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High Dose Melphalan in the Treatment of Advanced Neuroblastoma: Results of a Randomised Trial (ENSG-1) by the European Neuroblastoma Study Group

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Background. High dose myeloablative chemotherapy (''megatherapy''), with haematopoietic stem cell support, is now widely used to consolidate response to induction chemotherapy in patients with advanced neuroblastoma. ***Procedure.*** In this study (European Neuroblastoma Study Group, ENSG1), the value of melphalan myeloablative ''megatherapy'' was evaluated in a randomised, multi-centre trial. Between 1982 and 1985, 167 children with stages IV and III neuroblastoma (123 stage IV > 1 year old at diagnosis and 44 stage III and stage IV from 6 to 12 months old at diagnosis) were treated with oncovin, cisplatin, epipodophyllotoxin, and cyclophosphamide (OPEC) induction chemotherapy every 3 weeks. After surgical excision of primary tumour, the 90 patients (69% of the total) who achieved complete response (CR) or good partial response (GPR) were eligible for randomisation either to high dose melphalan (180 mg per square meter) with autologous bone marrow support or to no further treatment. ***Results.*** Sixty-five (72%) of eligible children were actually randomised and 21 of these patients were surviving at time of this analysis, with median follow-up from randomisation of 14.3 years. Five year event-free survival (EFS) was 38% (95% confidence interval (CI) 21–54%) in the melphalan-treated group and 27% (95% CI 12–42%) in the ''no-melphalan'' group. This difference was not statistically significant ($P = 0.08$, log rank test) but for the 48 randomised stage IV patients aged >1 year at diagnosis outcome was significantly better in the melphalan-treated group-5 year EFS 33% versus 17% ($P = 0.01$, log rank test). ***Conclusions.*** In this trial, high dose melphalan improved the length of EFS and overall survival of children with stage IV neuroblastoma >1 year of age who achieved CR or GPR after OPEC induction therapy and surgery. Multi-agent myeloablative regimens are now widely used as consolidation therapy for children with stage IV disease and in those with other disease stages

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when the MYCN gene copy number in tumour cells is amplified. Because they are more toxic, complex, and costly these combination megatherapy regimens should be compared with single agent melphalan in randomised clinical trials. Pediatr Blood Cancer 2005;44:348–357.

Key words: high dose melphalan; randomised trial; stage 3 and 4 neuroblastoma; survival; toxicity

INTRODUCTION

Advanced neuroblastoma is one of the most aggressive forms of childhood cancer and patients aged >1 year at diagnosis with stage 4 disease have a particularly poor prognosis [1–4]. Induction chemotherapy schedules, usually cisplatin-based, developed in the 1980s and 1990s are associated with higher response rates and longer median survival than those achieved prior to that time [4,5]. Survival rates have also improved, especially in patients <1 year of age at diagnosis [6,7]. Some older children are also long-term survivors [8,9] but in most cases resistance to chemotherapy develops within 2 years from diagnosis and more than half of stage 4 patients >1 year of age at diagnosis still succumb to the disease.

The log-linear in vitro dose-response of cultured neuroblastoma cells to melphalan and other alkylating agents [10] provides a rationale for ''high dose consolidation'' chemotherapy (''megatherapy'') to try to overcome drug resistance [11]. Since its introduction into clinical practice in 1977, high dose melphalan has been used by many centres to treat patients with advanced neuroblastoma, often in combination with either total body irradiation (TBI) or other high dose cytotoxic drugs [12–14]. However, even with full supportive care including haematopoietic support with autologous bone marrow or peripheral blood stem cell transplantation, this treatment is toxic, resource-consuming, and expensive.

In the randomised controlled trial, known as ''ENSG1,'' reported here we decided to evaluate the impact of high dose melphalan as a single agent in patients with Evans [15] stages III and IV neuroblastoma who had achieved ''good partial'' response (GPR) or complete response (CR) after induction chemotherapy and surgery. Because of an encouraging 74% response rate to the combination of vincristine (oncovin), cisplatin, teniposide (an epipodophyllotoxin), and cyclophosphamide (known as ''OPEC'') given 3 weekly in a multicentre United Kingdom pilot study [4], we selected this regimen for induction therapy, with a lower dose of cisplatin per course to try to reduce renal and auditory toxicity. Surgical removal of the primary tumour was carried out prior to randomisation, and there was no radiation therapy. The principal objective was to establish whether or not high dose melphalan led to an increase in duration of event-free survival (EFS) and overall time to death. A supplementary aim was to establish whether or not the encouraging response rate to the ''OPEC'' regimen and surgery observed in the pilot study [4] could be replicated by a large international cooperative group. Preliminary results for some of the children in this study were reported in 1987 [16]. Here, we report the long-term results of ENSG1, with a minimum of 8.8 years off-treatment follow-up.

PATIENTS AND METHODS

The study was carried out and completed prior to the publication of the now widely-accepted ''International Criteria'' for diagnosis, staging, and response applied to patients with neuroblastoma [17]. However, the definitions used for ENSG1 were agreed unanimously by the participants as they were considered to be ''standard practice'' at the time of the trial.

Diagnosis, Staging, and Response Assessment

The diagnosis of neuroblastoma was based on either (a) histological appearances, confirmed by the trial pathologist (JRP) or (b) the presence of tumour cells in bone marrow and 24 hr urinary homovanillic acid (HVA) and/or vanilmandelic acid (VMA) and/or vanillyl glycol levels at least twice the normal values for age. Minimum staging investigations were: antero-posterior and lateral chest radiographs, ^{99}technetium isotope bone scan, abdominal ultrasonography, or computerised tomographic scan and bilateral iliac bone marrow aspirates and trephine biopsies [18]. Marrow aspirates were examined by conventional cytological methods without Ficoll separation. Meta-iodobenzylguanidine (mIBG) scanning was not used because it was available only in a few centres at the time of the study. Staging investigations that were ''positive'' at diagnosis were always repeated at the time-point when the response to ''OPEC'' and surgery was assessed, usually 5–8 months after the start of treatment.

Ethical Approval, Patient Selection, and Follow-Up

The study was approved in writing by the Research Ethics Committee of each of the 16 participating treatment centres and informed written consent was provided by the children's parents or guardians prior to randomisation. Children with stage IV disease [19] were excluded, but all children >6 months old at diagnosis with stages III or IV

neuroblastoma referred to each of the 16 centres were entered into the study, which recruited patients from January 1982 until March 1985. Patients' clinical characteristics are shown in Table I. Only those achieving CR or GPR after induction treatment were eligible for randomisation, but all eligible and ''ineligible'' children registered in the study (n = 167) were followed up at 6 monthly intervals for 5 years, then annually thereafter.

Induction Treatment and Surgery

Patients were scheduled to receive courses of 4-drug ''OPEC'' induction therapy (vincristine 1.5 mg per square meter and cyclophosphamide 600 mg per square meter on day 1, cisplatin 60 mg per square meter on day 2, and teniposide 150 mg per square meter on day 4) repeated every 21 days or as soon as possible afterwards if recovery of neutrophil and platelet counts was slow. Unjustified delay of more than 28 days between succeeding courses, or more than 33% increase or reduction in more than four individual drug doses, were defined as protocol deviations. Haematopoietic growth factors were not available for use at the time of the trial.

There were two early deaths from complications of progressive tumour, one before and one during the first course of OPEC. There were also 10 children in whom OPEC induction treatment could not be continued because of toxicity of individual agents—allergy to teniposide in seven instances and severe neuropathy from vincristine in one case. In addition, cisplatin was omitted altogether in one case because of tumour-related obstructive uropathy and in a second case because it was implicated in an

TABLE I. Balance of Randomised and Non-Randomised Groups According to Clinical Characteristics at Time of Diagnosis

	High dose melphalan group (n = 32)	No melphalan group (n = 33)	Group not randomised (n = 25)
Stage			
III	6	7	5
IV	26	26	20
Gender			
Boys	16	18	14
Girls	16	15	11
Ages			
6–12 months	3	2	4
13–24 months	8	9	8
>24 months	21	22	13
Centre			
Larger	18	18	8
Smaller[a]	14	15	17
Site of primary tumour			
Cervical	0	1	0
Thoracic	1	2	1
Thoraco-abdominal	1	1	2
Abdominal	28	28	19
Pelvic	0	0	2
Multiple	1	1	0
Unknown	1	0	1
Metastases (stage 4 only n = 73)			
Bone	21	19	12
Bone marrow	21	17	12
Liver	5	4	4
Distant lymph nodes	14	17	9
Pleura	1	5	0
Other	4	3	2
No. of induction chemotherapy courses received			
6	9	13	4
7–8	10	11	4
9–10	13	9	17
Type of response to OPEC			
Complete	17	19	14
Good partial	15	14	11

[a]These centres contributed fewer than 12 patients each.

episode of disseminated intravascular coagulation. Renal and auditory function were carefully monitored but, in contrast to the United Kingdom pilot study in which the dose of cisplatin (100 mg per square meter) was higher [4], never led to discontinuation of the induction regimen.

Surgical removal of the primary tumour was not attempted in the ''non-responders,'' in whom metastatic disease could not be controlled by ''OPEC,'' but ''responding'' patients then underwent surgical resection of the primary tumour. The intent of surgery was to ''debulk'' the tumour to the maximum safe extent, which usually entailed prolonged, meticulous piecemeal tumour removal from the retroperitoneum and the great vessels supplying and draining the abdominal organs. Regional lymph nodes were usually resected, without a formal ''block dissection.'' There was no post-operative irradiation or ''second look surgery.''

Tumour response was assessed after 6, 8, and 10 courses of induction chemotherapy and surgery. CR was defined as: disappearance of primary tumour, as judged by abdominal imaging, and of secondary deposits and normal urine catecholamine metabolite levels. GPR was defined as: (a) shrinkage of primary tumour by more than 50% in each of three dimensions, (b) reduction of urine catecholamine metabolite levels by more than 50% of the original values, and (c) disappearance of secondary deposits including bone marrow involvement. In both types of response, however, isotope bone scan appearances need not have normalised so long as there was improvement at all sites reported as abnormal on the scan performed at diagnosis, with no new lesions. This criterion was used because, prior to ENSG1, biopsies of ''improving'' isotope bone scan lesions in children with stage 4 neuroblastoma had shown no identifiable tumour in 8 of 8 instances (Breatnach F, Gordon I, and Pritchard J, unpublished observation). All other patients, including many with complete resolution of symptoms and clinical signs of the disease, were classified as ''non-responders.''

Eligibility and Procedure for Randomisation

The decision as to whether or not to proceed to randomisation was taken after 6, 8, or 10 courses of the OPEC induction treatment plus surgery, between 5 and 9 months from diagnosis. Parents' understanding of their child's illness was considerable by this time and informed written parental consent or refusal for randomisation was obtained in each case following careful discussions, which focused on the possible risks and benefits involved, with a senior clinical investigator. Randomisation was performed at the coordinating data centre by means of a minimisation technique [20] using (a) stages (III or IV) and (b) individual participating centres as stratification variables. The overall aim was to randomise at least 60 patients. The statistical power of a trial of this size to detect an improvement in 2-year survival to 40% in the ''high dose melphalan'' group from an anticipated 20% in the ''no melphalan'' group was calculated as 0.53.

Megatherapy/Autologous Bone Marrow Transplant

Two of the 32 patients randomised to receive melphalan relapsed after they had achieved GPR, but before melphalan was scheduled. In the remaining 30 children bone marrow harvesting was carried out under general anaesthesia the day before melphalan administration. The median total nucleated cell count in the 30 bone marrow harvests was 4.6×10^8 per kg (range $1.8–14.2 \times 10^8$ per kg). Marrow was stored at 4°C without any ''purging'' procedure. Melphalan was administered 4–8 weeks after surgery or the final course of induction chemotherapy, whichever was more recent, as a bolus dose of 180 mg per square meter, with pre- and post-hydration using 5% dextrose/0.45% sodium chloride. Twelve to 30 hr afterwards the bone marrow was returned intravenously over 2–4 hr. Subsequent isolation, nursing, and supportive procedures, including nutritional supplementation, followed the policy of each individual centre, though guidelines for blood product transfusion were provided and early parenteral feeding was recommended.

Relapse

Relapse was confirmed histologically, or by rising urinary HVA and/or VMA excretion when the clinical evidence was overwhelming.

Statistical Methods

Length of EFS (time to relapse or death prior to relapse) and survival to death (time to death from any cause), from randomisation, were displayed in the form of Kaplan–Meier life tables and analysed by the log rank test [21,22]. Accumulating data were inspected [23] at 6 monthly intervals by the trial statistician (DRJ), in consultation with an independent medical advisor. Interim analyses were presented to participating clinicians without identification of the treatment groups. In the final analyses, allowance for the effects of factors other than treatment (tumour stage, patient age, size of participating centre, number of courses of ''OPEC'' received and type of response—GPR or CR) was made by means of proportional hazards regression models [24]. The data were analysed overall and a retrospective decision was made to analyse separately the large sub-group of age >1 year patients with stage IV disease, because these characteristics were by that time widely agreed to identify the ''worst prognosis'' category of children with neuroblastoma [1–3,6].

Recruitment to the trial was terminated when the planned number of randomised patients was exceeded.

RESULTS

In the 39-month recruitment period for ENSG1, the 16 participating hospitals admitted a total of 167 patients aged 6+ months at diagnosis with stage III or IV neuroblastoma. The childrens' progress in the study is summarised in Figure 1. Non-compliance by clinicians at 2 of the 16 centres accounted for 25 of the 37 deviations during induction treatment, one of these centres withdrawing from the study after contributing 18 patients, only 1 of whom was randomised. The protocol deviations were as follows: (a) in 3 cases patients were eligible but not registered, (b) 1 stage IV patient was initially incorrectly staged, (c) 2 children died before chemotherapy could be administered, (d) 2 stage III cases had initial gross tumour resection but no further treatment, (e) in 10 cases the dose of cisplatin (>35% of the recommended dose) was not as per protocol, (f) in 1 patient there were excessive treatment delays, (g) in 14 cases—all in the centre that withdrew from the study—the physician stopped or changed induction treatment without evidence of treatment failure, and (h) in 4 cases the parents elected to stop induction treatment.

Response to OPEC and Surgery

Of the 130 patients who remained "on study," 90 (69.2%) were designated "responders"—44 CR and 46 GPRs to the OPEC regimen plus surgery. Of these patients, 26 had received 6 courses of OPEC, 24 had received 8 courses, and 40 had received 10 courses. The primary tumour was not detectable in one child, but the remainder underwent surgery. In 46 cases, resection was macroscopically incomplete and, although all visible tumour was resected from the remaining 43 children, only 5 (5.6% of 89) had clear post-surgical tumour margins. There were two deaths in the immediate post-operative period, one from uncontrolled bleeding at the abdominal primary tumour site in a child with a normal platelet count and coagulation studies, and the other for unexplained reasons.

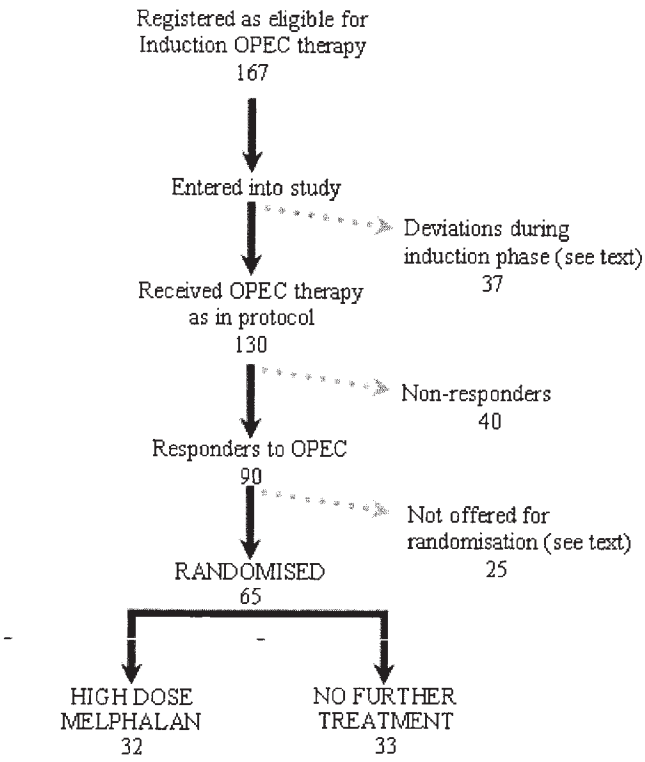

Fig. 1. Flow diagram of the study.

Twenty-five of the 90 eligible patients were not randomised for the following reasons; 3 patients died after documentation of response but before randomisation, 2 died in the immediate post-operative period, (as above) and one of unknown causes, suddenly at home. In the other 19 instances randomisation was declined, usually because the very substantial disparity between the two treatment options. Of the 65 patients successfully randomised (72% of the total "eligible"), 32 were allocated to high dose melphalan and 33 to no further treatment. Two children randomised to receive high dose melphalan relapsed without actually receiving this treatment, but both are included in the data, i.e., the analysis was by "intention to treat." Table I shows that the most influential prognostic variables were well balanced between the two randomised groups. Covariate analysis confirmed that the effect of any imbalances, for example, in the number of induction chemotherapy courses and the types of responses, were not statistically significant.

Toxicity of High Dose Melphalan

Despite autologous bone marrow transplantation, the toxicity of high dose melphalan was considerable (Table II). The 30 patients who received high dose melphalan were hospitalised for a median of 30 days. Each child had severe myelosuppression and unpleasant gastrointestinal symptoms. Fifteen patients had an otherwise uncomplicated recovery but amongst the other 15 children there were 21 episodes of life-threatening toxicity including 2 treatment-related deaths (7%), both due to septicemia, in children aged 20 months and 27 months at diagnosis. Additional morbidity was as follows. Of the five children with severe gastrointestinal or nutritional problems—two had severe oral mucositis, one suffered an episode of paralytic ileus, and two lost more than 10% body weight. A boy with severe esophageal ulceration developed a stricture that resolved only after 3 years of regular endoscopic dilatation. The three episodes of major haemorrhage included two children with haematemesis associated with melphalan-induced mucositis and one with bleeding into the pleural cavity. Renal function was sub-optimal at the time of high dose melphalan in four

TABLE II. Toxicity of High Dose Melphalan (n = 30)

	Duration (days)			
	n	%	Median	Range
(a) Inevitable events[a]				
Neutrophils $<1{,}000 \times 10^6$/mm[b]	30	100	16	9–41
Platelets $<100{,}000 \times 10^6$/mm[b]	30	100	30	19–70
Diarrhoea	28	93	10	2–40
Mucositis	30	100	10	5–45
(b) Serious clinical events			Fatalities	
Sepsis	7	23	2[c]	
Gastro-intestinal complications	5	17	0	
Bleeding	3	10	0	
Renal toxicity	4	14	0	
Hepatic veno-occlusive disease	2	7	0	
Convulsions	1	3	0	

[a]Temporary haemopoietic damage and mucositis were considered ''inevitable.''
[b]Conversion factor to SI units = 10^6.
[c]One caused by *Staphylococcus aureus*, the other by *Staphylococcus epidermidis*.

children because of previous cisplatin and aminoglycoside antibiotics, and deteriorated acutely. Function later stabilised without evidence of chronic renal failure in three cases, but the fourth required renal transplantation. One child had a single grand mal seizure but anticonvulsant therapy prevented recurrence.

Survival

At the time of analysis, there were 21 surviving children with a median follow-up of 14.3 years from randomisation (range 8.8–17.1 years). Median time from randomisation to relapse was 21 months for the high dose melphalan group and 7 months for the ''no melphalan'' group. Five year EFS (Fig. 2A) was 38% (95% confidence intervals (CI) 21–54%) in the melphalan-treated group and 27% (95% CI 12–42%) in the ''no melphalan'' group ($P = 0.08$, log rank test). Five year survival (Fig. 2B) was 47% (95% CI 30–64%) in the melphalan-treated group, including the two treatment related deaths, and 30% (95% CI 15–46%) in the ''no melphalan'' group ($P = 0.1$, log rank test). These differences were not significant, using a 2-sided analysis.

Significant differences in EFS (Fig. 2C) and survival (Fig. 2D) were, however, evident for children aged >1 year with stage IV disease. Twenty-four patients randomised to receive melphalan and 24 of those in the ''no-melphalan'' group fell into this category. The median time from randomisation to relapse was 18 months for the melphalan treated patients compared to 3 months for the ''no melphalan'' patients (5 year EFS 33% vs. 17%, $P = 0.01$ log rank test). Five year survival was also significantly better in the melphalan-treated children (46% vs. 21%, $P = 0.03$ log rank test). To demonstrate that incomplete randomisation of eligible patients did not affect the results, we conducted survival analyses for all 167 registered patients (Fig. 3A) and also for all 123 children aged >1 year with stage IV disease (Fig. 3B). These analyses strongly indicate that there was no systematic selection bias of patient entry into the trial.

EFS and survival to death and were better in stage III patients than in patients with stage IV tumours, and in those <1 year compared with those >1 year of age at diagnosis. Other variables of potential prognostic importance were also investigated but no clear relationship was established between EFS or time to death and any of the following; gender, individual treatment centre, year of treatment, type of response or number of ''OPEC'' courses received. These findings were also reflected in the results of multifactorial analyses in which only treatment had a clearly demonstrable effect on EFS. When stage, treatment centre, and the two ''stratification variables'' were added to the list of ''explanatory variables'' included in the Cox's proportional hazards model they had little influence on the estimates of treatment effect. Similarly, deviations from the proportional hazards assumption and interactions between treatment and other variables were not significant.

Most relapses occurred within 2 years and most deaths within 5 years from randomisation (Fig. 2). However, there were four patients (all aged >1 year with stage IV disease at diagnosis) who relapsed and died after 5 years from randomisation. One patient in the ''no melphalan'' group relapsed with recurrent neuroblastoma after 7 years and died 10 months later and a second child in this group relapsed at 7 months from randomisation achieved a second remission after further therapy, but died 12 years later after a second relapse. Similarly, two patients in the melphalan group died 6 years from randomisation, having first relapsed at 26 and 45 months, respectively.

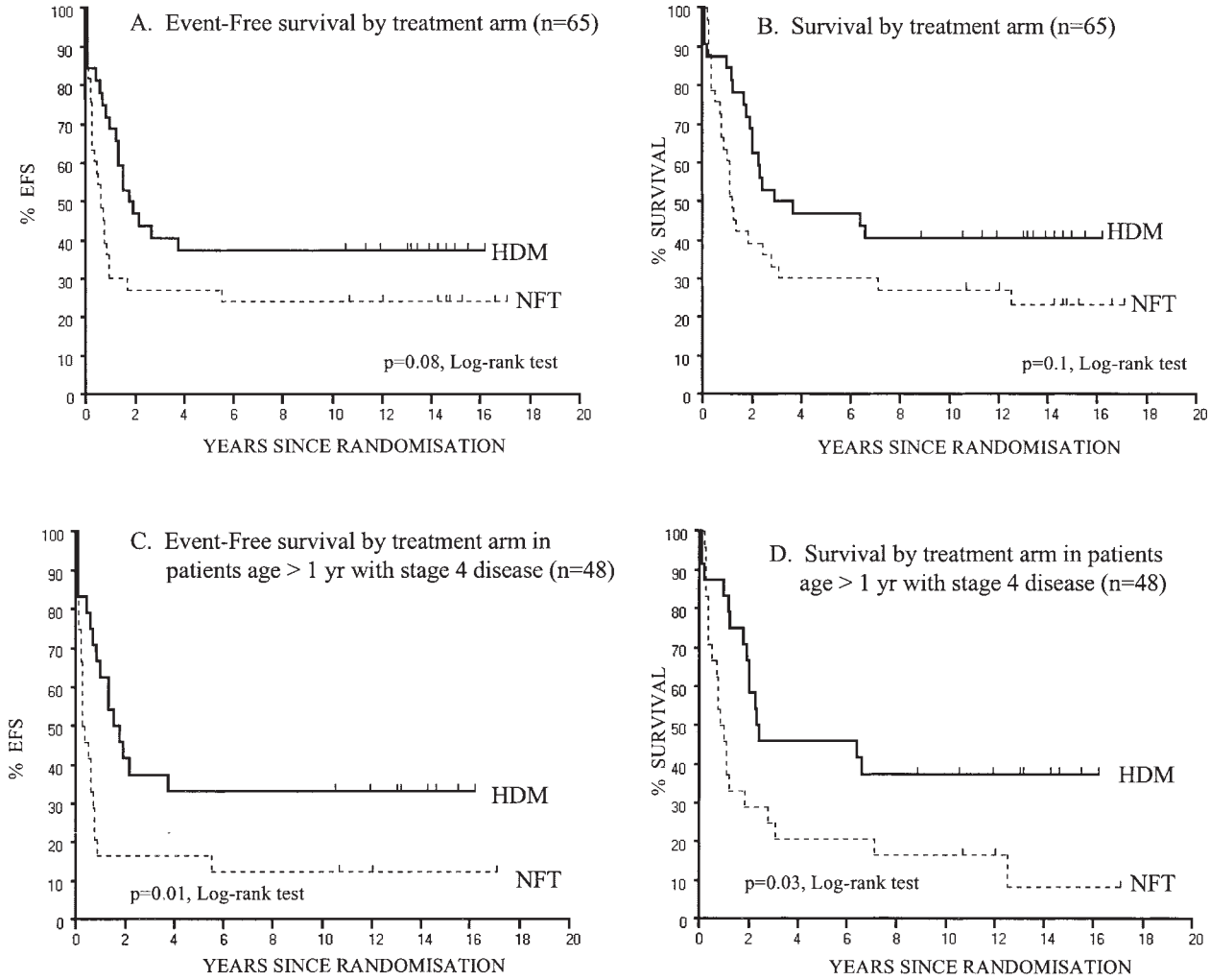

Fig. 2. Survival and event-free survival (EFS) from randomisation to death by treatment group. HDM, high dose melphalan; NFT, no further treatment (no melphalan). **A**: EFS by treatment arm (n = 65); (**B**) survival by treatment arm (n = 65); (**C**) EFS by treatment arm in patients aged >1 year with stage IV disease (n = 48); (**D**) survival by treatment arm in patients aged >1 year with stage IV disease (n = 48).

DISCUSSION

In the 1980s, single agent melphalan was an attractive choice for high dose therapy because its major dose-limiting toxicities—myelosuppression and gastro-intestinal damage—could be offset, respectively, by bone marrow "rescue" and nutritional support [11]. Renal and central nervous system damage, though reported after high dose melphalan, were unusual and fatalities rare. Other high dose drugs and TBI have been used, in a variety of combinations with melphalan in the "megatherapy" treatment of neuroblastoma since then [9,25–31] but at the time this study was designed, the toxicity and efficacy of these combinations was unknown and the members of the ENSG, which included small centres treating 1 or 2 patients each year, favoured the least complex option. Why was melphalan administration scheduled after 5–9 months of induction chemotherapy? Despite initial good clinical responses to conventional dose induction chemotherapy, such as the "OPEC" combination and surgery, patients with advanced neuroblastoma usually relapsed after the completion of this treatment, 6–18 months from diagnosis [1–4]. The hypothesis under test in ENSG1 was that high dose melphalan might lead to additional tumour cell kill, reflected by longer survival and perhaps an increased cure rate. By analogy with acute leukaemia [32] this hypothesis was best tested when tumour burden was at a minimum, during first clinical remission.

Neuroblastoma is a rare disease and single centre studies involve relatively few cases. In ENSG1 this difficulty was overcome by involving 16 centres in 6 European countries. The formation of the ENSG to enable international collaboration in this way was original and unique at the time ENSG1 commenced. The obligatory registration procedure (see "Patients and Methods"), to which all centres were obliged to adhere, was intended to eliminate

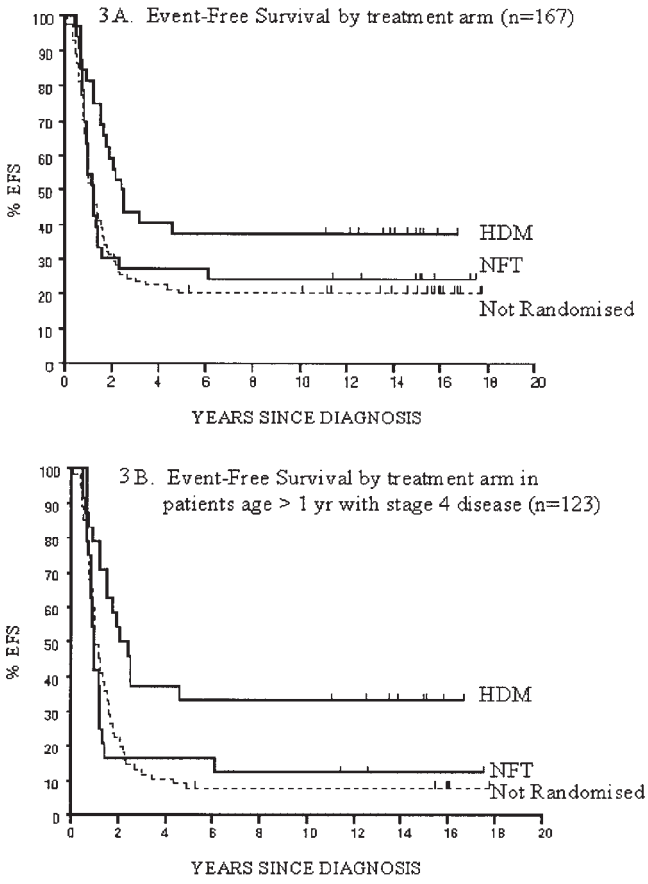

Fig. 3. EFS from randomisation to death for randomised patients, by treatment group, and for eligible patients not randomised (see text). HDM, high dose melphalan; NFT, no further treatment (no melphalan). **A**: EFS (all patients) HDM versus NFT $P = 0.08$, NFT versus not randomised $P > 0.1$, log rank test; (**B**) EFS (patients age >1 year with stage IV tumours) HDM versus NFT $P = 0.01$, NFT versus not randomised $P > 0.1$, log rank test.

selection bias of patients entering the study. The response rate to ''OPEC'' and surgery was 69%, a result comparable to that achieved in the pilot study [4]. Thus, one of the secondary objectives of ENSG1 was achieved successfully.

Only 65 of a possible 90 responding patients were actually randomised but comparable difficulties are often encountered in randomised studies in which there is a major disparity between treatment alternatives (see, for example [33,34]). Despite autologous bone marrow transplant and appropriate nutritional support, the toxicity of high dose melphalan was considerable, with some long-term sequelae. Nevertheless, there was an apparent EFS and survival advantage for patients in the high dose melphalan group compared with those who received no melphalan, and the difference was statistically significant in patients with stage IV disease aged >1 year at diagnosis. The number of ''induction'' cycles needed for patients to achieve the required response to treatment varied slightly between the two randomised groups (Table I) but this difference was taken into account in the multivariate analyses and had no significant prognostic effect. The even balance between other, potentially influential prognostic factors (Table I) together with the results of the multivariate modelling strongly suggests that the observed differences in outcome were genuinely a consequence of high dose melphalan therapy and not of any confounding factor. Long-term survival of several melphalan-treated patients whose bone marrow had been heavily infiltrated by tumour at diagnosis indicates that it is not always necessary to ''purge'' the harvested marrow of these patients prior to haematopoietic stem cell support.

If ''megatherapy'' is effective in only a modest proportion of children with advanced neuroblastoma, there must be a very chemotherapy-resistant tumour cell population in most other patients. Expression of both P-glycoprotein and the multidrug resistance protein (MRP) are predictive of disease progression in neuroblastoma [35,36] but alkylating agents are not eliminated from cells by these mechanisms so other drug-resistance pathways must also be involved. Drug resistance might be constitutive or might evolve during the first few months after diagnosis, during ''induction'' chemotherapy. The ENSG has recently completed a randomised trial, known as ''ENSG5,'' to determine whether ''rapid ''COJEC'' (J = carboplatin) induction chemotherapy delivered at high dose-intensity, regardless of myelosupression, followed by surgery and high dose melphalan, can give better EFS and overall survival rates than standard ''OPEC'' induction delivered at 3 week intervals. The ''rapid'' regimen has been shown to be superior, both in terms of EFS and disease-free survival (DFS) [37]. The question therefore arises: since the ''moderately resistant'' tumour cell population eradicated by melphalan may be much the same cell population also eradicated by the higher dose-intensity rapid induction regimen, do patients who have responded to ''rapid COJEC'' benefit from high dose melphalan to the same degree as those treated with standard ''OPEC''? Another large scale randomised trial of high dose melphalan, versus no further treatment, following response to ''rapid COJEC'' and surgery, would be required to resolve this important question.

Follow-up for the 21 survivors in ENSG1 is very long, with a minimum of 8.8 years and median of >14 years. All four ''late deaths'' after 60 months from diagnosis were of patients who were aged >1 at diagnosis with stage IV disease, three of whom had relapsed once already. Stage IV, age >1 year and prior relapse were identified as significant risk factors for ''late events'' in a much larger series of ENSG patients recorded in its ''registry'' [38].

ENSG1 enrolled patients with stage III or IV disease, including children aged 6–12 months of age, because at the time the trial was designed these children were collectively considered by ENSG centres to be at high risk

of relapse, with survival expectations of <50%. In retrospect, a small number of patients with a relatively good prognosis—stage III and stage IV patients with MYCN non-amplified tumours—may have been included. However, ENSG1 was designed and conducted before analysis of cytogenetics and ''molecular'' variables (tumour ploidy, MYCN amplification, 1p36 deletion, 17q gain and TRK-A expression) were available in participating institutions. We, therefore, have no data on the relationship between these characteristics, response to melphalan and survival. Nowadays these molecular studies are crucial in enabling us to define in different prognostic groups at the time of diagnosis [39–41].

Over the past 20 years many other centres have used melphalan in combination with other drugs in high dose, with or without TBI [25–31], to determine whether multi-agent myeloblative regimens achieve more tumour cell kill than melphalan alone but there is no clear-cut difference in the outcome for stage 4 patients who are >1 year of age at diagnosis. These disappointing results are at least partly due to the greater toxicity and mortality of the multi-agent ''megatherapy'' regimens, compared with high dose melphalan alone. Similarly, the outcome after two sequential high dose melphalan-containing treatments seem no better than after one treatment [25] and results are not obviously superior in centres where ''purging''—to try to deplete the reinfused bone marrow of tumour cells—is used, compared with those in which it is not. Allogeneic and autologous marrow transplantation give similar results, with no evidence of an allogeneic ''graft-versus-tumour'' effect [42].

The Children's Cancer Group conducted a randomised trial (CCG-3891) examining the value of ''megatherapy'' in children >1 year old with stage 4 (INSS) neuroblastoma [9]. This trial demonstrated significant improvement in EFS for those randomised to receive TBI and autologous bone marrow ''rescue'' purged of neuroblastoma cells, compared with three cycles of intensive, but non-myeloblative, chemotherapy. Colleagues in the German Pediatric Oncology Group (GPOG) have recently completed a study [43], similar in design to that of CCG-3891, in which ''megatherapy'' was also randomly compared with ''maintenance chemotherapy.'' These two studies and ENSG1 are the only three randomised trials yet conducted to gauge the effects of ''megatherapy'' in children with advanced neuroblastoma. ENSG1 was conducted >10 years before either CCG-3891 or the GPOG study, when supportive care was less advanced, and there were also differences between ENSG1 and the two other studies in terms of staging and eligibility criteria, length of follow-up, and trial design. The CCG-3891 trial also included a second randomisation either to 13-*cis*-retinoic acid or to placebo. Patients receiving *cis*-retinoic acid had a better outcome than those in the placebo group. As regards ''megatherapy,'' the outcome of the three trials was similar but the toxicity of multi-agent regimens was inevitably greater, even though contemporary supportive care was used, with a procedure-related mortality of 5–10%. In ENSG1, when several of the centres were unfamiliar with high-dose melphalan therapy, mortality was 6%, but since then—for example, in the ENSG5 study—the figure has fallen to around 1% (Pearson ADJ, personal communication). It may be, therefore, that any minor gain in tumour cell kill by multi-agent ''megatherapy'' regimens is offset by an increase in fatal complications. The greater patient discomfort, staffing requirements, and costs demanded by multi-agent regimens also have to be carefully considered. We therefore recommend that randomised trials are now carried out to compare the risk:benefit ratio of melphalan only versus multi-agent consolidation ''megatherapy,'' without TBI, for >1 year old children with stage 4 neuroblastoma.

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