

ORIGINAL ARTICLE

Long-term outcomes in children with high-risk neuroblastoma treated with autologous stem cell transplantation

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We retrospectively analysed the outcomes of children transplanted for high-risk neuroblastoma (NB) at a single institution predominantly transplanted with total body irradiation and chemotherapy. The aims of this study were to determine the prognostic impact of clinical and biological features and to document long-term health outcomes. Forty patients were transplanted with a single unpurged autograft. Fourteen patients died from disease progression and two from late complications of treatment. Twenty-three patients are alive at a median of 4.6 years from diagnosis. Kaplan–Meier estimates of overall survival at 2, 5 and 10 years are 76 ± 7.0 , 60.2 ± 8.4 and $54.7 \pm 9.3\%$ following transplant. Response to induction therapy was significantly associated with survival ($P < 0.01$). Long-term complications included growth (100%) and pubertal failure (83%), hearing impairment (73%), orthopaedic complications (63%), renal impairment (47%) and thyroid abnormalities (36%). Intrinsic and acquired resistance to chemotherapy remains the major obstacle to improving outcomes in high-risk NB. Although patients with chemo-sensitive disease are less likely to experience a relapse, substantial therapy-related toxicities result in poor long-term health outcomes for survivors.

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plantation (ASCT) and biological agents, outcomes for high-risk neuroblastoma (NB) remain suboptimal with survival approximating 30%.^{1–11} Features associated with an improved prognosis include younger age^{5,7} and absence of bone marrow involvement at diagnosis,¹² early disease response¹³ and a complete or near complete response following induction therapy.^{6,7,10,14} A poorer prognosis has been associated with an elevated serum ferritin (> 143 ng/ml),⁶ bone and BM metastasis,⁶ detectable circulating NB cells at diagnosis,^{15,16} persisting skeletal and BM disease at the end of induction therapy,^{1,2} circulating NB cells in patients otherwise apparently disease-free¹⁶ and high-level of NB contamination at marrow harvest.^{14,15} Intensive induction therapy and ASCT have improved the outcomes of older children and those with *MYCN* amplified disease.^{6,10} Late regimen-related toxicities have increasingly been recognized and include second malignancy,¹⁷ hearing loss^{18–20} and multiple endocrinopathies.^{21–24} Despite this, neuropsychological and adaptive functioning appear to be well preserved.^{25,26}

Since 1985, high-risk NB patients at our hospital have been transplanted with an unpurged autologous graft. A previous analysis showed an excellent disease-free survival²⁷ and the objectives of this study were to update the long-term outcome of these children. We demonstrate here that initial chemo-responsiveness correlated with a lower relapse risk but long-term survivors have an unacceptably high burden of long-term health consequences.

Introduction

Despite the widespread adoption of intensive induction chemotherapy, autologous haematopoietic stem cell trans-

Patients and methods

The study was approved by the human research ethics committee of the South Eastern Sydney and Illawarra Area Health Service. Inclusion criteria were 40 consecutive children who received an ASCT for NB at the Sydney Children's Hospital (SCH), between 1 June 1985 and 31 December 2003. The cohort includes 33 patients who were treated from diagnosis at SCH and 7 patients who were referred for ASCT after receiving induction therapy at outside centres. There were three patients who were offered ASCT after relapse or progression of intermediate risk

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disease. The treatment plan was to obtain a remission with combination chemotherapy followed by delayed primary tumour resection, collect an autograft, treat the tumour bed and sites of active disease with local radiotherapy and administer conditioning therapy followed by ASCT. Successive, published, induction chemotherapy regimens were employed to achieve the best tumour response.^{27–31} Stem cell collection was scheduled following the maximum response to chemotherapy and after resection of the primary tumour. The median time between diagnosis and tumour resection was 131 days (range 93–328) and stem cells were collected at a median of 177 days (range 72–408) from diagnosis. Autografts were collected by BM harvest until January 1994, following which, peripheral blood stem cells (PBSC), mobilized with chemotherapy and G-CSF, were collected. Local radiotherapy, 18 Gy in 12 fractions, was administered to the tumour bed and sites of active disease. A modified VAMP-TBI (cisplatinum 80 mg/m², teniposide 130 mg/m², adriamycin 30 mg/m² and melphalan 120 mg/m² with 12 Gy TBI in six fractions) conditioning was administered to 34 patients,^{27, 3} received TEC (thiotepa 810 mg/m², etoposide 1800 mg/m² and cyclophosphamide 200 mg/kg) and 3 CEM (carboplatinum 1700 mg/m², etoposide 1352 mg/m² and melphalan 210 mg/m²). The median duration between diagnosis and ASCT was 231 days (range 144–443). Tumour cell purging was not performed and all autografts were infused without manipulation. From 1999 onwards, all patients received 13-*cis*-retinoic acid (160 mg/m² per day administered for 14 consecutive days in a 28-day cycle for 6 months) following ASCT.⁶ The presenting features, INSS staging, INRC response, immediately before ASCT, and outcome of the 40 patients are summarized in Table 1.

Post-ASCT evaluation

All survivors were reviewed post-ASCT to detect relapse and therapy-related complications. Thirteen have undergone detailed endocrine evaluations, which have included, where appropriate, measurement of thyroid function (free T4 (fT4), TSH), dynamic growth hormone (GH) stimulation testing (glucagon and clonidine) or 24 h GH profile and sex hormones (follicle-stimulating hormone (FSH), luteinising hormone (LH) and either oestradiol or testosterone). Limited endocrine evaluations have been performed on two other survivors and no endocrine evaluations in six others. The following definitions were used for endocrinopathies: hypothyroidism: an fT4 <9.8 pmol/l; GH deficiency: a peak GH concentration <10 mU/l; primary hypogonadism: failure to progress through puberty without hormone replacement therapy or a raised FSH or LH with no clinical signs of puberty in girls > 12 years and boys > 13 years. Height measurements were converted into sex-matched standard deviation scores using the 1978 CDC/WHO growth reference curves.³²

Statistical analysis

Progression-free survival (PFS) and overall survival (OS) were calculated from diagnosis according to the method of Kaplan and Meier. Univariate analyses of survival were performed and the log-rank test was used to identify

Table 1 Patient characteristics

Number	40
Median age (years) at diagnosis (range)	2.69 (birth–10.8)
Male:female	26:14 (65:35%)
<i>INSS staging</i>	
III	2 (5%)
IV	36 (90%)
IVs	2 (5%)
<i>MYCN status</i>	
Single copy	18 (45%)
Amplified	10 (25%)
Not known	12 (30%)
<i>INRC response</i>	
CR or VGPR	24 (60%)
PR	12 (30%)
MR, NR and PD	4 (10%)
<i>Survival</i>	
Alive at last follow-up	23 (57.5%)
Median follow-up	4.6 years (0.6–17.8)
Estimated 5-year PFS ± s.e.	59.4 ± 8.9%
Estimated 5-year OS ± s.e.	60.2 ± 8.4%

Abbreviations: ASCT = autologous stem cell transplant; INRC = International Neuroblastoma Response Criteria; CR = complete response; VGPR = very good partial response; PR = partial response; NR = no response; MR = mixed response; PD = progressive disease; INSS = International Neuroblastoma Staging System; OS = overall survival; PFS = progression free survival; s.e. = standard error.

significant differences. Disease progression was defined as an increase in the size of any existing lesion, the appearance of any new lesion or an increase in urine catecholamines despite treatment. Patients who had no response or progressive disease despite induction therapy were deemed to have progressed at the time of diagnosis. For infants with relapsed disease, PFS was measured from the diagnosis of relapse rather than from original diagnosis. The Student's *t*-test and the Mann–Whitney rank sum tests were used to test normally and non-normally distributed data respectively. The χ^2 and Fishers exact test were used to assess the significance of differing proportions within patient groups. Reported *P*-values are two-sided and a *P*-value of 0.05 was considered to indicate statistical significance.

Results

ASCT, complications and outcome

Forty patients received an ASCT and the median time to neutrophil engraftment was 15 days (range 9–54 days), but was significantly shorter for recipients of PBSC (14 days, 95% CI 11.0–16.1) compared to BM grafts (19 days, 95% CI 15.0–32.4) (*P* = 0.003). One patient died of progressive disease without being discharged. Fourteen patients died owing to progressive NB with a median time to progression and death following ASCT of 182 days (range 25–1410) and 416 days (range 55–1760), respectively. Twenty-three patients were alive with no evidence of NB progression with a median follow-up of 4.6 years (range 0.6–17.8) from diagnosis and 3.8 years (range 0.1–17.2) following ASCT.

Of the 34 patients conditioned with TBI, 19 are alive and disease-free while 12 died owing to disease recurrence and 3 died while in remission. The median survival of these patients is 4.2 years (range 0.6–18.2) from diagnosis and 3.7 years (range 0.1–17.2) from ASCT.

Six patients received chemotherapy-only conditioning and three are alive and disease-free. One patient is alive with stable disease (VGPR) and two have died owing to recurrent disease. The median survival of these patients is 1.7 years (range 1.2–3.8) from diagnosis and 0.9 years (range 0.6–3.4) from ASCT. Two of three infants, who were offered ASCT for relapsed disease, died of progressive disease. One infant remains alive with stable disease (VGPR).

Survival analysis

The 5-year OS and PFS survival rates for the cohort were 60.2 ± 8.4 and $59.4 \pm 8.9\%$ following ASCT (Figure 1). ASCT patients who achieved a CR/VGPR had significantly better survival (Figure 1 and Table 2). There was no significant difference in the survival of patients based on *MYCN* status, age, ferritin level, sex, type of harvest or period of transplant (Table 2).

Long-term complications following ASCT. There have been three deaths in remission: one patient developed acute myeloid leukaemia, one patient died from complications of renal failure and one committed suicide. In all, 26 ASCT patients developed at least 1 complication arising from their treatment, some of whom subsequently relapsed and died of progressive disease (Table 3).

Ten of 21 patients tested (47%) have renal impairment indicated by a persistent elevated creatinine or a decreased GFR (<90 ml/min/1.73 m²) following ASCT. Two patients have been dialysed and one subsequently received a living related donor kidney transplant. One patient with mild renal impairment was treated for recurrent gout before death. Of the remaining seven patients with renal complications, two were diagnosed with post transplant nephropathy, one with an obstructive uropathy secondary to the

tumour arising in the pelvic retroperitoneum, one with a combination of post-infectious glomerulonephritis and tubular dysfunction, one with recurrent urinary tract infections complicated by pyelonephritis and one with an unknown cause of renal impairment.

Growth has been a substantial problem in this cohort with a significantly lower median height s.d.-score following ASCT (-2.0 , $n = 17$) compared to diagnosis (0.4 , $n = 34$) (mean difference -2.43 , 95% CI -1.82 to -3.04 , $P < 0.0001$) with growth failure found in all 13 patients (who received TBI) with matched height measurements (median s.d.-score at diagnosis 0.4 versus -1.8 post-ASCT, mean difference -2.5 , 95% CI -2.0 to -3.2 , $P < 0.001$). GH has been measured in nine, but only one was found to be GH deficient. Four patients, none with GH deficiency, have received exogenous GH. Hypothyroidism was identified in 4 of 14 (28.6%) patients tested and a goitre found in one euthyroid survivor. Of the six pubertal patients (five males and one female), primary hypogonadism has occurred in five of six (83.3%) patients, all of whom are males.

Eleven of 15 patients tested (73%) have a bilateral high-frequency, static, hearing loss but only 5 patients currently require hearing aids. Ten patients had abnormal hearing tests during induction therapy. Twelve patients have developed cataracts.

Twelve of 19 patients (63%) have experienced a musculoskeletal complication. Osteochondromas have been found in eight patients, all of whom received VAMP-TBI. One patient with a thoracic primary developed a thoracic kyphoscoliosis. Two patients have slipped capital femoral epiphyses one of whom also has a leg length discrepancy. Four patients have experienced a fracture following the transplant, including fractures of a vertebrae, leg, ankle and arm.

Three second malignancies have been diagnosed including one acute myeloid leukemia, a plexiform fibrohistiocytic tumour of the chest wall and a basal cell carcinoma.

Discussion

The survival rates for this cohort of patients receiving an unpurged ASCT for high-risk NB (60.2 ± 8.4 and $59.4 \pm 8.9\%$ at 5 years for OS and PFS) compare favourably with other studies,^{1–6,9–11} but surviving patients had significant late effects of therapy. This study reports the outcome of children who received an ASCT and therefore the survival analysis reflects the exclusion of those patients who died of disease progression during induction therapy and were not transplanted. These results have important implications for the choice of treatment for high-risk NB patients, and demonstrate the very narrow therapeutic index inherent with conventional chemo-radiotherapeutic treatment strategies.

Although the cohort is heterogenous owing to accrual over a long period, successive dose intensive chemotherapy protocols, with similar remission induction rates,^{27–31} were used and we do not believe that this has had a substantial influence on the post transplant outcomes. The only factor predictive of survival was the quality of the response to

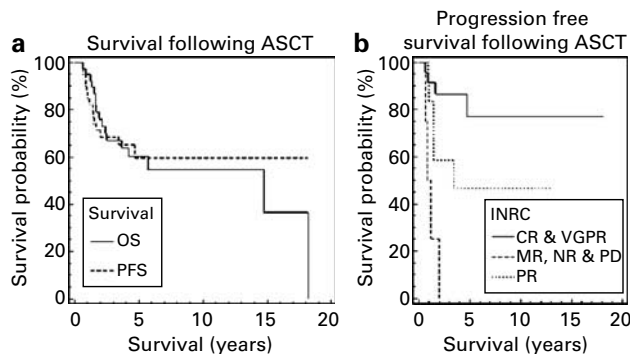


Figure 1 Survival following ASCT for high-risk NB. (a) OS and PFS for the cohort following ASCT. (b) PFS according to INRC response immediately before ASCT. ASCT = autologous haematopoietic stem cell transplantation; INRC = International Neuroblastoma Response Criteria; NB = neuroblastoma; OS = overall survival; PFS = progression-free survival.

Table 2 Univariate survival analysis for ASCT patients

<i>INRC response (n = 40)</i>	<i>CR or VGPR (n = 24)</i>	<i>PR (n = 12)</i>	<i>MR, NR or NR (n = 4)</i>	
OS	80.2 ± 8.9	45.7 ± 15.5	0 ± 0	<i>P</i> < 0.0001
PFS	76.9 ± 11.1	46.7 ± 15.4	0 ± 0	<i>P</i> = 0.0001
<i>MYCN status (n = 28)</i>	<i>Not amplified (n = 18)</i>	<i>Amplified (n = 10)</i>		
OS	66.1 ± 12.5	46.7 ± 16.6		<i>P</i> = 0.26
PFS	61.9 ± 14.7	50.0 ± 14.7		<i>P</i> = 0.16
<i>Ferritin level (n = 29)</i>	<i>< 143 ng/ml (n = 7)</i>	<i>≥ 143 ng/ml (n = 22)</i>		
OS	71.4 ± 17.1	55.9 ± 11.1		<i>P</i> = 0.76
PFS	68.6 ± 18.6	49.7 ± 11.4		<i>P</i> = 0.77
<i>Age (n = 40)</i>	<i>< 18 months (n = 8)</i>	<i>> 18 months (n = 32)</i>		
OS	72.9 ± 16.5	57.1 ± 9.5		<i>P</i> = 0.69
PFS	62.5 ± 17.1	60.6 ± 9.6		<i>P</i> = 0.45
<i>Graft type (n = 40)</i>	<i>Bone marrow (n = 23)</i>	<i>PBSC (n = 17)</i>		
OS	63.5 ± 10.4	55.1 ± 14.0		<i>P</i> = 0.80
PFS	62.1 ± 11.0	58.0 ± 13.6		<i>P</i> = 0.69
<i>Sex (n = 40)</i>	<i>Female (n = 14)</i>	<i>Male (n = 26)</i>		
OS	51.6 ± 14.6	65.4 ± 10.0		<i>P</i> = 0.18
PFS	39.7 ± 15.8	71.3 ± 9.2		<i>P</i> = 0.12
<i>Period of ASCT (n = 40)</i>	<i>Before 15/6/1994 (n = 22)</i>	<i>After 15/6/1994 (n = 18)</i>		
OS	57.3 ± 10.9	65.2 ± 12.9		<i>P</i> = 0.70
PFS	56.0 ± 11.4	69.1 ± 11.6		<i>P</i> = 0.77

Abbreviations: ASCT = autologous stem cell transplant; CR = complete response; INRC = International Neuroblastoma Response Criteria; MR = mixed response; NR = no response; OS = overall survival; PBSC = peripheral blood stem cells; PD = progressive disease; PFS = progression-free survival; PR = partial response; VGPR = very good partial response.

Table 3 Long-term complications following ASCT for NB

<i>Complication</i>	<i>Number (%)</i>	<i>Notes</i>
Deaths in remission	3	AML, renal failure, suicide
Second cancers	3	AML, basal cell carcinoma, plexiform fibrohistiocytic tumour
Renal impairment	10 of 21 (47%)	Two dialysed, one subsequently received renal transplant
Hearing impairment	11 of 15 (73%)	Five using hearing aids
Cataracts	12	
Growth failure	13 of 13 (100%)	All received TBI
GH deficiency	1 of 9 (11%)	Four treated with growth hormone
Hypothyroidism	4 of 14 (29%)	One patient with goitre
Hypogonadism	5 of 6 (83%)	
Musculoskeletal	12 of 19 (63%)	Osteochondromas (8), kyphoscoliosis (1), slipped capital femoral epiphysis (2), leg length discrepancy (1), fractures (4). Three patients had > 1 musculoskeletal complication

induction therapy with no survivors among those who failed to respond. Children who achieved a CR/VGPR had excellent outcomes compared to those who attained a PR (OS 80.2 ± 8.9 versus 45.7 ± 15.5% and PFS 76.9 ± 11.1 versus 46.7 ± 15.4% at 5 years). Matthay and co-workers have shown an association between a CR/VGPR and a lower relapse rate following ASCT,^{6,14} a finding replicated in the LMCE3 cohort⁷ and the German Society of Paediatric Oncology and Hematology NB97 trial.¹⁰

The long-term health outcomes in our patient cohort have been disappointing, with chronic problems particularly related to disordered growth and endocrine function. The majority of patients received TBI-based conditioning therapy and we have only short-term follow-up data on the few patients who received chemotherapy-only conditioning. There is little published information on the long-term outcomes of survivors of high-risk NB, and although our data should be interpreted in the light of a retrospective study, we believe that it provides valuable information to guide health management. All long-term survivors have experienced at least one long-term health complication, similar to findings of the Memorial Sloan Kettering Cancer Center cohort.²⁴ Two deaths in remission are directly attributable to long-term complications, one a secondary malignancy and the other due to the complications of end-stage renal failure. The suicide of the third patient may have been related to the long-term health consequences.

Marked growth failure has been described following TBI-containing conditioning regimens for NB but is less evident in patients transplanted with chemotherapy-only regimens.^{21–23} We documented GH deficiency in only one of nine survivors tested, indicating that factors other than growth hormone insufficiency, such as radiotherapy fields encompassing vertebral bodies and endocrinopathies involving thyroid³³ and sex hormone secretion, contribute to growth failure in these patients. Pubertal failure has been a significant problem among our cohort and has been noted in NB survivors²⁴ and recipients of fractionated TBI for transplant conditioning.³⁴ Previously, we and others have reported osteochondromas arising in patients following ASCT for high-risk NB.^{35,36} Since our previous report of

five cases,³⁶ we have identified a further three cases. Other musculoskeletal complications including fractures and slipped capital femoral epiphyses were observed in six patients. Only one fracture occurred in a pubertal patient; however, given the high incidence of pubertal failure in the adolescent survivors, we are concerned about their fracture risk and risk of osteoporosis in the longer term. The relatively high burden of renal complications is not surprising owing to the combination of nephrotoxic insults throughout the course of therapy for high-risk NB. Hearing loss was documented in 73% of patients tested, 45% of whom required hearing aides. The majority of patients had hearing loss documented before ASCT. NB patients are particularly vulnerable to hearing loss, not only from the cumulative cisplatin dose at a young age³⁷ but also from the other ototoxic insults, including aminoglycoside antibiotics and diuretics. Our result is not substantially different from other high-risk NB cohorts.^{18,20} Kushner and co-workers documented moderate-to-severe high-frequency hearing loss (Brock grade 2–4) in 84% of high-risk NB patients following induction therapy and ASCT.²⁰

Although the post-transplant relapse rate is reduced in patients with responsive disease compared to historical series,^{1–3,5} the overall burden of therapy is high and the range of toxicities in long-term survivors excessive. We believe that TBI is a major contributor to their genesis. Despite the limitations of this study, a retrospective analysis of a small cohort accrued over a long period, these results allow an improved focus on important issues facing clinicians who treat patients with high-risk disease. First, while children with chemo-responsive disease have the opportunity of long-term survival, the most common cause of death remains disease progression. Intrinsic and acquired resistance to therapy remains the most important barrier to achieving improved outcomes for children with high-risk disease. Second, survivors face long-term morbidities that result in significant impairment in the quality of life and they would benefit from more effective, but less toxic therapy, if available.

High-risk NB patients, transplanted with a TBI-containing conditioning regimen, face a high risk of unacceptable long-term side effects. A number of approaches could be taken to improve survival and reduce the toxicity of therapy. Since relapse remains the most common cause of death, the identification of novel agents, which could be used during all phases of treatment (induction therapy, consolidation therapy and post-remission), is an important goal. High-dose consolidation therapy, ASCT and graft purging have been emphasized in recent studies, and despite substantial differences in induction and ASCT conditioning regimens and the use of graft purging, recent randomized trials of ASCT for NB have produced similar long-term results.^{6,10,11} Alternative consolidation strategies, such as tandem transplantation, may provide a survival benefit for high-risk patients but are likely to come with a risk of long-term side effects if they contain TBI.³⁸ George and co-workers have reported promising long-term survival following tandem transplantation with a regimen containing 12 Gy TBI.³⁸ Although a comprehensive analysis of late effects in this tandem transplant cohort was not reported, the authors noted a similar range of late effects, including

growth failure, lack of pubertal progress and hypothyroidism in their surviving patients.³⁸ We suggest that the omission of TBI from NB transplant conditioning therapy may be important to achieve an acceptable balance between the chance of long-term cure and the risk of late side effects. Based on the observation that children who respond well and achieve a CR/VGPR before ASCT have the best chance of cure (data presented here and from the CCSG, LMCE3 and the German Society of Paediatric Oncology and Hematology NB97 cohorts),^{6,7,10,14} we believe that trialling novel induction agents, used in upfront window studies to improve the pretransplant response rate, should be considered in future studies of high-risk NB.

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