the running of all trials within the institute. The knowledge that a site visit could occur should encourage closer protocol adherence and better standards of data management. Such a programme can only improve the credibility of clinical trials. Its development could be hastened if peer-reviewed journals insisted that consideration of papers for publication be dependant on agreement by centres to a programme of random checks by an independent Quality Assurance Group. The most important result of such a development should be improved standards of care for patients with cancer.

1. Pinedo HM, Klenis Y. Chemotherapy of advanced soft tissue sarcoma in adults. *Cancer Treat Rev* 1977, **4**, 67–86.
2. Mauer JK, Hoth DF, Macfarlane DK, Hammershaimb LD, Wittes RE. Site visit monitoring program of the clinical cooperative groups: results of the first 3 years. *Cancer Treat Rep* 1985, **69**, 1177–1187.
3. Sunderland M, Kuebler S, Weiss G, Coltman C. Compliance with protocol: quality assurance data from the Southwest Oncology Group (SWOG). *Proc Am Soc Clin Oncol* 1990, **9**, 229.
4. Bramwell VHC, Mouridsen HT, Santoro A, *et al.* Cyclophosphamide versus ifosfamide: final report of a randomized Phase II trial in adult soft tissue sarcomes. *Eur J Cancer Clin Oncol* 1987, **23**, 311–321.
5. O'Bryan RM, Baker LH, Gottlieb JE, *et al.* Dose response evaluation of adriamycin in human neoplasia. *Cancer* 1977, **39**, 1940–1948.
6. Mansi JL, Fisher C, Wiltshaw E, MacMillan S, King M, Stuart-Harris R. A phase I–II study of ifosfamide in combination with adriamycin in the treatment of adult soft tissue sarcomas. *Eur J Cancer* 1988, **24**, 1439–1443.
7. Vantongelen K, Steward WP, Blackledge G, Verweij J, van Oosterom AT. EORTC joint ventures in Quality Control: Treatment-related variables and data acquisition in chemotherapy trials. *Eur J Cancer* 1991, **27**, 201–207.
8. De Vita VT. Dose–response is alive and well. *J Clin Oncol* 1986, **4**, 1157–1159.

Eur J Cancer, Vol. 29A, No. 7, pp. 947–956, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

Indication and Limits of Megatherapy and Bone Marrow Transplantation in High-risk Neuroblastoma: A Single Centre Analysis of Prognostic Factors

Ruth Ladenstein, Marie Favrot, Christine Lasset, Eric Bouffet, Irene Philip, Valerie Combaret, Frank Chauvin, Maud Brunat-Mentigny, Pierre Biron and Thierry Philip

76 patients with high risk neuroblastoma were treated with one (41 patients) or two consecutive courses (35 patients) of megatherapy. Autologous bone marrow transplantation was scheduled after each megatherapy. Univariate analysis confirmed two prognostic factors in this heterogeneous study population: no bone lesions before megatherapy and age at diagnosis of less than 2 years with 5-year progression-free survival rates of 51% ($P < 0.0007$) and 53% ($P < 0.025$), respectively. Both factors were shown to be of independent prognostic significance using the Cox proportional hazard model. Identification of prognostic factors should help to define the interest and limits of megatherapy. We consider that elective megatherapy followed by innovative treatments appears justified in patients with persisting bone disease. In contrast, megatherapy has to be re-evaluated for patients showing a more favourable response pattern and/or young age, ideally in a randomised, prospective trial. *Eur J Cancer*, Vol. 29A, No. 7, pp. 947–956, 1993.

INTRODUCTION

NEUROBLASTOMA is the most common childhood solid tumour before the age of 5 with a prognosis closely related to disease extension and age at diagnosis. In 30% of the cases neuroblastoma presents as localised disease (stage 1, 2 and 3); the prognosis is generally good with survival rates ranging from 40 to 90% and is influenced by the degree of local disease extension and the quality of surgical excision [1–3]. In 5% of cases, neuroblastoma is observed in infants of less than 1 year of age with a very particular disease pattern (stage 4s); although these patients present with metastatic spread to liver, skin and bone marrow but without bone lesions, the survival is more than 80% at 5 years [4, 5]. In contrast, 65% of neuroblastomas present at diagnosis as high-risk stage 4 metastatic disease involving most frequently bone marrow and bones. Major efforts have been undertaken to improve their prognosis. Historical control groups had a survival expectancy of only 10% at 3 years with conventional multimodality treatments [6–8]. More intensive induction

Correspondence to T. Philip.
T. Philip, R. Ladenstein, E. Bouffet, I. Philip, M. Brunat-Mentigny and P. Biron are at the Bone Marrow Transplantation Department; C. Lasset and F. Chauvin are at the Biostatistic Department; and M. Favrot and V. Combaret are at the Immunology Laboratory, Centre Léon Bérard, 28 rue laennec, 69373 Lyon, cedex 08, France.
Revised 17 Aug. 1992; accepted 21 Sep. 1992.

EJC 29:7-C

treatments [9–14] as well as megatherapy followed by bone marrow transplantation (BMT) [15–19] have been introduced to therapy schedules during the past decade. Overall survival now reaches 45% at 2 years but decreases to 20% at 5 years due to late relapses [17, 18].

Integration of biological parameters such as N-*myc* amplification, DNA ploidy, lactate dehydrogenase (LDH), ferritin and neuron specific enolase (NSE) appear to further modify prognosis within previously identified prognostic groups such as age or disease extension. These factors are at the centre of interest in ongoing studies which either try to further define the impact of such factors within a given treatment strategy in a prospective manner, or aim to eliminate their impact on prognosis by risk-adapted treatments.

76 patients had been treated at the Centre Léon Bérard (CLB) by megatherapy followed by BMT between July 1982 and October 1990. It is currently difficult to define the group of patients who benefit from such aggressive therapy. Biological parameters as outlined above are not yet available in a sufficient number of patients. However, the identification of clinical prognostic factors in patients treated with megatherapy, as shown in this series, should help to define the interest and limits of megatherapy for the time being and to discuss new therapeutic strategies.

PATIENTS AND METHODS

From July 1982 to October 1990, 76 high-risk neuroblastoma patients (47 males, 29 females) received one or two courses of megatherapy in our institute followed by BMT. Patients considered to be at risk in terms of long term survival were patients with stage 4 disease over 1 year of age at diagnosis as well as treatment failures including relapse or disease progressions of stage 1 to 4 neuroblastoma.

Patients' characteristics and treatment inclusion criteria.

33 patients had been treated from diagnosis at the CLB with standardised induction therapy, surgery and megatherapy followed by BMT as consolidation therapy. A second group of 43 patients is more heterogeneous in terms of pre-megatherapy treatment and/or disease status at megatherapy. Most of them had been pretreated elsewhere and were referred to our centre for megatherapy only due to our specialisation in such a therapeutic approach. This group thus includes stage 4 neuroblastoma referred in complete response (CR) or partial remission (PR) after first line therapy (10 patients), stage 4 neuroblastoma referred due to delayed response to second or third line therapy (19 patients) and metastatic or localised neuroblastoma in relapse (14 patients). The different therapeutic protocols and the main characteristics of the patients in each treatment group are described in Tables 1 and 2. Details of initial disease presentation, pre-megatherapy disease status, response and follow-up are summarised in Table 3. Parents of all children gave informed consent according to the laws for clinical research in France and other countries. The LMCE protocols were reviewed and approved by the "Comité d'Ethique de l'Université Claude Bernard" at Lyon in France.

The LMCE1 multicentre protocol (Lyon, Marseille, Institut Curie, East of France) was initiated in September 1982 and was closed in March 1987. Results have been published in detail elsewhere [17], and we will consider here only the 18 CLB patients. All patients had stage 4 neuroblastoma at diagnosis and were eligible for this single megatherapy protocol after achieving CR or PR status with first line induction treatment.

The LMCE2 study, also initiated by the LMCE group, was a pilot study conducted from April 1985 to August 1988 to test the efficacy of a double megatherapy procedure and involved a selected group of 27 high risk patients at the CLB who were not eligible for the LMCE1 study [20]. 17 patients had stage 4 neuroblastoma with only minor response to first line therapy and 10 were in relapse from stage 3 or 4 neuroblastoma. All patients received second line treatment (or more) including etoposide, cisplatin or carboplatin in an attempt to improve their response status before inclusion in the megatherapy protocol.

The LMCE3 study was initiated in March 1988 and is built on the experience of the previous protocols (LMCE1 and LMCE2). 15 patients were included at the CLB up to October 1990. Induction is of shorter duration than in the LMCE1 study and megatherapy was given as consolidation treatment. According to response status patients in CR or very good partial remission (VGPR) are consolidated with one megatherapy whereas PR patients received repeated megatherapies [19].

Another group of 16 patients had only one megatherapy. They had all been referred to our institute for this consolidation treatment. 12 patients had stage 4 neuroblastoma; first line induction therapy was thus different but well defined [10–13]. 4 patients were treated in relapse either from stage 4 neuroblastoma (1 patient) or stage 1 or 2 disease (2 patients); the last patient in relapse from stage 3 neuroblastoma had progressive disease at the time of megatherapy.

Staging of disease and response

Disease assessment of patients received at our centre at diagnosis included abdominal and chest computed tomography (CT) scans and ultrasounds of the primary tumours. Bone disease was evaluated until the end of 1985 with ^{99}Tc scans only (28 patients) and was consecutively replaced by meta-iodobenzylguanidine (mIBG) scans. 17 patients had both, ^{99}Tc scans and mIBG scans, whereas in the more recent patients (n=22) only mIBG scans have been performed. Assessment of bone marrow included four aspirates and four core biopsies from the anterior and posterior iliac crest. Measurement of urinary catecholamine metabolites (VMA and HVA) was also performed. Before megatherapy, all patients were re-evaluated by a full diagnostic work-up regarding residual tumour size, urinary catecholamine excretion, bone lesions and bone marrow infiltration by diagnostic means as outlined above. At this time ^{99}Tc scans were performed in 19 patients, mIBG scans in 33 and 14 had both. Improved, single residual hot spots on ^{99}Tc scans have been biopsied to rule out active disease. Staging was performed 1–2 weeks before megatherapy, 2–3 months after megatherapy and thereafter every 6 months or whenever progression or relapse was suspected. Applied response criteria have been published in detail previously [21].

Before entering the megatherapy programme 54 patients (19 in the single procedure and 35 in the double approach) had measurable disease and were thus evaluable for response. 22 patients with CR and VGPR pre-megatherapy were not evaluable. Neither were early toxic deaths considered for response rates since complete post-megatherapy staging was not done in most of them.

Bone marrow harvest

A total of 111 bone marrows (107 autologous and 4 allogeneic) reinfusions were performed. Autologous bone marrows (n=107) (harvest 1=75, harvest 2=32) were evaluated according to CLB standards [22] using both cytological and double fluorescence

Table 1. Patients' characteristics and treatments

	LMCE1	LMCE2	LMCE3	Others	Total
Patients' number	18	27	15	16	76
Boys	11	17	7	12	47
Girls	7	10	8	4	29
Median age at diagnosis (range, months)	36.5 (16–93)	50 (8–187)	45 (14–116)	38 (11–296)	44 (8–296)
Median age at first graft (range, months)	50 (20–102)	76 (18–223)	49 (15–120)	52 (25–315)	54 (15–315)
Median time interval from diagnosis to BMT (range, months)	7 (3–11)	15 (4–80)	4 (4–7)	12 (4–89)	
Stage at diagnosis:					
1, 2, or 3	0	4	1	3	8
4	18	23	14	13	68
Pregraft therapy:					
First line treatment	PE/CADO × 2	Heterogeneous* radiochemo therapy ± surgery	VP16–CDDP/ CADO × 2 (± G CSF) surgery	Heterogeneous† radiochemo therapy ± surgery	
	surgery				
	PE/CADO × 2				
Second (or third) line treatment		VP16–CDDP × 2 (VP16–CBDCA × 2)			
Megatherapy	VCR L-PAM TBI	BCNU VM26 CDDP (or CBDCA) BMT1	CR as LMCE1 PR as LMCE2 (± GM CSF)	Heterogeneous‡ megatherapy	
	BMT			BMT	
		VCR,L-PAM,TBI BMT2			

Pregraft treatments: PE/CADO: Cisplatin (CDDP: 100 mg/m², day 1) and tenioposide (VM26: 160 mg/m², day 3) followed by cyclophosphamide (CYC: 300 mg/m², days 1–5), doxorubicin (DXR: 60 mg/m², day 5) and vincristine (VCR: 1.5 mg/m², day 1 and 5) [9]. VP16–CDDP (–CBDCA): Etoposide (VP16: 100 mg/m², days 1–5) and cisplatin (CDDP: 40 mg/m², days 1–5/or: CBDCA: 200 mg/m², days 1–5) [11].

*LMCE2: Different multicentre protocols used as first line treatments: D-NB 85 [12]/3 patients, AEIOP 85/2 patients [27], N4SE [10]/2 patients, ENSG 3C [13]/1 patients, PE/CADO [9]/1 patient and 4 patients had different other pretreatments.

†Others: pretreatments: F-NB87 [19]/3 patients, ENSG1 [16]/2 patients, D-NB85/2 patients, AEIOP/1 patient, F-NB84 [9]/1 patient and other multiagent chemotherapies/4 patients. ‡For details see statistical methods.

analysis of the bone marrow before and after the purging procedure; bone marrows were purged in 91/107 of the cases (84%). Eighty-nine (harvest 1=62, harvest 2=27) were purged with the immunomagnetic depletion procedure as previously published [23]. Two marrows (harvest 1 and 2) were purged with 6 OH-DOPA. Sixteen (harvest 1=11, harvest 2=5) autologous bone marrows (16%) were reinfused without *in vitro* treatment. Allogeneic bone marrows ($n=4$) were harvested and purged with a cocktail of T-monoclonal antibody (1 patients of the group "other" and 3 patients of the LMCE2 study at the second graft).

Table 2. Megatherapy regimens used in the LMCE1, 2 and 3 studies

		−7	−6	−5	−4	−3	−2	−1	0
VCR	1.5 mg/m²		×						
	0.5 mg/m²		×	×	×	×	×		
L-PAM	180 mg/m²							×	
TBI	2 × 2 Gy*			//	//	//			
ABMT									×
BCNU	300 mg/m²	×							
VM26	250 mg/m²		×	×	×	×			
CDDP or	40 mg/m²		×	×	×	×	×		
CBDCA	250 mg/m²		×	×	×	×	×		

*Lung protection at 10 Gy

Megatherapy regimens

Megatherapy regimens used in the LMCE1, 2 and 3 study are given in detail on Table 2. In the "others" group megatherapy regimens were more heterogenous since modifications were necessary due to previous treatments. The following combinations were used: Vincristine (VCR), L-phenylalanine mustard (L-PAM), total body irradiation (TBI) (7 patients); L-PAM, carboplatin (CBDCA) (5 patients); L-PAM, CDBCA, teniposide (VM26) (1 patient); carmustine (BCNU), cisplatin (CDDP) (1 patient).

All patients were treated under simple reverse barrier isolation conditions. Right venous double lumen catheters were inserted in all patients and all patients received parenteral feeding. Platelet transfusions and red cell concentrates were administered when indicated. All blood products were irradiated with 25 Gy before transfusion. Prophylactic oral non-absorbable antibiotics

Table 3. (a) Patients' characteristics at diagnosis, disease status before megatherapy, response and follow-up (single megatherapy)

	Diagnosis						Treatment		Status at MGT1					
No.	Age	Sex	Stage	Pri. Tu.	Meta.		LMCE	Surg.	Stbl	B	O	Cat.	Stal	Follow-up
1	50	F	IV	A	B		1	PT1	CR	−	−	−	CR	DOD
2	93	M	IV	A	BO		1	PT3A	CR	−	−	−	NE	DOT
3	81	F	IV	A	BO		1	PT1	CR	−	−	−	CR	DOT
4	51	M	IV	A	BO		1	PT3A	VGPR	−	+	−	CR	DOD
5	37	M	IV	AT	BO		1	PT1	VGPR	−	+	−	NE	DOT
6	35	M	IV	T	BOH		1	PT3B	VGPR	−	−	−	PR	DOD
7	17	M	IV	A	BO		1	PT3A	VGPR	−	+	−	NE	DOT
8	33	M	IV	T	BO		1	PT1	VGPR	−	+	−	VGPR	DOD
9	16	M	IV	A	BOH		1	PT1	PR	−	+	−	CR	ADF
10	49	F	IV	A	BO		1	PT3A	PR	+	+	−	CR	DOT
11	49	F	IV	/	BOH		1	/	PR	−	+	+	CR	DOD
12	83	F	IV	A	BO		1	PT1	PR	+	+	+	SD	AIR
13	43	M	IV	A	BOH		1	PT1	PR	−	+	−	SD	DOD
14	23	M	IV	A	BON		1	PT3B	PR	+	+	−	PD	DOD
15	34	M	IV	A	B		1	PT3A	PR	+	−	−	CR	ADF
16	36	F	IV	A	BON		1	PT3B	PR	−	+	+	PD	DOD
17	18	F	IV	A	BOC		1	PT3B	PR	+	+	−	PD	DOD
18	16	M	IV	A	BH		1	PT1	PR	−	+	−	CR	DOD
19	36	M	IV	A	OAH		0	PT1	CR	−	−	−	died	DOT
20	18	F	IV	A	OH		0	PT3A	CR	−	−	−	CR	ADF
21	15	M	IV	A	BH		0	PT3A	CR	−	−	−	CR	ADF
22	246	M	IV	T	BO		0	/	CR	−	−	−	CR	ADF
23	71	F	IV	A	BOHN		0	PT3A	VGPR	−	+	−	VGPR	DOD
24	23	M	III	AT	/		0	PT3A	VGPR	−	−	−	CR	ADF
25	31	M	IV	A	BOH		0	PT1	PR	+	−	−	CR	DOT
26	42	M	IV	/	BONL		0	/	PR	+	−	−	PR	DOT
27	296	M	IV	A	BON		0	PT3B	PR	−	+	+	SD	AWD
28	25	M	III	A	/		0	PT3C	MR	−	+	/	PD	DOT
29	38	F	IV	A	BON		0	PT1	MR	+	+	−	PR	AIR
30	21	F	IV	A	OA		0	PT3B	MR	−	+	+	SD	AWD
31	86	M	I	O	/		0	/	SR	−	+	−	SD	DOD
32	63	M	II	A	/		0	PT1	RR	+	+	−	SD	DOD
33	159	M	II	T	N		0	/	RR	−	+	/	SD	DOD
34	106	M	IV	T	BO		0	PT3B	R	+	+	−	died	DOD

were given. Febrile episodes were promptly treated with broad spectrum intravenous (i.v.) antibiotics.

Statistical methods

Sex, age, surgical results, quality and sites of response, treatment groups, treatment intensity (single versus double megatherapy) and type of diagnostic methods used to evaluate bone disease were analysed for their impact on overall survival and progression-free survival (PFS). The number of patients with data available on N-*myc* was too small for evaluation in this analysis. Probability of survival was analysed according to the Kaplan–Meyer method and overall survival curves were compared using the log-rank test [24]. A multivariate regression model (Cox proportional hazards regression model) taking into account the different potential prognostic variables simultaneously and developed for censored data was used to determine the most important risk factors for survival [25]. The date of bone marrow reinfusion was assumed as more accurate than the date of diagnosis for measurement of megatherapy impact on pre-megatherapy disease status in a heterogeneous patient group with different lengths of pretreatments. Overall survival includes all causes of death (disease-related, toxic deaths), PFS indicates the interval between date of bone marrow reinfusion in the single graft or the first graft in the double graft procedure and the time to document progressive disease; toxicity related deaths are not regarded as an event of progression.

RESULTS

The group of 76 patients achieved an overall survival of 35% at 2 years and of 24% at 5 years. The PFS was 40% at 2 years and 28% at 5 years, respectively (Fig. 1). For the 62 patients treated in first remission the PFS was 41% at 2 years and 30% at 5 years.

Treatment groups

Response to megatherapy and follow-up of patients are detailed in Table 3. Overall and progression-free survivals (PFS) are summarised in Table 4 for the different treatment groups and indicates the absence of significant variations. Procedure-related toxic deaths are summarised in Table 5.

The response to megatherapy was 44% in the LMCE1 group ($n=18$). Procedure-related deaths occurred in 4 patients (22%). The median postgraft follow up of this group is 84 months (range, 55–112 months).

The LMCE2 group ($n=27$) showed a response rate of 59% to

Table 3. (*b*) *Patients' characteristics at diagnosis, disease status before megatherapy* 1 *and* 2, *response and follow-up* (*repeated megatherapy*)

	Diagnosis						Treatment		Status at MGT1					Status at MGT2					
No.	Age	Sex	Stage	Pri. Tu.	Meta.		LMCE	Surg.	Stb1	B	O	Cat.	Sta1	Stb2	B	O	Cat.	Sta2	Follow-up
35	150	M	III	A	/		2	PT3A	CRR	−	−	−	CR	SR	−	−	−	CR	ADF
36	67	M	?	A	?		2	PT1	SR	−	−	/	PD	SR	−	−	−	PR	DOD
37	26	M	IV	A	ON		2	/	SR	−	−	−	CR	SR	−	−	−	CR	DOD
38	36	M	III	A	N		2	PT3B	SR	−	−	−	PR	SR	−	−	−	PR	DOT
39	187	M	IV	A	BONL		2	/	SR	−	+	−	SD	SR	−	+	−	NE	DOT
40	8	F	IV	A	BO		2	PT1	SR	−	−	−	SD	SR	−	−	−	CR	DOD
41	37	M	IV	A	B		2	/	SR	−	−	−	CR	SR	−	−	−	CR	DOT
42	136	F	IV	A	P		2	PT1	SR	+	−	−	CR	SR	−	−	−	NE	DOT
43	71	M	IV	A	BO		2	PT3	RR	+	+	−	PR	SR	−	+	−	PR	ADF
44	70	M	III	A	/		2	PT3A	RR	−	−	−	PR	SR	−	−	−	CR	ADF
45	36	F	IV	A	BON		2	PT1	CR	−	−	−	CR	CR	−	−	−	CR	DOD
46	57	F	IV	/	BOH		2	/	PRm	+	+	/	PR	PR	−	+	−	CR	DOD
47	81	M	IV	A	O		2	PT1	PRm	−	+	−	PR	PR	−	+	−	SD	DOD
48	97	M	IV	A	BO		2	/	PRm	+	+	+	SD	PR	+	+	+	SD	DOD
49	34	M	IV	A	BO		2	PT1	PRm	+	−	+	CR	CR	−	−	−	CR	ADF
50	46	F	IV	A	BO		2	PT3B	PRm	+	−	−	PR	PR	+	−	−	NE	AWD
51	94	F	IV	A	BO		2	PT3C	PRm	+	+	/	SD	PR	+	+	−	PR	DOD
52	74	M	IV	A	BONHL		2	PT3C	PRm	+	+	+	PR	PR	+	−	+	PD	DOD
53	50	M	IV	A	BO		2	PT3A	PRm	+	−	−	SD	PR	+	−	−	SD	DOT
54	15	F	IV	A	NV		2	PT3B	PRm	−	−	−	PR	PR	−	−	−	PR	AWD
55	19	M	IV	A	BO		2	PT3A	PRm	+	+	−	PR	PR	−	+	−	R	DOD
56	88	F	IV	A	BO		2	/	PRm	−	−	−	PR	CR	−	−	−	CR	DOD
57	26	M	IV	A	BO		2	PT3B	PRm	+	+	−	PR	PR	−	+	−	PR	DOD
58	27	M	IV	A	BO		2	PT3C	PRm	+	+	+	PD						DOD
59	60	F	IV	A	BON		2	PT3B	PRm	+	−	−	PR	PR	−	−	−	PR	AWD
60	23	F	IV	A	BON		2	PT1	PRm	−	−	−	SD	PR	−	−	−	CR	ADF
61	48	M	IV	A	BON		2	PT1	PRm	+	−	−	NE	CR	−	−	−	NE	ADF
62	51	M	IV	A	BO		3	PT1	CR	−	−	−	CR						AIR
63	17	F	IV	A	B		3	PT3B	CR	−	−	−	CR	CR	−	−	−	CR	ADF
64	11	F	IV	A	BON		3	PT1	VGPR	−	−	−	CR						DOD
65	45	F	IV	A	BN		3	PT3B	VGPR	−	−	−	NE						DOD
66	24	F	IV	T	O		3	PT3B	VGPR	−	−	−	NE						AWD
67	83	M	IV	A	BO		3	PT3A	VGPR	−	−	−	VGPR						AWD
68	36	M	IV	A	BON		3	PT3A	PR	+	+	+	SD						AWD
69	37	M	IV	A	BOHN		3	PT3B	PR	−	+	−	PR	PD	+	−	−	PR	DOT
70	57	M	IV	A	BON		3	PT3B	PR	+	−	+	SD	PR	+	−	/	SD	AIR
71	14	F	IV	A	BON		3	PT3B	PR	−	−	+	PR	CR	−	−	+	CR	ADF
72	116	F	IV	A	BON		3	PT3A	PR	+	+	+	PR	PR	+	−	+	PR	DOT
73	47	M	IV	A	BON		3	PT3B	MR	+	+	+	MR						AWD
74	41	F	IV	A	BNS		3	PT3B	MR	+	−	+	PR	PR	−	−	+	PD	DOD
75	62	F	IV	A	BON		3	PT3B	MR	+	+	+	SD	MR	+	+	+	PR	DOD
76	50	M	IV	A	BONS		3	PT1	MR	+	+	+	PR	PR	+	+	+	PR	DOD

M: male, F: female, A: abdominal primary tumour, T: thoracal primary tumour, B: bone marrow, O: bones, N: lymph-nodes, L: lung metastases, H: Hutchinson's Syndrom, V: liver metastases; S: malignant cells in the peripheral blood, Surg: surgery, PT1: complete surgical resection, PT3A: microscopic tumour residual, PT3B: macroscopic tumour residual, PT3C: surgical removal impossible or biopsy only, Stb/Sta: status before/after megatherapy, Cat: catecholamines, CR: complete remission, VGPR: very good partial remission, PR: partial remission, PRm: delayed partial remission (previous minor response), MR: minor response, SR: sensitive relapse, RR: resistant relapse, UR: untreated relapse; SD: stable disease, DOD: dead of disease, DOT: dead of toxicity, ADF: alive disease free, AWD: alive with disease, AIR: alive in relapse.

megatherapy 1 with a CR rate of 18.5%. 1 patient died with progressive disease before megatherapy 2. 26 patients received a second BMT procedure (autologous: 23 cases/allogeneic: 3 cases) after a median interval of 3 months (range, 2–6 months). At this time patients presented with a disease status as follows: 6 CR, 18 PR, 2 progressive disease (PD). 5 patients still had positive bone marrows, 8 positive bone metastases and 2 elevated catecholamines excretion. The overall response rate after two procedures was 74% with a final CR rate of 33.3%. The median follow up since megatherapy 1 is 62 months (range, 34–85 months) at the time of analysis.

The LMCE3 group (n = 15) included 7 patients in CR or VGPR after induction therapy; thus they had a single megatherapy procedure only and were non-evaluable for response. In this treatment arm no disease progressions or toxicity related deaths occurred. 8 patients with PR status after first line treatment entered the double megatherapy arm. The response rate to megatherapy 1 was 50%. None was improved to CR or VGPR. 5

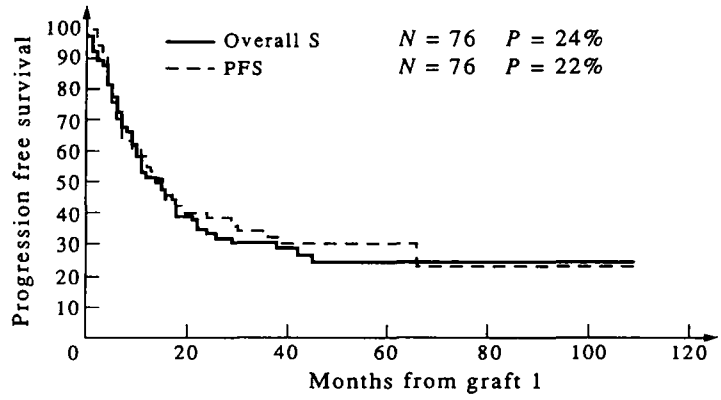

Fig. 1. Overall and progression free survival of 76 patients treated at the CLB.

Table 4. Univariate analysis of possible prognostic factors

Object of analysis	No. of pts	% Overall survival 2 (5) years		% Progression free survival 2 (5) years	
Sex					
Boys	47	36 (18)	NS	38 (23)	NS
Girls	29	33 (33)		38 (38)	
At diagnosis					
Age < 2 years	16	69 (49)	S	73 (53)	S
Age ⩾ 2 years	60	27 (19)	$P = 0.017$	27 (22)	$P = 0.025$
Bone lesions positive	56	31 (17)	NS	33 (23)	NS
vs. negative at diagnosis	20	39 (39)		56 (48)	
Marrow positive	59	32 (21)		35 (27)	NS
vs. negative at diagnosis	17	45 (nr)		52 (nr)	
Surgery in 1st remission (62 patients)					
Complete resection	22	41 (23)	NS	42 (28)	NS
Microscopic residual	15	38 (36)		59 (47)	
Macroscopic residual	19	51 (36)		44 (36)	
Biopsy/no surgery	4	25 (nr)		25 (nr)	
Response before MGT					
Complete remission	9	44 (nr)	NS	62 (nr)	NS
Very good partial response	11	12 (nr)		22 (nr)	
Partial remission	34	47 (29)		46 (35)	
Minor response	8	nr		nr	
After relapse	14	21 (nr)		34 (nr)	
Bone lesions positive	38	22 (7)	S	22 (4)	S
vs. negative before megatherapy	38	44 (41)	$P = 0.015$	51 (51)	$P = 0.0007$
Marrow positive	31	25 (22)	NS	34 (27)	NS
vs. negative before megatherapy	45	36 (26)		48 (40)	
Treatment groups					
LMCE 1	18	33 (17)	NS	43 (21)	NS
LMCE 2	27	37 (33)		42 (38)	
LMCE 3	15	33 (nr)		26 (nr)	
Others	16	22 (nr)		34 (nr)	
Single grafts	41	30 (19)	NS	35 (23)	NS
vs. double grafts	35	37 (31)		42 (38)	

No. of pts: number of patients, NS: non significant, S: significant.

Table 5. Toxic deaths

	LMCE 1 $n=18$	LMCE 2 $n=27$		LMCE 3 $n=15$		Other $n=16$	Total
		MGT1	MGT2	MGT1	MGT2		
Sepsis	1	/	/	/	2	2	5
Interstitial pneumonitis (± cardiac arrest)	/	/	3	/	/	/	3
Veno-occlusive disease (± cardiac arrest)	2	/	1	/	/	/	3
Haemorrhage	/	/	2	/	/	/	2
No engraftment	1	/	/	/	/	/	1
Total	4 (22%)		6 (19%)		2 (13%)	2 (13%)	14

MGT1: megatherapy 1, MGT2: megatherapy 2.

still had positive bone marrows, 2 positive bone lesions and 5 elevated catecholamines excretion before the second procedure. Median interval between megatherapy 1 and megatherapy 2 was 3 months (range, 3–6 months). The observed response rate after megatherapy 2 was again 50% in these patients. The median follow up since the single procedure or the first of the double procedures is 34 months (range, 17–49 months) at the time of analysis.

In the "others" patient group (n = 16) 6 patients had residual disease but none of the patients responded to megatherapy. The median follow up was 69 months (range: 18–110 months) at the time of analysis.

Autologous bone marrow harvest

At first harvest 30 marrows contained malignant cells and 12 marrows still were positive at the second harvest. After purging, none of the autograft samples contained any residual neuroblastoma cells detectable by immunological analysis (detection limit: 10^{-5} tumour cells).

Quality of bone marrows was evaluated at the day of reinfusion and showed a median value of 0.71×10^8 mononuclear (MN) cells/kg (range, $0.12–3.83 \times 10^8$/kg) and a median value of 3.2×10^4 colony forming units (CFU)/kg (range, $0.1–32 \times 10^4$/kg).

Engraftment

Engraftment characteristics are summarised in Table 6. The delayed recovery of platelets in the LMCE1 and LMCE3 single graft patients is comparable with the recovery of platelets after the second graft in the LMCE2 and the double graft arm of the LMCE3 study. Nevertheless, engraftment of all cell lineages was in general slower with the second of two grafts. No major difference in engraftment characteristics was observed in the allogeneic patient group.

Univariate and multivariate analysis of possible prognostic factors

The results of this analysis are detailed in Table 4. Only residual bone metastases before megatherapy (Fig. 2) and age at diagnosis of less than 2 years (Fig. 3) influenced outcome significantly. In contrast, bone metastases and bone marrow involvement at diagnosis did not distinguish any prognostic subgroups.

Analysis of residual metastatic disease, summarising bone marrow, bones, lymph nodes and other distant sites before megatherapy, clearly demonstrated that residual disease in the primary site, in metastatic sites as well as isolated bone marrow involvement or positive lymph nodes, were not predictive for the patient outcome. In contrast, bone metastases alone are a prognostic factor, since in those patients the PFS was of only 4% at 5 years (Fig. 2). Comparing actuarial survival and PFS curves related to bone metastases according to the applied diagnostic method (^{99}Tc scan, mIBG scan, or both) no statistical difference was found either at diagnosis or before megatherapy (Fig. 4). Of 34 patients with residual bone lesions, 5 were classified as VGPR (due to one or two residual positive bone lesions); all of them died, 3 with progressing disease and 2 with megatherapy related toxicity. Patients of less than 2 years at diagnosis (with two of them of less than 1 year: 1 stage 4 patient and 1 relapse patient) had a better outcome. 9 had no residual bone metastases when they received megatherapy and 2 only died of disease. However,

Table 6. Duration of engraftments according to treatment groups in days

Study	> 1000 WBC	> 200 PNC	> 500 PNC	> 50 000 PLTS
LMCE 1 (single graft group)				
Median	23	21	25	58
Range	13–43	11–48	13–62	35–128
LMCE 3 (single graft group)				
Median	16	20	28	26
Range	12–67	12–41	16–67	15–67
LMCE 3 (double graft group)				
Graft 1				
Median	23	21	26	26
Range	14–27	13–26	7–30	18–39
Graft 2				
Median	38	35	45	68
Range	17–67	21–70	27–73	41–80
LMCE 2 (double graft group)				
Graft 1				
Median	18	20	23	27
Range	0–35	13–36	13–60	10–78
Graft 2				
Median	27	40	43	65
Range	3–81	15–95	15–129	27–600
Others (single graft group)				
Median	24	27	n.d.	33
Range	12–51	21–71	n.d.	22–116

WBC: white blood cells, PNC: polynucleated cells, PLTS: platelets.

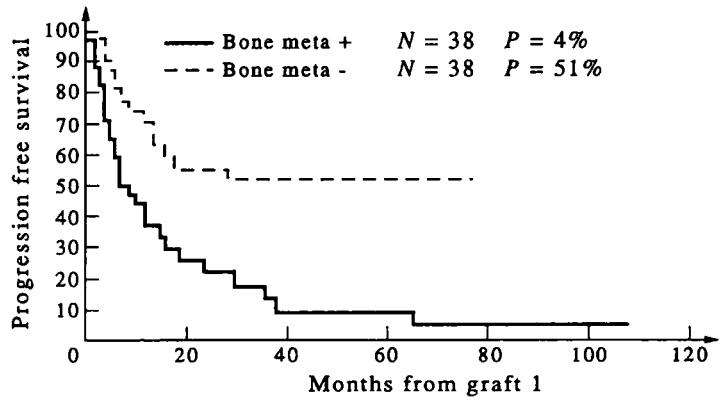

Fig. 2. Progression free survival related to bone metastases before megatherapy ($P = 0.0007$).

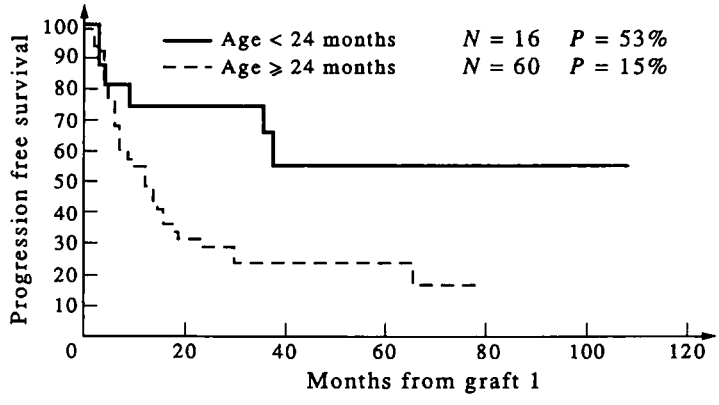

Fig. 3. Progression free survival related to age at diagnosis ($P = 0.025$).

7 had residual bone metastases, 1 died of toxicity, 4 relapsed but 2 are alive 15 months and 9 months post megatherapy.

All clinical factors of potential predictive value also were included in a multivariate analysis where residual bone disease ($P = 0.0068$) and age ($P = 0.035$) were confirmed as independent significant prognostic factors. A stronger influence was demonstrated for bone disease.

DISCUSSION

In this single centre analysis the PFS for high-risk neuroblastoma patients amounts to 40% at 2 years and 28% at 5 years. This is an improvement in comparison to the prognosis of about 10% at 2 years seen in the historical LMCE control group as previously reported [17] and may be due to both modern dose escalating induction therapy and/or megatherapy. The role of megatherapy as consolidation treatment versus no further treatment was also clearly demonstrated in the only randomised trial, the ENSG 1 study [16].

Procedure-related toxicity associated with aggressive dose escalation is one of the limits to achieving better survival with respect to the higher toxic death rate associated in particular with the second consecutive course of megatherapy in our series. Thus pre and post megatherapy schedules to diminish toxicity are of major importance. Prophylactic heparin infusion during the continuous vincristine infusion schedule was able to markedly lower the incidence of venous occlusive disease (VOD) at

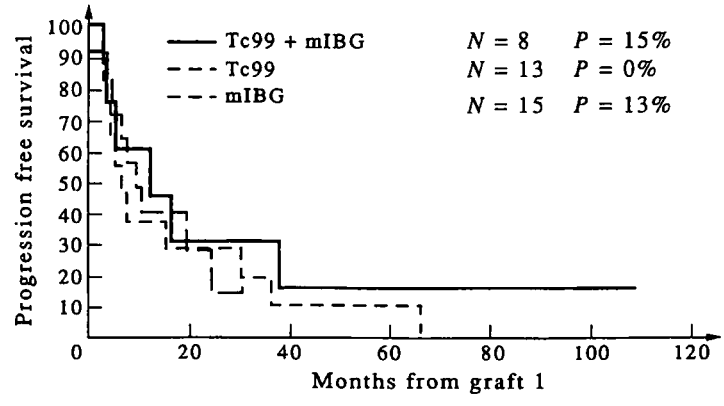

Fig. 4. Progression free survival related to positive bone metastases according to the applied diagnostic method (Tc-99 scan, mIBG scan, or both).

the CLB, which was a high treatment related risk factor during the LMCE1 study [17]. A retarded platelet recovery was observed in the LMCE1 and appears to be related to TBI. The retarded engraftment after the second megatherapy in the double procedure programmes may be related to a disturbed microenvironment at the second harvest. Thus, haematopoietic growth factors were introduced in the LMCE3 study [during induction (G-CSF) and after megatherapy (GM-CSF)] to shorten the periods of bone marrow aplasia [26].

Detection of residual disease both in the bone marrows and the autograft was the rational basis for purging. The first course of megatherapy (BCNU-VM26-CBDCA) in the repeated megatherapy protocol aiming to achieve an additional *in vivo* purge effect, was not efficient enough to eliminate residual disease in the patients since most of them still had positive marrows at the time of the second harvest. The clinical value of purging is difficult to prove, since the potential danger of reinfused neuroblastoma cells still has not been clearly demonstrated but this could become more evident if more successful treatments are available to eliminate residual disease in the patient [27].

Development and adaptation of primary treatment and megatherapy schedules over an 8 year period as in this study introduces a set of variables that could bias results. Nevertheless, this study confirmed the importance of previously detected prognostic factors which can help to stratify patients and to evaluate the benefit of different therapeutic modalities in future prospective randomised studies [28]. Hartmann previously observed a better prognosis in stage 4 neuroblastoma patients treated at the Institute Gustave Roussy [18] for patients with disappearance of all metastases and negative catecholamines before surgery and thus before megatherapy. Further the positive correlation concerning the response of bone disease before megatherapy was shown in LMCE1 study [17]. The LMCE1 study identified a small subgroup of stage 4 patients showing complete regression of bone metastases before megatherapy achieving a PFS rate of 30% at 5 years versus 12% in the positive-bone scan patients. The significant impact of healed bone metastases achieved by first or second line treatments before megatherapy is now confirmed by a PFS plateau at 51% from 2 to 5 years in our single centre analysis. Since evaluation of bone metastases were not homogeneous over the years we compared the three different groups diagnosed by ^{99}Tc scans or mIBG scans or both. There was no detectable statistical difference. Thus we concluded that judgement on residual bone disease was accurate, but evidently this should be confirmed in a prospective manner comparing the different diagnostic means. Although mIBG scanning at diagnosis cannot distinguish focal bone marrow disease from cortical bone metastases it appears that it adequately discriminates after induction therapy when bone marrows contain only minimal residual disease in most patients. The different value of imaging methods for detection of active bone marrow disease was recently highlighted by Corbett and coworkers [29] comparing mIBG and MRI with iliac crest biopsy and aspirate specimens. Indeed, positive mIBG spots that we would have interpreted as bone metastasis appeared more as bone marrow disease on MRI scans and both methods were in line with iliac bone marrow disease in only 44% of the cases. So the discussion which methods should define bone or bone marrow metastases is not settled and will need a more homogenous definition than given by recent International Neuroblastoma Staging System criteria to better compare future studies and to evaluate the prognostic impact of what is detectable with the different imaging methods. It is noteworthy that patients with "local" VGPR and PR status and no active bone disease are part of this good prognosis group, suggesting that residual primary tumour or bone marrow disease were not a major factor influencing survival duration in this patient group. Furthermore, these results indicate that VGPR as previously defined is not a homogeneous group in terms of prognosis. While VGPR patients with minimal local disease do have an outlook close to CR patients, the prognosis for patients with residual bone disease is poor suggesting that these patients would be better grouped within the PR group.

Age of less than 2 years at diagnosis was the second prognostic factor isolated in this study. The distribution of remission status before megatherapy is similar in both age groups. However, and, as described in the results one must note that this favourable outcome was observed in those young children only when bone metastases were cleared by chemotherapy before megatherapy. This observation is confirmed by the multivariate analysis. A cut-off point at 2 years was also previously chosen in the report of Evans *et al.* [5] in a series of 69 stage 3 and 4 neuroblastoma patients just failing to reach significance (P-value of 0.064). We do not have enough results in this series of patients on the analysis of genetic and molecular characteristics but it is of importance to note that the age of 2 years was also recently described by Look and co-workers as the threshold for the prognostic value of N-*myc* and DNA ploidy [30]. Further studies will have to show if intensive treatment as employed in megatherapy and BMT may abrogate the prognostic value of such biological features in relationship to age. Only 1 patient in this series was a borderline case in terms of age with 11 months at diagnosis and was not excluded. This patient eventually died with disease progression. Thus these results are not biased by the superior outlook given for infants with stage 4 neuroblastoma of less than 12 months of age. No other age limits were able to regroup patients in a prognostic manner.

Different treatment groups and thus treatment intensity in terms of one or two consecutive megatherapy regimens did not influence outcome in this single centre analysis. Nevertheless, the higher toxicity associated with the second megatherapy has to be taken into account. Interestingly, no advantage was detected for better quality surgical procedures nor did remission status before megatherapy influence outcome.

Taking our own data and the reports of others together, a group of "good prognosis" stage 4 neuroblastoma may well be defined by an age of less than 2 years, no N-*myc* amplification or DNA hyperdiploidy and healed bone metastases after induction treatment. This finding could be of major importance considering procedure related toxicity in this very young age group and megatherapy related toxic deaths.

In the context of megatherapy related toxicity and the cost of a transplant procedure, eligibility criteria have to be re-evaluated and we agree with others that megatherapy should be compared with other treatments on the basis of prospectively randomised trials [28]. The poor prognosis associated with residual pre-megatherapy bone disease was demonstrated in a homogenous group of prospectively treated patients, the LMCE1 study, and is now confirmed in this report in more heterogeneous patients. Such poor prognosis patients need very intensive treatments, e.g. elective megatherapy but this should be followed by innovative approaches to eliminate residual disease.

1. Evans AE, D'Angio GJ, Koop CE. The role of multimodal therapy in patients with local and regional neuroblastoma. *J Ped Surg* 1984, **19**, 77–80.

2. Carlsen NLT, Christensen IJ, Schroeder H, *et al.* Prognostic value of different staging systems in neuroblastoma and completeness of tumor excision. *Arch Dis Child* 1986, **61**, 832–842.
3. Nitschke R, Smith Ei, Altshuler G, *et al.* Postoperative treatment of non-metastatic visible residual neuroblastoma: A pediatric oncology group study. *J Clin Oncol* 1991, **9**, 1181–1188.
4. Stephenson RS, Cook BA, Mease AD, Ruymann FB. The prognostic significance of age and pattern of metastases in stage IVs neuroblastoma. *Cancer* 1986, **58**, 372–375.
5. Evans AE, D'Angio GJ, Propert K, Anderson J, Hann HWL. Prognostic factors in neuroblastoma. *Cancer* 1987, **59**, 1853–1859.
6. Hartmann O, Scopinario M, Tournade MF, Sarrazin D, Lemerle J. Neuroblastomes traités à l'Institute Gustave Roussy de 1975 à 1979. Cent soixante-treize cas. *Arch Fr Pediatr* 1983, **40**, 15–21.
7. Shafford EA, Rogers DW, Pritchard J. Advanced neuroblastoma: Improved response rate using a multiagent regimen (OPEC) including sequential cisplatin and VM26. *J Clin Oncol* 1984, **2**, 742–747.
8. Rosen EM, Cassady JR, Frantz CN, Kretschmar C, Levey R, Sallan SE. Neuroblastoma: The Joint Center for Radiation Therapy/Dana Farber Cancer Institute/Children's Hospital Experience. *J Clin Oncol* 1984, **2**, 719–732.
9. Bernard JL, Philip T, Zucker JM, *et al.* Sequential cisplatinum/VM26 and vincristine–cyclophosphamide–doxorubicin in metastatic neuroblastoma. An effective alternating non-cross resistant regimen? *J Clin Oncol* 1987, **5**, 1952–1959.
10. Kushner BH, Helson L. Coordinated use of sequential escalated cyclophosphamid and cell-cycle specific chemotherapy (the N4SE protocol) for advanced neuroblastoma: Experience with 100 patients. *J Clin Oncol* 1987, **5**, 1746–1751.
11. Hartmann O, Pinkerton CR, Philip T, Zucker JM, Breatnach F. Very high dose Cisplatin and VP16 in children with untreated, advanced neuroblastoma. *J Clin Oncol* 1988, **6**, 44–50.
12. Berthold F, Burdach S, Kremens B, *et al.* The role of chemotherapy in the treatment of children with neuroblastoma stage IV: The GPO (German Pediatric Oncology Society) experience. *Klin Pädiatr* 202, 1990, **4**, 262–269.
13. Pinkerton CR, Zucker JM, Hartmann O, *et al.* Short duration, high dose, alternating chemotherapy in metastatic neuroblastoma. (ENSG 3C induction regimen). *Br J Cancer* 1990, **62**, 319–323.
14. Cheung NK, Heller G. Chemotherapy dose intensity correlates strongly with response, median survival and median progression-free survival in metastatic neuroblastoma. *J Clin Oncol* 1991, **9**, 1050–1058.
15. August CA, Serota FT, Koch PA, *et al.* Treatment of advanced neuroblastoma with supralethal chemotherapy, radiation and allogeneic or autologous marrow reconstitution. *J Clin Oncol* 1984, **2**, 609–616.
16. Pritchard J, Germond D, Jones D, Debraker J, Love S. 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* 1986, **5**, 205, (abstr).
17. Philip T, Zucker JM, Bernard JL, *et al.* Improved survival at 2 and 5 years in the LMCE1 unselected group of 72 children with stage IV neuroblastoma older than 1 year of age at diagnosis: Is cure possible in a small subgroup? *J Clin Oncol* 1991, **9**, 1037–1044.
18. Hartmann O, Benhamou E, Beaujean F, *et al.* Repeated high dose chemotherapy followed by purged autologous bone marrow transplantation as consolidation therapy in metastatic neuroblastoma. *J Clin Oncol* 1987, **5**, 1205–1211.
19. Zucker JM, Philip T, Bernard JL, *et al.* Single or double consolidation treatment according to remission status after initial therapy in metastatic neuroblastoma: first results of LMCE3 study in 40 patients. In Evans AE, D'Angio GJ, Knudson AG, Seeger RC, eds. *Advances in Neuroblastoma Research* 3, New York, Wiley-Liss Inc, 1991, 543–551.
20. Philip T, Ladenstein R, Zucker JM, Pinkerton R, Bouffet E, Louis D, *et al.* Double megatherapy and autologous bone marrow transplantation for advanced neuroblastoma: The LMCE2 study. *Br J Cancer* (in press).
21. Philip T, Helson L, Bernard JL, Zucker JM, Kremens B, Favrot MC, Hartmann O. Definition of response and remission in children over one year of age with advanced neuroblastoma. *Ped Hemat Oncol* 1987, **4**, 25–31.
22. Favrot MC, Frappaz D, Maritaz O, *et al.* Histological, cytological and immunological analyses are complementary for the detection of neuroblastoma cells in the bone marrow. *Br J Cancer* 1986, **54**, 637–641.
23. Kemshead JT, Heath L, Gibson FM, *et al.* Magnetic microspheres and monoclonal antibodies for the depletion of neuroblastoma cells from bone marrow: experiences, improvements and observations. *Br J Cancer* 1986, **54**, 771–778.
24. Peto E, Pike MC, Armitage P, *et al.* Design and analysis of randomised clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Br J Cancer* 1977, **35**, 1–39.
25. Cox R. Regression models and life tables (with discussion). *JR Stat Soc (B)*, 1972, **34**, 187–202.
26. Philip T, Michon J, Bernard JL, *et al.* 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 Peter WP, ed. *The Comparative Effects of Recombinant Myeloid Colony Stimulating Factors in Man* 1992 (in press).
27. Garaventa A, Ladenstein R, Chauvin F, *et al.* ABMT in advanced stage IV neuroblastoma. Comparison between two non-randomized groups treated with the same conditioning regimen and receiving purged or unpurged ABMT. *Eur J Cancer* (in press).
28. Shuster JJ, Cantor AB, McWilliams N, Pole GJ, Castleberry RP, Marcus R, *et al.* The prognostic significance of autologous bone marrow transplant in advanced neuroblastoma. *J Clin Oncol* 1991, **9**, 1045–1049.
29. Corbett R, Olliff J, Fairley N, *et al.* A prospective comparison between magnetic resonance imaging, meta-iodobenzylguanidine scintigraphy and marrow histology/cytology in neuroblastoma. *Eur J Cancer* 1991, **27**, 1560–1564.
30. Look T, Hayes FA, Shuster JJ, *et al.* Clinical relevance of tumor cell ploidy and N-myc gene amplification in childhood neuroblastoma: A pediatric oncology group study. *J Clin Oncol* 1991, **9**, 581–591.

Acknowledgements—Ruth Ladenstein is a recipient of the Erwin Schrödinger Stiftung/Austria. The LMCE group is supported by Institut National de la Santé et de la Recherche Médical (INSERM, réseau LMCE no 48.60.22) and the San Salvatore Fondazione. The authors thank the nurses and staff physicians for their efforts on behalf of the patients. They also thank C.R. Pinkerton for reviewing the manuscript.