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Original Paper

Treatment of Neuroblastoma Stage 4 with ^{131}I-meta-iodo-benzylguanidine, High-dose Chemotherapy and Immunotherapy. A Pilot Study

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Disseminated neuroblastoma after infancy has a prognosis of approximately 10–20% with conventional therapy. We investigated the role of high-dose chemotherapy (HDCT) with peripheral blood stem cell (PBSC) rescue in combination with ^{131}I-metaiodobenzylguanidine ([^{131}I-m]IBG). 11 children with neuroblastoma stage 4 were pretreated within the German Neuroblastoma Trial NB90 and included in a high-dose concept for consolidation. Remission was documented by ultrasound, CT, NMR, or [^{123}I-m]IBG scanning. HDCT was a combination of melphalan (180 mg/m^2), carboplatin (1,500 mg/m^2) and etoposide (40 mg/kg). All children were treated by [^{131}I-m]IBG (0.58 GBq/kg) prior to high-dose treatment. All 11 children were additionally treated with antiGD2 murine- or chimeric-antibody (ch14.18). 4 children had no change to their remission status but three achieved a complete response (from a partial response to first line) and one a partial response (from no response to first line). The other 3 children progressed, 2 dying of their disease. Using Kaplan–Meier analysis, the probability of progression-free survival was 0.70 ± 0.15 with a median observation time of 19 months. 9/11 children are alive, 8 without progression or relapse, whilst 2 have died of their disease. The combination of mIBG plus high-dose chemotherapy with PBSC support supplemented by immunotherapy with antiGD2 antibody appears to be a feasible and effective treatment regimen for disseminated neuroblastoma in this limited series. Larger numbers of patients should be treated to confirm these results. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

NEUROBLASTOMA STAGE 4 in children over 1 year of age is still an unresolved problem in paediatric oncology. Even with highly intensified chemotherapy plus maintenance treatment the outcome was [1, 2] and is [3] poor. Results are still unsatisfactory with myeloablative megatherapy [4, 5] and even with a double megatherapy regimen [6]. Neither adding interleukin 2 (IL2) treatment [7] nor allogeneic transplantation have resolved this problem [8]. For some time, we have used and shown the efficacy of mIBG treatment [9, 10], high-dose therapy [11, 12] and immunotherapy [13, 14]. Therefore, we decided to combine these treatment modalities in a pilot study, which is described here.

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PATIENTS AND METHODS

Patient characteristics

From January 1991 to June 1997 11 patients with neuroblastoma stage 4 over 1 year of age at diagnosis were included in the pilot study (Table 1). All patients were study patients of the German Neuroblastoma Study NB90 [15]. Within this study, the decision for high-dose treatment was optional. The patients were referred from four centres (including our own). Age at diagnosis ranged from 1.6 to 9.8 years with a median of 3.3 years (mean of 3.8 ± 2.3 years); age at treatment was at a median 4.2 years with a range from 2.2–10.7 years (mean

Table 1. *Patients characteristics*

Patient	Age (years) at DX	Age (years) at HDTX	Body weight kg	Response	mIBG [Gbq/kg]	Bone lesions	Courses of MAb treatment
1	3.0	4.2	19.0	NR	0.58*	n	2
2	1.6	2.2	10.0	PR	0.74	y	2
3	5.2	6.0	21.0	VGPR	0.49	y	2
4	2.4	3.4	14.3	CR	0.78	y	5
5	3.2	4.2	18.8	PR	0.51	y	4
6	3.3	4.0	16.0	VGPR	0.45	y	5
7	4.9	5.4	13.6	NR	0.82	y	5
8	8.1	9.1	30.0	PR	0.37	y	5
9	4.5	5.6	17.2	PR	0.65	y	5
10	3.3	4.3	15.4	PR	0.72	y	3
11	9.8	10.7	37.8	CR	0.29	y	3
Median	3.3	4.2	17.2		0.58		
Mean ± SEM	3.8 ± 2.3	5.0 ± 2.5	19.4 ± 7.6		0.58 ± 0.16		

DX, diagnosis; HDTX, high-dose treatment; Response, Response on previous treatment; bone lesions, mIBG-positive bone lesions at diagnosis; mIBG, high-dose mIBG, *received mIBG treatment in spite of having no bone lesions because of a huge abdominal rest tumour; MAB, monoclonal antibody; ± SEM, standard error of the mean.

5.0 ± 2.5). Body weight at apheresis and transplantation ranged from 10 to 37.8 kg with a mean of 19.4 ± 7.6 kg.

Diagnostic parameters and risk features

In Tables 2 and 3 diagnostic parameters and risk features of the patients are listed. In most patients catecholamines in urine ($n = 11$) and/or serum ($n = 9$), serum-ferritin ($n = 6$), serum-lactate dehydrogenase (LDH) ($n = 7$) and serum-neuron specific enolase (NSE) ($n = 10$) were elevated. The bone marrow was involved in 10 cases, positive bone marrow immunology was obtained in 7 cases. Bone lesions were documented by conventional X-ray in 5 of 7 examined cases, by technetium-scans in 9 of 11 cases and by mIBG-scans in 11 of 11 cases.

First line treatment

First line treatment of all patients was according to the German Neuroblastoma Trial NB90 [15] with four to eight courses of protocol therapy. The treatment consisted of two alternating combinations (4×PEV: cisplatin 5×40 mg/m^2, etoposide 4×125 mg/m^2, vindesine 1×3 mg/m^2; 4×VDIA: vincristine 2×1.5 mg/m^2, actinomycin-D 5×200 mg/m^2, ifosfamide 5×1.5 mg/m^2, doxorubicin 2×30 mg/m^2). 2 patients received 4 or 5 treatment blocks of NB90, 1 had 6 blocks and 8 had 8 blocks. 10 patients received 1 and 1 patient received 2 surgical interventions (1 biopsy only, 5 total resection, 2 incomplete resection microscopically, 2 incomplete resection macroscopically, 1 no tumour microscopically). 7 patients received no first line radiotherapy, 1 received a 30 Gy dose to the primary tumour and 3 received 30 Gy dose for metastases.

2 children were in complete first remission (CR1) when included in the study, 2 in first very good partial remission (VGPR1), 5 in first partial remission (PR1) and 2 were treated after non-response (NR1). All 11 children were treated as consolidation. Remission criteria followed international definitions [16].

Stem cell mobilisation and harvest

The treatment schedule was the same in all patients: after finishing the conventional first line treatment, the mobilisation and harvest of peripheral blood stem cells was organised. Mobilisation has been done either directly following protocol chemotherapy by adding G-CSF (Neupogen®, Amgen, Munich. Germany) at a dose of 10 μg/kg or after an additional treatment with 4 g/m^2 cyclophosphamide. Mobilisation and harvesting have been described in detail previously [12].

Preparation regimen and stem cell transplantation

Preparation started in all children with high-dose [131I-meta]Iodobenzylguanidine (HD-mIBG) [9, 10]. Inclusion criteria for HD-mIBG treatment were informed consent (the consent for mIBG was separated from the consent for high-

Table 2. *Risk criteria at primary diagnosis*

Parameter	No. of patients Normal	Pathological	n.d.
Catecholamines			
Urine	–	11	–
Serum	–	9	2
Serum ferritin	1	6	4
Serum NSE	–	10	1
Serum LDH	4	7	–
Bone marrow cytology	1	10	–
Bone marrow immunology	–	7	4
X-ray skeleton	2	5	4
Technetium scan	2	9	–
mIBG scan	–	11	–

n.d., not done.

Table 3. *Values at primary diagnosis*

Parameter	Mean	SEM	Min	Max	*n*
VMA serum (ng/ml)	172.0	101.7	17	294.4	7
HVA serum (ng/ml)	235.8	152.5	69.3	438.0	7
Ferritin (ng/ml)	307.8	235.7	99	808	6
NSE (ng/ml)	155.6	146.6	21	559	10
LDH (U/l)	809.96	718.3	124	2498	11

SEM, standard error of the mean.

dose treatment) a minimum age and cooperation of the patient and initial and/or actual mIBG-positive lesions (Table 1). HD-mIBG therapy was performed in the treatment unit of the nuclear medicine department as described in detail previously [9]. HD-mIBG dosage was 0.58 ± 0.16 GBq/kg body weight with a range of 0.29 to 0.82 GBq/kg bw. The defined maximum of legally accepted total activity/patient was 11.1 GBq. That upper limit caused different doses relatively to body weight with lower doses for children with higher body weights (Table 1). Children usually stayed 6–7 days in the isolation unit and left the unit when the radiation dose/h had fallen below the legally defined thresholds. Directly thereafter, preparation was started with melphalan (180 mg/m^2 in 4 equally divided doses as intravenous (i.v.) push), carboplatin (1500 mg/m^2 in 3 equal doses as short time infusion over 4 h) and etoposide (40 mg/kg as 4 h infusion). Melphalan was administered at days -8 to -5, etoposide at day -4 and carboplatin at days -4 to -2. After a pause on day -1 PBSC were thawed and directly infused without further manipulations as described before [12]. Post transfusion all children were treated by cytokines either GM-CSF (250 µg/m^2; $n = 3$) or G-CSF (10 µg/kg: $n = 8$) until achieving a stable WBC. In 8 children before re-transfusion positive selection of PBSC by immunoadsorption was performed ($n = 3$ CellPro®, Munich, Germany; $n = 5$ MACS® Milteny, Germany) [17].

Monoclonal antibody treatment

After recovery from high-dose therapy a renewed informed consent was necessary for antibody treatment. We used a chimeric monoclonal antibody (MAb) (ch14.18) provided by Dr Reisfeld, Scripps Clinic, La Jolla. Previously, we have detailed the properties of this antibody [13, 14]. Antibody treatment was given for 5 days with 20 to 40 mg/m^2 antibody infusion over 8 h. Abdominal pain made supportive treatment with morphine hydrochloride necessary.

Statistics

Life table analysis and graphical plotting was done using the Kaplan–Meier algorithm calculating the probability of progression free survival from the day of stem cell support.

RESULTS

Recovery

All 11 patients completed the preparation regimen and could be rescued by the thawed stem cells. In the first 3 patients grafted with unselected PBSC a mean of $2.7 \pm 0.3 \times 10^8$ (range 2.3–3.1) MNC/kg bw was administered, whilst in the next 8 patients a mean of $2.6 \pm 1.4 \times 10^6$ (range 0.2–5.0) CD34$^+$ cells/kg bw were given. Time to achieve > 500 ANC/µl was similar in both groups and ranged from 8 to 14 days with a mean of 11.6 ± 1.7 days (Table 4). Stable platelet levels were achieved in all patients but took longer to achieve than the time in hospital.

Toxicity

Toxicity is reported as an NCI score (Table 4). Main extrahaematological toxicity was mucositis, infection, disturbance of renal function and diarrhoea. Mucositis occurred in all children at least as grade 2, but was more severe in 5 patients (2 had grade 3; 3 had grade 4). Signs of infection occurred in all children as fever of unknown origin (grade 3 in only 1 child). All children had been pretreated with nephrotoxic agents so it was not unexpected to observe grade 3 nephrotoxicity events. Grade 2 diarrhoea was observed in 9 and grade 4 in 2 children. There was no fatal event during and following hospitalisation caused by a non-relapse event.

Side-effects of antibody treatment have been reported earlier in detail [14]. The pattern of side-effects was no different in our patients.

Survival

The main endpoints of our study were response and treatment toxicity. However, in Table 5 progression-free survival (PFS) is presented at the day of the analysis (1 January 1998). It ranges from 0.43 to 5.61 years (mean 2.15 ± 1.79 years). The Kaplan–Meier estimate (with a median observation time of 19 months) for the whole cohort is 0.70 ± 0.15 (Figure 1) calculated from the day of stem cell rescue.

Response to treatment (Table 5) in 4 children was 'no change of remission state' (pt 11 was already in CR before start of treatment, pts 8 and 9 were in partial remission and pt 6 was in very good partial remission). In 4 children an amelioration of remission state from PR to CR (pts 2, 3, 5) or from NR to PR (pt 7) was observed. 3 children progressed

Table 4. Time to achieve > 500 ANC/µl and toxicity

		Toxicity (NCI)			
Patient	> 500 ANC (d)	Mucositis	Infection	Renal	Diarrhoea
1	+11	2	2	0	2
2	+8	3	2	0	2
3	+12	y	2	0	2
4	+14	2	2	2	4
5	+13	2	3	0	2
6	+11	2	2	2	2
7	+13	4	2	3	4
8	+10	2	2	0	2
9	+13	4	2	3	2
10	+11	3	2	2	2
11	+12	4	2	2	2

> 500 ANC; day at which an absolute neutrophil count of > 500/µl was achieved; toxicity, grade of toxicity according to the NCI score modified by SIOP for children.

Table 5. Response to overall treatment

Patient	Overall response	Patient status (survival in years)
1	PROG-dod	dod (1.19)
2	⇒CR	a/w (+5.61)
3	⇒CR	a/w (+5.21)
4	REL-dod	dod (1.33)
5	⇒CR	a/w (+3.15)
6	CVGPR	a/w (+2.25)
7	⇒PR	a/w (+1.89)
8	CPR	a/w (+1.56)
9	CPR	a/w (+1.13)
10	PROG. alive	rel (+0.54)
11	CCR	a/w (+0.43)

Changes are marked by ⇒. CCR, continuous complete remission; CPR, continuous partial remission; PROG, progression; REL, relapse; dod, dead of disease; a/w, alive and well; rel, relapse (date of event or when last seen).

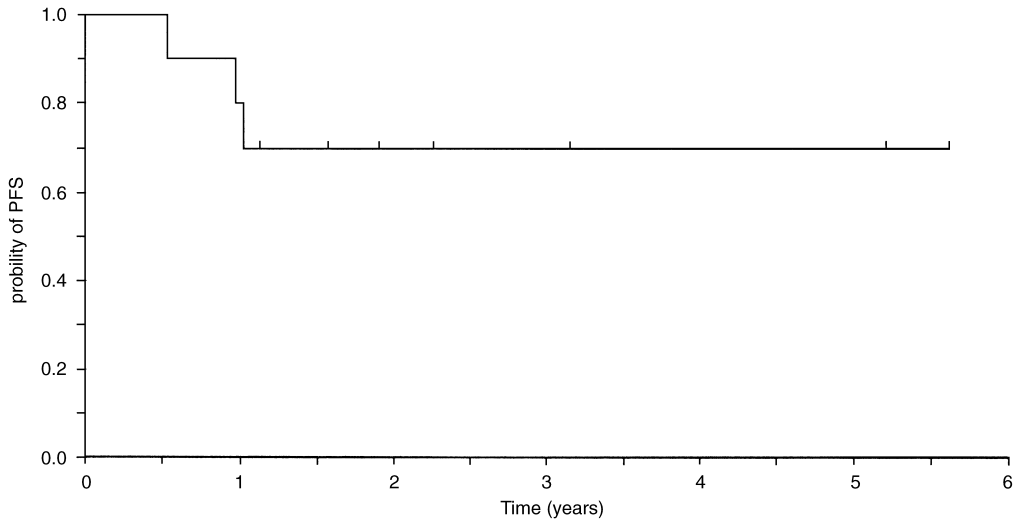

Figure 1. Probability of progression free survival (PFS) for the study group calculated from the day of stem cell rescue; PFS 0.7 SEM 0.145. $n = 11$.

(pts 1, 4, 10) and 2 of them died from disease (pts 1, 4) of risk factors.

DISCUSSION

We treated 11 children after a standard induction treatment with a combination of HD-mIBG. HD-chemotherapy and MAb therapy. All patients were more than 1 year of age at diagnosis. The risk features of our patients indicate a group with the well known pattern of elevated NSE, LDH and ferritin [2]. Berthold [2] has demonstrated the role of LDH as an important risk parameter with patients with stage 4 neuroblastoma and elevated LDH having 13% survival at 6 years. 7 of our patients had an elevated LDH-level. Other biological risk factors could not be sufficiently documented, but do not seem to add additional information compared with localised disease [18]. In fact, the patients which received mIBG plus high-dose chemotherapy at our BMT unit belonged to a high-risk group, with only 2 of 11 children in complete first remission. Of the three children who progressed, 1 was a non-responder, 1 had PR and 1 a CR to first line treatment—the failure of therapy for the NR and PR patients was, therefore, not unexpected.

Due to the small group of patients, an analysis of risk factors is not possible. The side-effects were tolerable and not therapy limiting. In comparison with other studies, the outcome in the presented study is favourable. It was reported [4], in 28 patients > 1 year at diagnosis, the survival of only 1 patient after high-dose melphalan and autologous bone marrow transplantation. In the Spanish Neuroblastoma Group, the probability of survival with stage IV was 0.24 at 5 years. Philip and colleagues [6] reported an outcome of 32% survival at 5 years for patients given a highly intensive megatherapy study. The toxic death rate in this group of 33 patients was 24%. In the European registry, overall survival after 5 years was 33% for stage 3 and stage 4 patients [5]. Persistence of bone lesions indicated a high risk, but no advantage for allogeneic versus autologous transplantation was observed, as was seen in the study of Matthay [8]. It is difficult to explain our more favourable results. The melphalan dose in [4] was much higher than in others without a better result. The role of mIBG seems to be important considering the outcome and the low toxic death rate. Similar to our results, Matthay and colleagues [19] observed a response of children to mIBG treatment after relapse. Additionally, the same authors [20] showed the superiority of high-dose mIBG over normal dose scanning in detecting persisting bone lesions. Gaze and colleagues reported feasibility and tolerability of combined mIBG/high-dose treatment in 5 children with advanced disease [21]. We assume, that most patients with neuroblastoma demonstrating a negative mIBG scan after completing standard therapy are, in fact, not in remission [22]. These patients may benefit from the irradiation of lesions that would not have been identified otherwise and consequently would not have been treated without mIBG. McCowage and colleagues also treated children with neuroblastoma stage 4 with a modified VAMP-TBI and yielded a disease-free survival rate of 87% [23]. The difference with our study is the use of TBI, which appears to cause a higher rate of long-term toxicity in these small children. More important is the selection of CR and VGPR, which could have played a role in the favourable results achieved. Another important study came from the Children's Cancer Group [24] which compared two studies and found a benefit for high-dose treatment with autologous bone marrow rescue. High-dose therapy in these studies included TBI, and also carboplatin, melphalan and etoposide in the later phase of the trial. In the retrospective analysis, superiority of the high-dose arm was found especially for children who had not completely responded to the induction treatment. The results of this analysis are in line with our experience.

In conclusion, our study seems to demonstrate a benefit for patients treated with the multimodality regimen. However, it appears essential to re-evaluate these results in a larger study.

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