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Double megatherapy and autologous bone marrow transplantation for advanced neuroblastoma: the LMCE2 study

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Summary In the LMCE1 study using a single course of megatherapy most of the relapses occurred during the first 2 years after autologous bone marrow transplantation. A second pilot study (LMCE2) was therefore set up using a double harvest/double graft approach with two different megatherapy regimens. Objectives were to test the role of increased dose intensity on response status, relapse pattern and overall survival.

Thirty-three patients (20 boys, 13 girls) with a median age of 53 months at first megatherapy (range, 17-202 months) entered this study. They were cases either with refractory disease in partial response after second line treatment for stage 4 neuroblastoma ($n = 25$) or after relapse from stage 4 ($n = 5$) or stage 3 disease ($n = 3$).

All patients received Etoposid and/or Cisplatinum (or Carboplatin) containing treatments before megatherapy. The first megatherapy regimen was a combination of Tenoposid, Carmustine and Cisplatinum (or Carboplatin), the second applied Vincristin, Melphalan and Total Body Irradiation. The first harvest was scheduled 4 weeks after the last chemotherapy, the second 60 to 90 days after megatherapy. All marrows were purged *in vitro* by an immunomagnetic technique. Median follow up time since first megatherapy is 56 months.

Response rates for evaluable patients were 65% (complete response rate:16%) for megatherapy 1 and 60% (complete response rate:25%) for megatherapy 2. Considering that only patients with delayed response or relapse were eligible for this pilot study the overall survival was encouraging with 36% at 2 years and still 32% at 5 years. The costs for these survival rates were high in terms of morbidity (four early and four late toxic deaths; toxic death rate:24%). Double harvesting may have the disadvantage of delayed engraftments related in part to a disturbance of marrow microenvironment by megatherapy 1. This double megatherapy approach achieved a prolonged relapse free interval (median 11 months, range 2-31 months) in patients reaching megatherapy 2 and justifies further evaluation of concepts with consecutive dose-escalation.

Stage 4 Neuroblastoma in children over one year of age at diagnosis is the most common childhood malignancy before the age of five (Carlsen *et al.*, 1986). Historical groups only had a survival expectancy of about 10% at 3 years with conventional chemotherapy (Shafford *et al.*, 1984; Hartmann *et al.*, 1983; Letourneau *et al.*, 1985; Rosen *et al.*, 1984). More intensive induction treatments (Shafford *et al.*, 1984; Bernard *et al.*, 1987, Philip *et al.*, 1987*b*; Hartmann *et al.*, 1988; Berthold *et al.*, 1990; Pinkerton *et al.*, 1990; Kushner *et al.*, 1987; Philip *et al.*, 1988) are now able to achieve initial response rates up to 90%. Megatherapy (MGT) and other consolidation modalities further contributed to improve survival rates (Pritchard *et al.*, 1982; Graham-Pole *et al.*, 1984; August *et al.*, 1984; D'Angio *et al.*, 1985; Pritchard *et al.*, 1986; Pinkerton *et al.*, 1987; Graham-Pole 1991*a*; Dini *et al.*, 1991; Zucker *et al.*, 1991; Philip *et al.*, 1985, Philip *et al.*, 1987*a*; Philip *et al.*, 1991; Hartmann *et al.*, 1986; Hartmann *et al.*, 1987, Seeger *et al.*, 1991). Although survival at 2 years has clearly improved to approximately 40%, the long-term survival remains unlikely for at least 75% of the patients due to later disease progressions or relapse. (Philip *et al.*, 1991).

Our group has previously reported an intensified induction treatment (Bernard *et al.*, 1987) and consolidation with Vincristin (VCR), Melphalan (L-PAM) and Total Body Irradiation (TBI) followed by purged Autologous Bone Marrow Transplantation (ABMT) in 72 consecutive unselected patients with stage 4 neuroblastoma over one year of age at diagnosis (Philip *et al.*, 1987*a*; Philip *et al.*, 1991). A significant impact on progression-free survival at 2 years was achieved, but it is not possible to differentiate if this overall improvement is related to intensified induction or MGT followed by BMT or both. No relapses were observed before 4 months post-MGT in the LMCE1 study (Philip *et al.*, 1991) with the majority occurring during the following two years.

These results served as the background to a pilot study in a group of patients who were not eligible for the LMCE1 study (Bernard *et al.*, 1987; Philip *et al.*, 1987*a*; Philip *et al.*, 1991). These patients showed delayed response to second or third line induction treatments or entered the study after relapse. In an attempt to further improve response and to decrease the incidence of early relapse, patients received two different courses of MGT within a 3 months interval. Bone marrow was harvested twice, before and after the first MGT (MGT1). The objectives of this study were to assess the effect of increased dose intensity on response as well as on relapse pattern and overall survival.

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Patients and methods

Methods

Parents of all children gave informed consent according to the Laws for clinical research in France or other countries. The LMCE2 protocol was reviewed and approved by the Comité d'Ethique de l'Université Claude Bernard at Lyon, France. Patients for this phase II study were selected on the basis of an estimated poorer outcome due to initial minor response to multicenter protocols or previous treatment failure. This study population is heterogenous due to patients inclusion criteria and bias of case selection cannot be excluded since this is a non-randomised study with a high percentage of transferred patients.

Definition of disease status

Patient evaluation at diagnosis was heterogenous since patients were not included in a common protocol at treatment start. Primary tumours were evaluated with CT-scans and some with ultrasound in addition. Assessment of metastases included iliac bone aspirates (one to four sites) and core biopsies (one to four sites). At diagnosis applied diagnostic methods for evaluation of bone disease were heterogenous. TC99-scan only was used in 12 patients, mIBG in eight, both in 11 patients and two patients showed bone lesions on radiographs (but had no further evaluation). Standarised cytohistology was performed in all patients.

At time of inclusion in the protocol, patients were uniformly reevaluated in all centers by a full diagnostic work up regarding residual tumour size, urinary catecholamine excretion, bone lesions and bone marrow infiltration. Primary tumour sites and other residual soft tissue masses were evaluated by CT-scans and ultrasound. As a rule, four bone marrow aspirates and four trephine biopsies were performed at inclusion, 1 to 2 weeks before MGT and 2 to 3 months after MGT. Bone marrows were evaluated by immunohistomorphology but a result of positive immunology was taken into account only when clumps of tumour cells were diagnosed. Assessment of bone disease was performed with mIBG-scans in 29 patients (88%) and 14 had additional Tc99-scans. Four patients had Tc99 scans only. Residual improved, but positive Tc99 lesions were biopsied to rule out active disease. Partial response (PR) was defined as previously published in detail (Philip *et al.*, 1987*c*) as 50% or more improvement of at least two criteria (initial tumour, bone marrow and bone). Sensitive relapse (SR) refers to the same PR criteria as above. Only one SR in 2nd complete response (CR) is included in this series. Resistant relapse (RR) defines minor response (< 50%) or disease progression. Patients were evaluated for response 2 weeks before MGT1, 60 to 90 days post MGT1 and before the second harvest. Final evaluation was scheduled 2 months after MGT2 and every 6 months during the first 2 years (then as indicated clinically).

Bone marrow harvest

Autologous bone marrows were evaluated according to standards as previously published (Combaret *et al.*, 1989*a*) using both cytological and double fluorescence analysis of the bone marrow. Both bone marrow harvests were treated *ex-vivo* by the immunomagnetic depletion procedure as previously published in detail (Combaret *et al.*, 1989*b*; Favrot *et al.*, 1987, 1989) with no detectable residual tumour cells after the procedure (detection limit 10^{-5} tumour cells). Patients receiving allogenic BMT are not included in this analysis.

Treatments

First line chemotherapy is the original treatment protocol which the patients entered at diagnosis. Second line chemotherapy refers to the change in treatment policy due to minor response to first line treatment. Surgical attempts to remove the primary tumour were undertaken in all but one patient. Results of surgery as part of pre-MGT treatments are given in detail on Table I. In fact, complete excision of primary tumours was achieved (PT1/PT3A) in only 8 patients of the PR group. For relapse patients results of surgery are detailed for first line treatments and was performed only in five during second line treatment. Three patients had received additional local radiotherapy before entering the LMCE2 study. A summary is given in Table I.

Pretreatments duration of < 12 months was observed in 17 patients and included one stage 4 relapse patient not previously grafted. Sixteen patients had longer intervals between diagnosis and date of entry in the double graft programm. Nine of them were patients that never had achieved complete remission and their pretreatments varied from 12 to 20 months. Seven patients in relapse had intervals from 15 to 52 months (exact relapse free interval not reported).

Following inclusion, two courses of Etoposid (VP16) and Cisplatinum (CDDP) ($n = 10$) or VP16 and Carboplatin (CBDCA) ($n = 8$) were given to increase response. Fifteen patients had pretreatment with other CDDP containing combinations. As shown in Table II, a first harvest was scheduled 4 weeks after the last chemotherapy. The first MGT regimen (MGT1), was a combination of Tenoposid (VM26), Bichloronitrosourea (BCNU) and CDDP or CBDCA and was given within 2 weeks after harvest. A second harvest was performed 60 to 90 days post-MGTI. The second MGT regimen (MGT2) was a combination of VCR, L-PAM and TBI (Philip *et al.*, 1987*a*) (Table II). Seven of the 33 patients had received granulomacrophage colony stimulating factor (GMCSF) either at MGT1 (one patient or MGT2 (6 patients) as part of a randomised study of this agent (Philip *et al.*, 1992).

Statistical methods

Probability of survival was analysed according to the Kaplan-Meier Method (Kaplan & Meier, 1958) regarding the date of first ABMT as day 0 for survival estimation. All events of death (toxicity or disease related) were included for survival estimations. The median follow up time is 70 months since diagnosis and is 56 months after MGT1.

Patients

Thirty-three patients were included in this LMCE2 pilot study from 04/85 to 08/88. Twenty were males and 13 females with a median age of 53 months (range: 17–202 months) before first graft.

A total of 25 patients with stage 4 neuroblastoma at diagnosis were in PR after at least second line induction treatments. Thirteen patients showed only minor response to first line protocols and were consequently improved to PR status with courses of VP16 and high dose CDDP or CBDCA as previously reported (Philip *et al.*, 1987*b*; Hartmann *et al.*, 1988; Philip *et al.*, 1988). None of these patients were eligible for the LMCE1 study in which a single MGT regimen is given. Twelve patients received pretreatments outside France: Germany (four cases), Israel (two cases), Italy (two cases), Switzerland (two cases), USA (one case), England (one case) and joined the LMCE2 protocol also in delayed PR after more than first line treatments.

Nineteen of 25 had bone marrow involvement at the time of the first harvest and the first MGT. Fourteen had bone involvement, 7/23 had elevated catecholamine secretion (two patients not tested) and 14 had primary, locoregional residual tumour (patient no 9 showed a local progression just before MGT).

Eight patients were included in the LMCE2 protocol after relapse from stage 4 (five patients) or stage 3 disease (three patients). Seven patients were still sensitive to the VP16-CDDP rescue regimen as previously described (Philip *et al.*, 1987*b*) and were defined as SR and received MGT1 in sub-

Table I Details of 33 patients before and after first autologous bone marrow transplantation

Patient's Number	*Modes of treatment*		*Results of Surgery*	*Status of Disease prior to ABMT1*				*Catechol.*	*Hematological Recovery (No of days)*				*Evaluation POST-ABMT 1*
	1st line	*2nd line*		*Status*	*B.M.*	*Bone*	*Primary tumour*		*<1000 WBC*	*<200 PNC*	*<500 PNC*	*<50.000 PLTS*	
1	CS	C	PT3B	PR	+	+	–	ND	24	24	24	30	PR
2	C	C	ND	PR	+	+	–	–	26	NE	26	22	PR
3	CSR	C	PTI	PR	+	+	–	–	22	24	33	25	SD
4	CSR	C	PT3A	OR	+	+	–	–	19	0	21	16	SD
5	CS	C	PT1	PR	–	+	–	–	14	13	19	15	PR
6	C	C	ND	PR	+	+	+	+	21	18	21	27	SD
7	CS	C	PT3B	PR	+	+	+	–	21	23	25	21	SD
8	CS	C	PT3C	PR	+	+	+	+	22	NE	31	22	PR
9	CS	C	PT3A	PR[1]	–	–	+	–	26	NE	19	NE	PR
10	CS	C	PT1	PR	+	–	–	+	21	21	23	30	CR
11	CS	2	PT3B	PR	+	–	+	–	20	19	22	23	PR
12	C	C	PT3C	PR	+	+	+	ND	15	15	26	18	SD
13	C	C	PT3C	PR	+	+	+	+	18	15	19	22	PR
14	CS	C	PT3A	PR	+	–	–	–	24	24	26	39	SD
15	CS	C	PT3B	PR	–	–	+	–	15	NE	20	21	PR
16	CS	C	PT3A	PR	+	+	–	–	12	14	15	15	PR
17	C	C	PT3C	PR	–	–	+	–	15	17	19	16	PR
18	CS	C	PT3B	PR	+	+	–	–	33	21	33	37	PR
19	CS	C	PT1	PR	+	–	–	+	13	16	20	15	PD
20	CS	C	PT3B	PR	+	–	+	–	NE	26	NE	25	CR
21	CS	C	PT3B	PR	–	+	+	+	NE	14	NE	NE	NE[2]
22	C	C	PT3C	PR	+	+	+	+	35	31	60	ND	PD
23	CS	C	PT3B	PR	+	–	+	–	16	16	18	20	PR
24	CS	C	PT3B	PR	–	–	+	–	18	22	28	16	SD
25	CS	C	PT1	PR	+	–	–	–	21	29	60	78	PR
26	CS	C	PT1	SR	–	–	+	ND	12	14	16	10	PD
27	CSR	CSR	PT3B	SR	–	–	+	–	0	17	23	17	CR
28	CS	C	PT3B	SR	–	–	+	–	24	30	31	40	PR
29	CS	C	PT1	SR	–	–	+	–	13	20	22	ND	SD
30	CS	CS	PT3C	SR	–	–	+	–	NE	NE	NE	NE	CR
31	CS	CS	PT3A	SR[3]	–	–	–	–	12	ND	13	ND	CR
32	CS	CS	PT1	SR	+	–	–	–	17	13	17	45	CR
33	CS	CS	PT3A	RR	–	–	+	–	14	ND	27	15	PR

Patients 1 to 25:MGT in first PR; patients 26 to 33:MGT in relapse;
C = Chemotherapy, S = Surgery; R = Radiotherapy; CR = Complete Response, PR = Partial Response; SR = Sensitive Relapse; RR = Resistant Relapse; SD = Stable Disease; PD = Progressive Disease; NE = Not Evaluable. PR[1] = progressed locally before BMT; NE[2] = toxic death; SR[3] = 2nd CR. ND = Not Done; PT1 = totally excised, PT3A = microscopic residual disease, PT3B = macroscopic residual disease; PT3C = biopsy only or surgical excision impossible ABMT = Autologous Bone Marrow Transplantation; Nb = Number; B.M. = Bone Marrow; Catechol. = Urinary catecholamines; WBC = White Blood Cells; PNC = Polymorphonuclear cells; PLTS = Platelets; + = Positive; – = Negative for Hematological recovery: NE = Not Evaluable (not reached or data not reported)

Table II LMCE 2 protocol in advanced neuroblastoma

-B.M. Harvest 1 + Purge			
BCNU	300 mg m^{-2}	Day 1	
VM26	250 mg m^{-2}	Day 2-4	
CDDP	200 mg m^{-2}	Day 2-4	ABMT 1
or			
CBDCA	250 mg m^{-2}	Day 2-6	
-B.M. Harvest 2 + Purge			
VCR	1,5 mg m^{-2} IV Bolus	Day 1	
	0,5 mg m^{-2} CI	Day 1-5	ABMT 2
TBI	12 Gy/6 fractions[a]	Day 2-4	
Melphalan	180 mg m^{-2}	Day 6	

[a]Lung protection at 10 Gy B.M. = Bone Marrow; IV = Intra-Venous; CI = Continuous Infusion; TBI = Total Body Irradiation; VCR = Vincristine; CBDCA = Carboplatin; CDDP = Cisplatinum; BCNU = Bichloronitrosourea; VM 26 = Teniposid; ABMT = Autologous Bone Marrow Transplantation.

sequent CR (one patient) or PR (six patients). One patient was grafted when progressing on rescue protocol (with VP16 and CDDP) and was defined as a RR. In this group 1/8 had bone marrow involvement at the time of the first harvest and before MGT1, none had bone involvement, 0/7 showed ongoing catecholamine secretion (one patient not tested) and 6/8 locoregional residual tumour.

Biological criteria at diagnosis are not available (N-*myc*, NSE) in this group of patients or just for a very limited number (Ferritin and LDH was evaluated in five and three patients respectively and was not done in all the others).

Twenty-four patients were grafted in Lyon, nine patients in other centers (in France:3 at the Institut Curie/Paris, one at Hôpital Nord/St Priest en Jarez, one at Hôpital Timone/Marseille; in Germany:two at the Universitätsklinikum Charlottenburg/Berlin; in Switzerland:one at Hôpital Cantonal/Geneve, one at the Centre Hospitalo-Universitaire/Lausanne).

Results

First harvest and first ABMT

At harvest the median number of nucleated cells was 3.02×10^8 kg^{-1} ($0.35-10.5 \times 10^8$ kg^{-1}) and the median number of colony forming units (CFU) was 18×10^4 kg^{-1} ($1.1-130 \times 10^4$ kg^{-1}). Reinfused, purged bone marrow contained a median of 0.65×10^8 nucleated cells kg^{-1} ($0.29-1.96 \times 10^8$ kg^{-1}) and a median of 3×10^4 CFU kg^{-1} (0.2 to 14×10^4 kg^{-1}).

Thirty-one of 33 patients were evaluable for response to BCNU, VM26 and Platinum as shown in Table I; (two patients were not evaluable for response:one patient was in second CR before MGT1 and stayed in CR, and one PR patient died on day 14 post ABMT1 due to septicaemia). Of the 31 evaluable patients, five achieved CR and 15 stayed in PR but showed a reduction in their disease of 50% or more. Thus the response rate to MGT1 is 65% (CR rate:16%). Eight patients had stable disease and three patients had disease progression. Eight of the 20 positive marrows prior to MGT1 were normalised by MGT1. Residual tumour was observed in the bone in eight patients and locoregionally at the primary tumour site in nine.

Median time to reach 1000 white cells after ABMT1 was 18 days (0-35) and 19 days (0-31) to reach 200 polymorphonuclear cells (PNC), 23 days (13-60) to reach 500 PNC and 22 days (10-78) to reach 50,000 platelets. One toxic death was observed (disseminated cryptococcus) and one patient had significant morbidity (endocarditis). Median time to return home was 30 days post ABMT1.

Second harvest and second ABMT

A second bone marrow harvest was performed 60-90 days post MGT1 and 12 still contained tumour cells (before purging). At harvest, the median number of nucleated cells was 4.53×10^8 kg^{-1} ($0.95-10.6 \times 10^8$ kg^{-1}) and the median number of CFU was 24×10^4 kg^{-1} ($4.1-75 \times 10^4$ kg^{-1}). The median number of nucleated cells and CFU grafted were respectively 0.92×10^8 kg^{-1} ($0.22-2.92 \times 10^8$ kg^{-1}) and 3.4×10^4 kg^{-1} (0.3 to 12×10^4 kg^{-1}).

Patients characteristics before MGT2, marrow recovery and response to MGT2 are summarised in Table III. Thirty of the 33 patients reached MGT2:one patient died with toxicity after the first graft, two patients with disease progression after the first graft received no MGT2 (one due to rapid death with disease, the other was a parental refusal). Median time to reach MGT2 was 3 months (range, 2-6 months) post MGT1. Five patients had achieved CR with MGT1, two additional patients with only residual local disease achieved CR before MGT2 with surgery (patient no. 31 was in 2nd CR before MGT1 and is not evaluated for response). One patient (patient no 7) died of toxicity and was not evaluable after the second graft, patient no 25 was not evaluated. Thus only 20 of the 30 patients are evaluable for the response to VCR, L-PAM and TBI. Five achieved CR and seven patients were further improved by 50% or more (PR). The response rate of these 20 patients to MGT2 is thus 60% (CR rate:25%).

Duration of aplasia was longer after MGT2 and ABMT2. The median time to reach 1000 white cells was 30 days (12-120), to reach 200 PNC cells 25 days (10-124), to reach 500 PNC cells 43 days (15-120). Twelve patients reached 50,000 platelets at a median time of 65 days (27-297). Six patients died with procedure related complications before reaching 50,000 platelets and in 12 patients the date of recovery was not reported due to their return to their transferring centers. Two patients (no. 1 and no. 27) with delayed platelets recovery died with disease. One never became independent of platelets transfusions since he experienced early relapse 3 months after MGT2 and died with progression 4 months later. The other one (no. 1) became independent after 120 days but died with disease progression one year after MGT2. Two further patients (no. 2 and no. 25) with delayed platelets recovery are alive and well after a prolonged period of platelet substitution dependency (reaching stable counts of more than 50,000 at days 189 and 297). The median time to return home was 40 days.

Three deaths due to interstitial pneumonitis occurred within the first 100 post-transplant days in two CR patients (days 35 and 78) and in one PR patient (day 41). Four further late toxic deaths were observed due to haemorrhage associated with delayed platelet recovery (one PR patient:days 118 and one CR patient:day 121), lung fibrosis in a PR patient on day 138 and renal failure with incomplete marrow engraftment in a PR patient on day 180.

Late and long term complications in the 11 survivors were documented growth impairment in one and hemosiderosis in another. The other patients appear to have normal life quality comparable to their age companions with full reintegration in their social structure.

Survival

The overall survival for the 33 patients after a median follow up time of 56 months is 36% (Figure 1) at 2 years and shows an actuarial 32% plateau at 5 years. Survival of patients was not influenced by the interval between diagnosis and study entry since actuarial survivals are exactly the same for patients with intervals of less or more than 12 months.

Twenty-five patients receiving MGT1/2 in first partial remission after second line treatments (with previous minor response to first line treatment) achieved an overall survival of 40% at 2 years and an actuarial survival of 34% at 5 years. Eight patients treated with MGT1/2 for relapse (one second CR, 6 second PR and one minor response) had an overall survival of 25% at 2 years (5 years not reached) (Figure 2).

Table III Details of 30 patients before and after second autologous bone marrow transplantation

Patient's Number	*Status*	*Status of Diseases prior to 2nd ABMT1*				*Hematological Recovery (No of days)*				*Evaluation*	*Toxic Deaths*
		B.M.	*Bone*	*Primary tumour*	*Catechol.*	*<1000 WBC*	*<200 PNC*	*<500 PNC*	*<50.000 PLTS*	*POST-ABMT 2*	*(days post ABMT 2)*
1	PR	–	+	–	–	34	30	37	120	CR	
2	PR	+	–	–	–	24	22	24	189	CR	
3	SD	+	+	–	–	53	NE	NE	65	VGPR	
4	PR	+	+	–	–	40	27	60	NE	SD	
5	PR	–	+	–	–	21	23	24	47	SD	
6	PR	+	+	+	+	21	17	21	27	SD	
7	PR	+	–	+	+	ND	25	34	NR	NE	I.P. (day 41)
8	PR	+	–	+	+	120	120	NR	NR[a]	MR	R.F. (day 180)
9	PR	+	–	–	–	19	NE	30	63	CR	
10	CR	–	–	–	–	52	43	63	NE	CR	
11	PR	+	–	–	–	37	30	44	NE	NE	
12	PR	+	+	+	–	42	32	45	47	PR	
13	PR	+	–	–	+	48	39	52	NE	PD	
14	PR	+	–	–	–	NR	NR	NR	NR[a]	SD	H (day 118)
15	PR	–	–	+	–	27	27	NE	NE	PR	
16	PR	–	+	–	–	12	15	15	NE	PD	
17	CR[1]	–	–	–	–	22	17	21	29	CR	
18	PR	–	+	–	–	81	69	83	NE	PR	
20	CR	–	–	–	–	46	10	45	60	CR	I.P. (day 78)
23	PR	–	–	+	–	27	25	26	NE	PR	
24	PR	–	–	+	–	30	44	49	NE[a]	CR	
25	PR	+	–	–	–	24	124	100	297	NE	
26	PD	–	–	+	–	25	18	25	81	PR	
27	CR	–	–	–	–	50	44	50	230	CR	
28	PR	–	–	+	–	39	40	43	NE	PR	P. (day 138)
29	SD	–	–	–	–	30	43	43	NE	CR	
30	CR	–	–	–	–	NE	NE	NE	NE[a]	CR	H (day 121)
31	CR	–	–	–	–	24	19	95	NE[a]	CR	
32	CR	–	–	–	–	NE	NE	NE	NE[a]	NE	I.P. (day 35)
33	CR[1]	–	–	–	–	20	17	24	NE	CR	

CR = Complete Response, CR[1] = Due to surgery, VGPR = Very Good Partial Response, PR = Partial Response, SD = Stable Disease, MR = Minor Response, PD = Progressive Disease, NE = Not Evaluable. No = Number; B.M. = Bone Marrow; Catechol. = Catecholamine; WBC = White Blood Cells; PNC = Polymorphonuclear cells; PLTS = Platelets; ABMT = Autologous Bone Marrow Transplantation; + = positive; – = negative

For Hematology recovery: NE = Not Evaluable (not reached or data not reported); ND = Not Done; NR = Not Reached; [a] = additional GM-CSF treatment

For toxic deaths:

I.P.: interstitial pneumonitis (pt 20:Pneumocystic carinii positive, pts 8,32:cause not identified)

P.: pneumopathy (pt 28:lung fibrosis at autopsy being pneumocystis carinii positive 1 month before and myxovirus positive 2 days before death)

H.: hemorrhage (pt 14:failure of engraftment:2 bone marrows rescues given)

R.F.: renal failure:failure of engraftment (only unstable rise of WBC under GM-CSF treatment); BM fibrosis at autopsy.

Table IV Sites of disease progression after autologous bone marrow transplantation

Patient's Number	Primary Tumour Site	Status after ABMT 2	Duration of Progression Free interval post ABMT1 (in months)	ABMT2	Site of Progression after ABMT 2	Cause of Death or last Disease Status
(1) Grafted in PR:						
1	n.i.	CR	16	13	Bone Marrow, Skeleton	Disease
4	Adrenal	SD	8	6	Bone Marrow, Skeleton, Mediastinum, Lungs, Liver	Disease
5	Adrenal	SD	19	15	Retroperitoneum	Disease
6	Retrop.	SD	11	7	Bone Marrow, Skeleton, Retroperitoneum	Disease
12	Adrenal	PR	12	10	Liver, Skeleton	Disease
13	Adrenal	PD	6	3	Bone Marrow, Skeleton	Disease
16	Adrenal	PD	3	1	Retroperitoneum	Disease and Haemorrage
17	Retrop.	CR	13	9	Lungs	Disease
18	Adrenal	PR	17	13	Bone Marrow, Skeleton, Retroperitoneum	Disease
*19	Adrenal	PD	3		Retroperitoneum, Mediastinum	Disease
*22	Adrenal	PD	2		Bone Marrow, Skeleton, Retroperitoneum, Central Nervous System	Disease
(2) Grafted in relapse:						
26	Adrenal	PR	8	3	Mediastinum	Disease
27	Adrenal	CR	6	3	Mediastinum	Disease
29	Retrop.	CR	9	6	Retroperitoneum	Disease
31	Adrenal	CR	31	26	Skeleton	Alive in relapse

[a] = No second graft; CR = Complete Response; PR = Partial Response; SD = Stable Disease; PD = Progressive Disease; ABMT = Autologous Bone Marrow Transplantation. n.i. = not identified; Retrop. = Retroperitoneum.

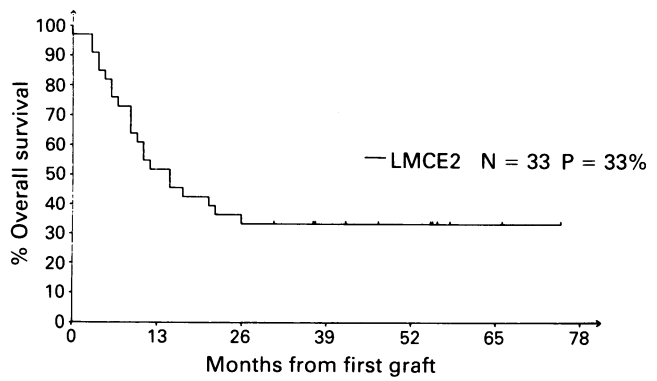

Figure 1 Overall survival of 33 study patients.

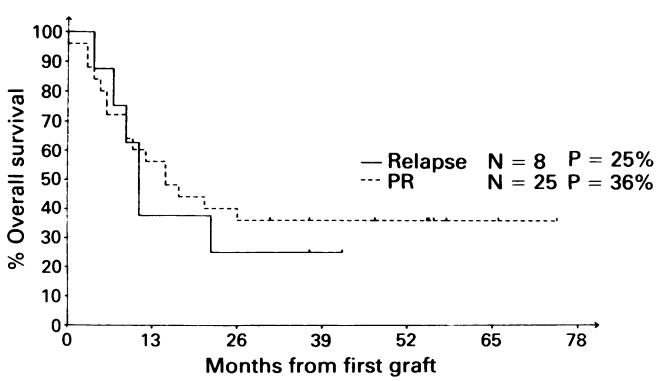

Figure 2 Survival of the 25 patients in PR and eight patients in relapse (seven SR, one RR).

Fifteen patients experienced relapse or disease progression. Two patients had very early metastatic and locoregional relapses at 2 and 3 months after MGT1 and the other 13 after MGT2. The sites of relapse or progression are detailed on Table IV. Nine patients showed distant relapse (two tumours recurred also in the primary site) whereas in three patients primary locoregional tumour was the first site of recurrent disease. The median duration of the progression free interval was 11 months (range, 2 to 31 months) post MGT1 for the 13 patients. Eight patients died from treatment related toxicity. Thus there are ten out of 33 patients surviving relapse-free 32 to 77 months after MGT1 and seven of them more than 4 years at the time of this report.

Discussion

MGT followed by ABMT as consolidation treatment in neuroblastoma has been investigated by several teams in very different study populations, treating either only patients in first CR after induction or in addition patients with residual locoregional disease and/or patients in PR after one line of induction treatment (Pritchard *et al.*, 1982; Hartmann *et al.*, 1986; Philip *et al.*, 1991; Graham-Pole *et al.*, 1991*a*; Dini *et al.*, 1991) but rarely patients with less sensitive disease with two or more lines of induction therapy.

We report on a subgroup of stage 4 neuroblastoma patients who only achieved delayed PR after at least second line treatments or had relapsed. All received consolidation treatment that consisted of two different MGT regimens (BCNU, VM26, CDDP/or CBDCA and VCR, L-PAM, TBI), given within a 3 months interval, each one preceded by harvesting and purging of the bone marrow.

This patient cohort fulfilled in our view poor prognosis criteria at the time of inclusion. In the group of PR patients, 19 (76%) still had bone marrow involvement at first harvest after intensive pretreatments and 14 (56%) still had active

bone disease. In fact only three patients had residual disease only at their primary tumor site, but one of them eventually showed local progression just before megatherapy (so in two of these one could in fact estimate a better prognosis than for the rest of patients). Further, there are eight relapse patients. Relapse signalises failure of previous treatments and tumours have a high chance to hold chemoresistant clones. This is in line with EBMT registry data showing rapid disease progression after MGT for most relapse patients. Only relapse patients in second complete remission have an actuarial survival of 32% post MGT (manuscript in preparation, unpublished EBMT data). Thus poor prognosis can be assumed also for the relapse patients, probably with the exception of one patient achieving CR before MGT who was not excluded from analysis.

We previously observed in the European Bone Marrow Transplantation Registry data an advantage for neuroblastoma patients with pretreatment durations of more than 12 months. This was found to be related to patients transferral to major transplant centers. To exclude such a bias we compared pretreatment durations in the LMCE2 study, but could not detect any influence on outcome. Nevertheless, patient transferral to major transplant centers always carries the risk of case selection and we therefore cannot exclude such a bias.

The toxicity of the MGT1 was acceptable (i.e. one toxic death and 30 days median time to return home) and produced a response rate of 65%. In contrast, the CR rate of only 16% is inadequate to rely only on this intensification regimen to increase survival. Moreover two patients showed progressive disease after MGT1 and did not reach the second procedure. The second MGT regimen was more effective, as previously reported for a different group of patients, (Pinkerton *et al.*, 1987), achieving a further 60% response rate with a CR rate of 25%. Complete remission in 11/33 patients (excluding 2 CR due to subsequent surgery) is hence encouraging for this patient cohort with either initial minor response or recurrent disease. Disease evaluation was done in all patients at certain time points after study entry as outlined previously. Undoubtedly, any response data taken at any time reflects comparison of consecutive evaluations during the course of treatment and serves as help to estimate effects. Additive effects of consecutive treatments cannot be separated and this has to be taken into account especially when regarding the additional effects of MGT2 in this series of patients. Ideally, future double MGT concepts would use more effective drugs for MGT1 but additive toxicity is the major problem. Thus different concepts of dose escalations using repetitive high dose monotherapies with growth factor support appear as the more interesting approach nowadays.

The probability of survival is 32% at 5 years with a median follow up time of 56 months. This is an equivalent outcome to patients entering MGT programmes after first line treatments and better initial response. However, as previously shown by various teams, efficacy and toxicity are not separable and the cost of CR in terms of toxic death was high in this study.

Concerning the double harvest approach we found that the second harvest was not a problem in terms of nucleated cells harvested. The number of GMCFU harvested was also very similar at first and second harvests. Also the number of nucleated cells was not different from one harvest to the other. Thus it does not appear that stem cell depletion due to toxicity to MGT1 was the cause for delayed engraftment after MGT2 as demonstrated by the clear delay in the time to return home, the time to reach 500 PNC cells and the platelet recovery.

We previously reported that immunomagnetic depletion was mildly toxic to bone marrow precursors as evaluated by their *in vivo* clonogenic efficiency although a substantial loss of bone marrow cells usually occurs (Combaret *et al.*, 1989*b*). The recovery of mononucleated cells after the purging procedure ranges from 32 to 42% in our overall experience with treated marrows with no difference between first and second harvests. The potential toxicity on hematopoetic progenitor cells of the immunomagnetic purging procedure or of the monoclonal antibodies used to target neuroblastoma cells (specially Thy1) must be considered. However, results observed in patients treated with only one MGT and reinfusion of *ex-vivo* treated marrow (Philip *et al.*, 1991; Lopez *et al.*, 1987; Gee *et al.*, 1987) do not favour such hypothesis.

The feasibility of repeated ABMT with mafosfamid treated marrow was previously reported as safe and effective for patients after intensive chemotherapy regimens by Hartmann and Beaujean (Hartmann *et al.*, 1987; Beaujean *et al.*, 1989). In contrast, this group had collected a single large marrow harvest before MGT1. Using this divided harvest approach observed a much shorter delay in engraftment after the second ABMT than observed in our study. Thus a possible explanation for the delay of the second engraftment is damage to the micro-environment by MGT1. This is in line with a recent and earlier reports stressing the impact of treatment intensity on bone marrow recovery (Graham-Pole *et al.*, 1991*b*, Kingston *et al.*, 1984). In addition, some of our LMCE2 patients with very late recovery showed an excess of CD8−Leu 7 cells and were thus given anti-CD8 monoclonal antibody. This was an effective treatment in some of these cases as previously reported (Favrot *et al.*, 1990).

Twenty-one of 33 patients included in this LMCE2 study and analysed at the time of the first harvest had residual bone marrow metastases and the question could be asked as to whether the first course of MGT (BCNU, VM26, CBDCA) was able to eliminate residual tumour cells in the bone marrow *in vivo*. This effect was observed in only eight of the 20 patients, with twelve patients still showing positive bone marrows at the second harvest. One patient with normal bone marrow before MGT1 even progressed with the appearance of bone marrow metastases after MGT1. *In 'vivo* purging' by MGT1 was not particularly efficient and therefore purging procedures appeared rational for the second harvest in most patients. Purging is however only of potential value in those cases where MGT achieves CR as any theoretical adverse effect of reinfusing tumour cells is irrelevant where significant marrow disease remains in the patient.

In summary, this treatment approach achieved an encouraging survival rate of 36% at 2 years with no late relapses at the time of this report in a subgroup of poor prognosis neuroblastoma patients. The delayed engraftments and the high toxic death rate after the seond graft supports the use of a single harvest approach. This study suggests that increased dose-intensity may improve the outlook for some patients with delayed reponse or relapse and it is the task of future randomised studies to evaluate the role of such multiple high dose procedures (Shuster *et al.*, 1991). Modification of the MGT regimens, the use of marrow growth factors and adjunctive peripheral blood stem cell transfusion could reduce morbidity to a more acceptable level.

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References

AUGUST, C.S., SEROTA, F.T., KOCH, P.A., BURKEY, E., SCHLESINGER, H., ELKINS, W.L., EVANS, A.E. & D'ANGIO, G.J. (1984). Treatment of advanced neuroblastoma with supralethal chemotherapy, radiation, and allogenic or autologous marrow reconstitution. *J. Clin. Oncol.*, **2**, 609–616.

BEAUJEAN, F., HARTMANN, O., BENHAMOU, E., LEMERLE, J. & DUEDARI, N. (1989). Hemopoietic reconstitution after repeated autologous transplantation with mafosfamide purged marrow. *Bone Marrow Transplant.*, **4**, 537–541.

BERNARD, J.L., PHILIP, T., ZUCKER, J.M., FRAPPAZ, D., ROBERT, A., MARGUERITTE, G., BOILLETOT, A., PHILIPPE, N., LUTZ, P., ROCHE, H. & PINKERTON, R. (1987). Sequential Cispaltinum-VM 26 and Vincristin-Cyclosphamid-Dorubicin in metastatic Neuroblastoma. An effective alternating non cross resistant regimen ? *J. Clin. Oncol.*, **5**, 1952–1959.

BERTHOLD, F., BURDACH, S., KREMENS, B., LAMPERT, F., NIETHAMMER, D., RIEHM, H., RITTER, J., TREUNER, J., UTSCH, S. & ZIESCHANG, J. (1990). The role of chemotherapy in the treatment of children with neuroblastoma stage 4: The GPO (German Pediatric Oncology Society) experience. *Klin. Pädiatr.*, **202**, 262–269.

CARLSEN, N.L.T., CHRISTENSEN, I.B.J., SCHROEDER, H., BRO, P.V., ERICHSEN, G., HAMBORG-PEDERSEN, B., JENSEN, K.B. & NIELSEN, O.H. (1986). Prognostic factors in neuroblastomas treated in Denmark from 1943 to 1080. A Statistical estimate of prognosis based on 253 cases. *Cancer*, **58**, 2726–2735.

COMBARET, V., FAVROT, M.C., KREMENS, B., PHILIP, I., BAILLY, C., FONTANIERE, B., GENTILHOMME, O., CHAUVIN, F., ZUCKER, J.M., BERNARD, J.L. & PHILIP, T. (1989*a*). Immunological detection of neuroblastoma cells in bone marrow harvested for autologous transplantation. *Br. J. Cancer*, **59**, 844–847.

COMBARET, V., FAVROT, M., PHILIP, I., CHAUVIN, F., COZE, C., CLAPISSON, G., MEZZIANE, F., BERNARD, J.L., ZUCKER, J.M., BRUNAT-MENTIGNY, M. & PHILIP, T. (1989*b*). Autologous bone marrow transplantation with an immunomagnetically purged bone marrow in neuroblastoma:analysis of 59 single ADMT and 32 double ABMT. In *Autologous Bone Marrows Transplantation, Proceedings of the 4th International Symposium.* Dicke, K.A., Spitzer, G., Jagannath, S. & Evinger-Hodges, M.J. (eds) p 549–557, Houston.

D'ANGIO, G.J., AUGUST, C., ELKINS, W., EVANS, A.E., SEEGER, R., LENARSKY, C., FEIG, S., WELLS, J., RAMSAY, N., KIM, T., WOODS, W., KRIVIT, W., STANDJORD, S., COCCIA, P. & NOVAK, L. (1985). Metastatic neuroblastoma managed by supralethal therapy and bone marrow reconstitution (BMRc). Results of a four-institution children's cancer study group pilot study. In *Advances in Neuroblastoma Research.* No 1, Evans, A.E., D'Angio, G.J., Knudson, A.G. & Seeger, R.C., (eds) p557–564, Alan R. Liss, Inc; New York.

DINI, G., LANINO, E., GARAVENTA, A., ROGERS, D., DALLORSO, S., VISCOLI, C., CASTAGNOLA, E., MANNO, G., BRISIGOTTI, M., ROSANDA, C., PASINO, M., RIVABELLA, L., BONI, L., MARCHESE, N., IVANI, G., RIZZO, A., FRANZONE, P., TRASINO, S. & DE BERNARDI, B. (1991). Myeloblative therapy and unpurged autologous bone marrow transplantation for poor prognosis neuroblastoma:report of 34 cases. *J. Clin. Oncol.*, **6**, 962–969.

FAVROT, M.C., PHILIP, I., COMBARET, V., MARITAZ, O. & PHILIP T. (1987). Experimental evaluation of an immunomagnetic bone marrow. Purging procedure using a Burkitt lymphoma model. *Bone Marrow Transplant.*, **2**, 59–66.

FAVROT, M.C., COMBARET, V., COZE, C., PHILIP, I. & PHILIP T. (1989). Is bone marrow purging efficient and necessary for ABMT in solid tumours? In *Bone Marrow Transplantation:Current Controversies*, Liss A.R. (ed), p 289–299.

FAVROT, M.C., PHILIP, T., COMBARET, V., LEMERLE, J., MARIE, J.P. & BERNARD, A. (1990). Effect of *in vivo* therapy with a CD8 monoclonal antibody on delayed engraftment after autologous bone marrow transplantation. *Bone Marrow Transplant.*, **5**, 33–38.

GEE, A.P., GRAHAM-POLE, J., LEE, C., BRUCE, K., PICK, T., HARVEY, W., WORTHINGTON-WHITE, D., HINTZ, M., JANSSEN, W. & GROSS, S. (1987). Transplantation for neuroblastoma using immunomagnetically purged autologous bone marrow. In *Autologous Bone Marrow Transplantation, Proceedings of the 3rd International Symposium.* Dicke, K.A., Spitzer, G. & Jagannath, S., (eds), p 425–431, Houston.

GRAHAM-POLE, J., LAZARUS, H.M., HERZIG, R.H., GROSS, S., COCCIA, P., WEINER, R. & STRANDJORD, S. (1984). High-dose Melphalan for the treatment of children with refractory neuroblastoma and Ewing's Sarcoma. *Am. J. Pediatr. Hematol. Oncol.*, **6**, 17–26.

GRAHAM-POLE, J., CASPER, J., ELFENBEIN, G., GEE, A., JANSSEN, W., KOCH, P., MARCUS, R., PICK, T., SHUSTER, J., SPRUCE, W., THOMAS, P. & YEAGER, A. (1991*a*). High dose chemoradiotherapy supported by marrow infusions for advanced neuroblastoma:a pediatric oncology group study. *J. Clin. Oncol.*, **9**, 152–158.

GRAHAM-POLE, J., GEE, A., EMERSON, S., GALLO, J., LEE, C., LUZINS, J., JANSSEN, W.E., PICK, T., WORTHINGTON-WHITE, D., ELFENBEIN, G., GROSS, S. & WEINER, R. (1991*b*). Myeloablative chemoradiotherapy and autologous bone marrow infusions for treatment of neuroblastoma:factors influencing engraftment. *Blood*, **78**, 1607–1014.

HARTMANN, O., SCOPINARO, M., TOURNADE, M.F., SARRAZIN, D. & LEMERLE, J. (1983). Neuroblastomes traités à l'Institut Gustave Roussy de 1975 à 1979, cent soixante-treize cas. *Arch. Fr. Pediatr.*, **40**, 15–21.

HARTMANN, O., KALIFA, C., BENHAMOU, E., PATTE, C., FLAMANT, F., JULIEN, C., BEAUJEAN, F. & LEMERLE, J. (1986). Treatment of advanced neuroblastoma with high dose melphalan and autologous bone marrow transplantation. *Cancer Chemother. Pharmacol.*, **16**, 165–169.

HARTMANN, O., BENHAMOU, E., BEAUJEAN, F., KALIFA, C., LEJARS, O., PATTE, C., BEHARD, C., FLAMANT, F., THYSS, A., DEVILLE, A., VANNIER, J.P., PAUTARD-MUCHEMBLE, B. & LEMERLE, J. (1987). Repeated high-dose chemotherapy followed by purged autologous bone marrow transplanation as consolidation therapy in metastatic neuroblastoma. *J. Clin. Oncol.*, **5**, 1205–1211.

HARTMANN, O., PINKERTON, R., PHILIP, T., ZUCKER, J.M. & BREATNACH, F. (1988). Very high dose Cisplatin and Etoposid in children with untreated advanced neuroblastoma. *J. Clin. Oncol.*, **6**, 44–50.

KAPLAN, E. & MEYER, P. (1958). Non parametric estimation from incomplete observations. *J. Am. Statis. Ass.*, **53**, 457–481.

KINGSTON, J.E., MALPAS, J.S., STILLER, C.A., PRITCHARD, J. & MCELWAIN, T.J. (1984). Autologous bone marrow transplantation contributes to haemopoietic recovery in children with solid tumours treated with high dose melphalan. *Brit. J. Haematol.*, **58**, 589–595.

KUSHNER, B.H. & HELSON, L. (1987). Coordinated use of sequential escalted cyclophosphamid and cell-cycle specific chemotherapy (the N4SE protocol) for advanced neuroblastoma:Experience with 100 patients. *J. Clin. Oncol.*, **5**, 1746–1751.

LETOURNEAU, J.N., BERNARD, J.L., HENDREN, H.W. & CARCASSONNE, M. (1985). Role of surgery in the treatment of neuroblastoma. *J. Pediatr. Surg.*, **20**, 244–249.

LOPEZ, M., ZUCKER, J.M., URRESOLA, R., DOUAY, L., QUINTANA, E., KEMSHEAD, J., GORIN, N.C. & VILCOQ, J.R. (1987). Influence of single and double immunomagnetic depletion on the hemopoietic capacity or marrow in patients with advanced neuroblastoma submitted to autologous bone marrow transplantation. *Bone Marrow Transplant.*, **2**, 413–419.

PHILIP, T., BERNARD, J.L., ZUCKER, J.M., SOUILLET, G., FAVROT, M., PHILIP, I., BORDIGONI, P., LUTZ, J.P., PLOUVIER, E., CARTON, P., ROBERT, A. & KEMSHEAD, J.T. (1985). Purged ABMT in 25 cases of very bad prognosis Neuroblastoma. *Lancet*, **2**, 576–577.

PHILIP, T., BERNARD, J.L., ZUCKER, J.M., PINKERTON, R., LUTZ, P., BORDIGONI, P., PLOUVIER, E., ROBERT, A., CARTON, R., PHILIPPE, N., PHILIP, I., CHAUVIN, F., FAVROT, M. (1987*a*). High dose chemoradiotherapy with BMT as consolidation treatment in Neuroblastoma:an unselected group of stage 4 patients over one year of age. *J. Clin. Oncol.*, **5**, 266–271.

PHILIP, T., GHALIE, R., PINKERTON, R., ZUCKER, J.M., BERNARD, J.L., LEVERGER, G. & HARTMANN, O. (1987*b*). A phase II study of high dose Cisplatin and VP 16 in Neuroblastoma. A report from the Société française d'Oncologie Pédiatrique. *J. Clin. Oncol.*, **5**, 941–950.

PHILIP, T., HELSON, L., BERNARD, J.L., ZUCKER, J.M., KREMENS, B., FAVROT, M.C. & HARTMANN, O. (1987*c*). Definition of response and remission in children over one year of age with advanced neuroblastoma. *Ped. Hemat. Oncol.*, **4**, 25–31.

PHILIP, T., GENTET, J.C., CARRIE, C., FARGE, A., MEZIANE, F., BOUFFET, E., ZUCKER, J.M. KREMENS, B. & BRUNAT-MENTIGNY, M. (1988). Phase II studies of combinations of drugs with high dose Carboplatin in Neuroblastoma (800 mg/m^2 to 1.150 g/m^2):a report from the LMCE group. In *Adances in Neuroblastoma Research.* no 2, Evans, A.E., D'Angio, G.J., Knudson, A.G. & Seeger, R.C., (eds) p 573–582, Alan R. Liss, Inc:New York.

PHILIP, T., ZUCKER, J.M., BERNARD, J.L., LUTZ, P., BORDIGONI, P., PLOUVIER, E., ROBERT, A., ROCHE, H., SOUILLET, G., BOUFFET, E., MICHON, J., LOPEZ, M., VILCOQ, J.M. GENTET, J.C., PHILIP, I., FAVROT, M.C., CHAUVIN, F. (1991). Improved survival at 2 and 5 years in the LMCE1 unselected group of 72 children with stage 4 neuroblastoma over 1 year of age at diagnosis:is cure possible in a small subgroup. *J. Clin. Oncol.*, **9**, 1037–1044.

PHILIP, T., MICHON, J., BERNARD, J.L., CHAUVIN, F., STERN, A., BOUFFET, E., ZUCKER, J.M. & RAYBAUD, C. (1992). Double-blind study of safety and efficacy of cho-cell granulocyte-macrophage colony stimulating factor (GM.CSF) given intravenously for 14 days in patients with neuroblastoma after autologous bone marrow transplantation compared to intravenous infusion of placebo. In *The Comparative Effects of Recombinant Myeloid Colony Stimulating Factors in Man.* Peters, W.P. (ed), (in press).

PINKERTON, C.R., PHILIP, T., BIRON, P., FRAPPAZ, D., PHILLIPE, N., ZUCKER, J.M., BERNARD, J.L., PHILIP, I., KEMSHEAD, J. & FAVROT, M.C. (1987). High-dose Melphalan, Vincristin and total body irradiation with ABMT in children with relapsed neuroblastoma. A phase II study. *Med. Ped. Oncol.*, **15**, 236–240.

PINKERTON, C.R., ZUCKER, J.M., HARTMANN, O., PRITCHARD, J., BROADBENT, V., MORRIS-JONES, P., BREATNACH, F., CRAFT, A.E., PEARSON, A.D.J. & WALLENDSZUS, K.R., PHILIP T. (on behalf of the European Neuroblastoma Study Group, ENSG). (1990). Short duration, high dose, alternating chemotherapy in metastatic neuroblastoma. (ENSG 3C induction regimen). *Br. J. Cancer*, **62**, 319–323.

PRITCHARD, J., MCELWAIN, T.J. & GRAHAM-POLE, J. (1982). High dose melphalan with autologous bone marrow for treatment of advanced neuroblastoma. *Br. J. Cancer*, **45**, 86–94.

PRITCHARD, J., GERMOND, S., JONES, D., DE KRAKER, J. & LOVE, S. (1986). Is high dose Melphalan of value in treating advanced neuroblastoma? Preliminary results of a randomized trial by the European Neuroblastoma Study Group. *Proc. Am. Soc. Clin. Oncol.*, **5**, 205.

ROSEN, E.M., CASSADY, J.R., FRANTZ, C.N., KRETSCHMAR, C., LEVEY, R. & SALLAN, S.E. (1984). Neuroblastoma:The Joint Center for Radiation Therapy/Dana Farber Cancer Institute/Children's Hospital Experience. *J. Clin. Oncol.*, **2**, 719–732.

SEEGER, R.C., VILLABLANCA, J.G., MATTHAY, K.K., HARRIS, R., MOSS, T.H., FEIG, S.A., SELCH, M., RAMSAY, N. & REYNOLDS, C.P. (1991). Intensive chemotherapy and autologous bone marrow transplantation for poor prognosis neuroblastoma. In *Advances in Neuroblastoma Research.* no 3, Evans. A.E., D'Angio. G.J. Knudson, A.G. Seeger, R.C., (eds) p 527–533, Alan R. Liss, Inc. New York.

SHAFFORD, E.A., ROGERS, D.W. & PRITCHARD, J. (1984). Advanced neuroblastoma:improved response rate using a multiagent regimen (OPEC) including sequential cisplatin and VM-26. *J. Clin. Oncol.*, **2**, 742–747.

SHUSTER, J.J., CANTOR, A.B., MCWILLIAMS, N., MCWILLIAMS, N., POLE, J.G., CASTLEBERRY, R.P., MARCUS, R., PIC, T., SMITH, E.I. & HAYES, F.A. (1991). The prognostic significance of autologous bone marrow transplantation in advanced neuroblastoma. *J. Clin. Oncol.*, **6**, 1045–1049.

ZUCKER, J.M., PHILIP, T., BERNARD, J.L., MICHON, J., BOUFFET, E., GENTET, J.C., LOPEZ, M., COZE, C., PHILIP, I., BORDIGONI, P., PLOUVIER, E., MAZINGUE, F., VOLCOQ, J.R. & ASSELAIN, B. (1991). Single or double consolidation treatment according to remission status after initial therapy in metastatic neuroblastoma: first results in LMCE 3 study in 40 patients. In *Advances in Neuroblastoma Research 3, Evans, D'Angio, Knudson, Seeger* (eds) p 543–551, New York, Wiley-Liss, Inc.