not significantly different, 46% for the autologous and 69% for the allogeneic ($P = 0.14$) [13].

Relapse sites after BMT

The most common sites of relapse in both the autologous and the allogeneic groups were primary tumour, bone, and bone marrow [13, 16]. There was no significant difference in the incidence of relapse sites in the two groups. However, in our study, there were no patients who had relapses in lung after allogeneic BMT, a site where one would expect tumour to lodge after infusion of contaminated bone marrow, but 7% of patients had relapses in lung after autologous BMT, a site not usually seen in neuroblastoma. One of these patients had a higher than normal tumour content at the time of purging, although after purging the marrow was tumour-free to a sensitivity of 1 tumour cell per 100,000 bone marrow cells. This may add further credence to the hypothesis that infusion of contaminated bone marrow is capable of establishing tumour recurrence. This is supported by our own and other reports of miliary lung metastases in a few cases after autologous BMT for neuroblastoma [18–20]. Other support for the importance of adequate tumour purging from bone marrow comes from the recent work of Brenner and colleagues showing that, after infusion of bone marrow marked with transduced neomycin resistance gene, tumour cells in the recurrence exhibit the genetic marker [21].

Retrospective comparison of autologous BMT to continued chemotherapy

There were 73 patients in the ABMT group, and 94 patients who continued progression-free on chemotherapy after 8 months, the median time to BMT. The relative risk of relapse after ABMT was significantly lower than after chemotherapy. This difference was most notable in the higher risk patients, including stage IV disease, age >2 years at diagnosis, bone metastases within the stage IV group, partial rather than complete response to induction, and tumour *MYCN* amplification.

DISCUSSION

The results of non-randomised pilot studies by the CCG suggest a modest prolongation of event-free survival for children with high risk neuroblastoma overall, treated with an intensive four drug chemotherapy regimen. In a non-randomised population, it appears that the addition of myeloablative chemoradiotherapy followed by autologous or allogeneic BMT may be of benefit to the highest risk subgroup of patients with stage IV neuroblastoma. This group includes patients in whom complete remission is not attained after 6–8 months of induction chemotherapy with surgery and local radiation, those who are greater than 2 years at the time of diagnosis, and those with tumour *MYCN* amplification.

Although these results are of interest, they must be interpreted with caution in a retrospective, non-randomised study. All decisions regarding transplantation were at investigator and parental choice, and unknown bias may have entered into the decision, although the overall remission status, bone involvement, bone marrow involvement, age distribution, and tumour *MYCN* amplification were comparable in the two groups [22]. This result differs from a previous retrospective non-randomised comparison made by the Paediatric Oncology Group, in which 49 patients not receiving ABMT were compared with 67 patients who did, but patients were not separated according to whether progression had occurred prior to BMT [23]. However, results from Philip and co-workers, like those presented here, have suggested that autologous BMT may benefit at least a subset of high risk neuroblastoma patients [4].

Only a prospective randomised trial can determine whether myeloablative therapy with purged bone marrow support can improve the outcome for high risk neuroblastoma. Without such a study, significant bias may enter into the selection of patients for different therapies. Such a study has been ongoing in the CCG since January 1991. The primary aims of this study are: (1)

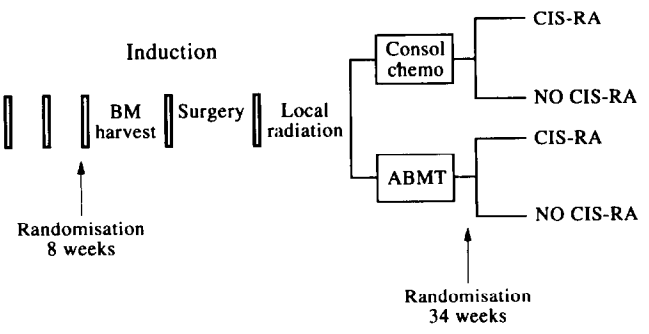

Figure 1. Schema for CCG-3891: chemotherapy versus ABMT for high risk neuroblastoma. Consol chemo, consolidation chemotherapy; CIS-RA, 13-*cis*-retinoic acid; ABMT, autologous bone marrow transplantation.

to determine, by prospective randomisation, the relative efficacy and toxicity of an intensive consolidation regimen with growth factors compared with myeloablative chemoradiotherapy with autologous purged BMT; (2) to compare, by prospective randomisation, the efficacy of maintenance biotherapy with the differentiating agent, 13-*cis*-retinoic acid, to no further therapy; and (3) to determine whether prognostic studies can predict response to intensive therapy. Thus far, as of July 1994, 367 patients have been accrued, including 304 stage IV, 50 high risk stage III, 2 *MYCN* amplified stage II, and 11 patients of initial stage I, II, or IVS who progressed without prior therapy. The overall schema is shown in Figure 1. On this protocol, patients receive 5 cycles of induction therapy, as in CCG-321P2 (Table 1). Randomisation between myeloablative chemoradiotherapy and intensive continuous infusion consolidation therapy with growth factors occurs at 8 weeks on study, to allow time for determination of medical and psychosocial eligibility for BMT. Induction includes cytoreductive surgery to all bulky disease, local irradiation to residual unresectable primary or metastatic tumour, bone marrow harvest with immunomagnetic purging in a central laboratory. All patients free of progression at the end of induction proceed to randomised ABMT or 3 cycles of consolidation with ifosfamide and continuous infusion cisplatin, doxorubicin and etoposide. At week 34, a second randomisation occurs on both arms for patients who are progression-free, between 13-*cis*-retinoic acid for 6 months and no further therapy. This study will determine whether autologous purged bone marrow transplantation preceded by ablative chemoradiotherapy prolongs survival and actually provides a long-term benefit in an unselected group of children with high risk neuroblastoma.

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