Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: a randomised controlled trial

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Summary

Background **Myeloablative megatherapy is commonly used to improve the poor outlook of children with high-risk neuroblastoma, yet its role is poorly defined. We aimed to assess whether megatherapy with autologous stem-cell transplantation could increase event-free survival and overall survival compared with maintenance chemotherapy.**

Methods **295 patients with high-risk neuroblastoma (ie, patients with stage 4 disease aged older than 1 year or those with *MYCN*-amplified tumours and stage 1, 2, 3, or 4S disease or stage 4 disease and <1 year old) were randomly assigned to myeloablative megatherapy (melphalan, etoposide, and carboplatin) with autologous stem-cell transplantation (n=149) or to oral maintenance chemotherapy with cyclophosphamide (n=146). The primary endpoint was event-free survival. Secondary endpoints were overall survival and the number of treatment-related deaths. Analyses were done by intent to treat, as treated, and treated as randomised.**

Findings **Intention-to-treat analysis showed that patients allocated megatherapy had increased 3-year event-free survival compared with those allocated maintenance therapy (47% [95% CI 38–55] *vs* 31% [95% CI 23–39]; hazard ratio 1·404 [95% CI 1·048–1·881], p=0·0221), but did not have significantly increased 3-year overall survival (62% [95% CI 54–70] *vs* 53% [95% CI 45–62]; 1·329 [0·958–1·843], p=0·0875). Improved 3-year event-free survival and 3-year overall survival were also recorded for patients given megatherapy in the as-treated group (n=212) and in the treated-as-randomised group (n=145). Two patients died from therapy-related complications during induction treatment. No patients given maintenance therapy died from acute treatment-related toxic effects. Five patients given megatherapy died from acute complications related to megatherapy.**

Interpretation **Myeloablative chemotherapy with autologous stem-cell transplantation improves the outcome for children with high-risk neuroblastoma despite the raised risk of treatment-associated death.**

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Introduction

The role of myeloablative megatherapy with autologous stem-cell transplantation in improving the outlook for children with high-risk neuroblastoma is poorly defined.[1,2] Myeloablative conditioning regimens, with or without total-body irradiation, followed by autologous stem-cell transplantation is widely used in the hope of overcoming resistance to standard polychemotherapy,[3] and can also be given in a tandem[4] or a triple[5] setting. Nonetheless, autologous stem-cell transplantation is expensive and carries a burden to the patient that is associated with death in 6–14% of patients.[3,6]

To our knowledge, only two randomised trials have been published of autologous bone-marrow transplantation for high-risk neuroblastoma. The European trial,[7] which used 180 mg/m^2 melphalan alone for myeloablation, assessed 48 patients aged older than 1 year with stage 4 neuroblastoma and showed a survival advantage for the transplantation group compared with no further treatment. A Children's Cancer Group study,[6] which assessed autologous bone-marrow transplantation versus continuing chemotherapy, and these treatments with or without retinoic acid, showed improved event-free survival, but not overall survival, for the 189 patients who were allocated autologous bone-marrow transplantation, particularly those assigned transplantation and retinoic acid. The transplantation regimen consisted of myeloablative chemotherapy (1000 mg/m^2 carboplatin, 640 mg/m^2 etoposide, and 210 mg/m^2 melphalan), total body irradiation (10 Gy), and reinfusion of purged autologous bone-marrow cells.

Other researchers have assessed prognostic factors[3,8–10] and the feasibility or effectiveness of various myeloablative regimens,[11–15] but have not made definitive conclusions about autologous bone-marrow transplantation. Furthermore, the improved outcome with autologous bone-marrow transplantation in the two randomised trials[6,7] must be viewed in the context of the complete therapy (eg, preceding chemotherapy, myeloablative regimen with or without total-body irradiation, stem cells, and type of continuation chemotherapy). Our comparison[16] of autologous bone-marrow transplantation with 1-year oral continuation therapy (assigned by investigators and not by

randomisation) suggested that event-free survival and overall survival were much the same in each group, but with a high frequency of second malignant disease in the etoposide-containing continuation-chemotherapy group. This result led to this prospective randomised comparison of myeloablative megatherapy including autologous stem-cell transplantation with modified oral, non-myeloablative maintenance chemotherapy in children, adolescents, and young adults with high-risk neuroblastoma.

Methods

Patients

339 individuals diagnosed at age 0–20 years with high-risk neuroblastoma in Germany and Switzerland and registered in the neuroblastoma study of the German Society of Paediatric Oncology and Hematology between April 28, 1997, and Oct 1, 2002, were eligible for the trial (NB97). According to data from the German Childhood Cancer Registry (Mainz, Germany), 99% of high-risk patients diagnosed in Germany participated in NB97. Therefore, there was no patient selection. NB97 was approved by the ethics committee of the University of Cologne, Germany, and of the cooperating institutions. Written informed consent was obtained from parents or legal guardians of every participant. All patients participated on a completely voluntary basis.

The diagnosis of neuroblastoma and the staging were done in accordance with the International Neuroblastoma Staging System (INSS) criteria.[17] High-risk neuroblastoma was defined as stage 4 disease in

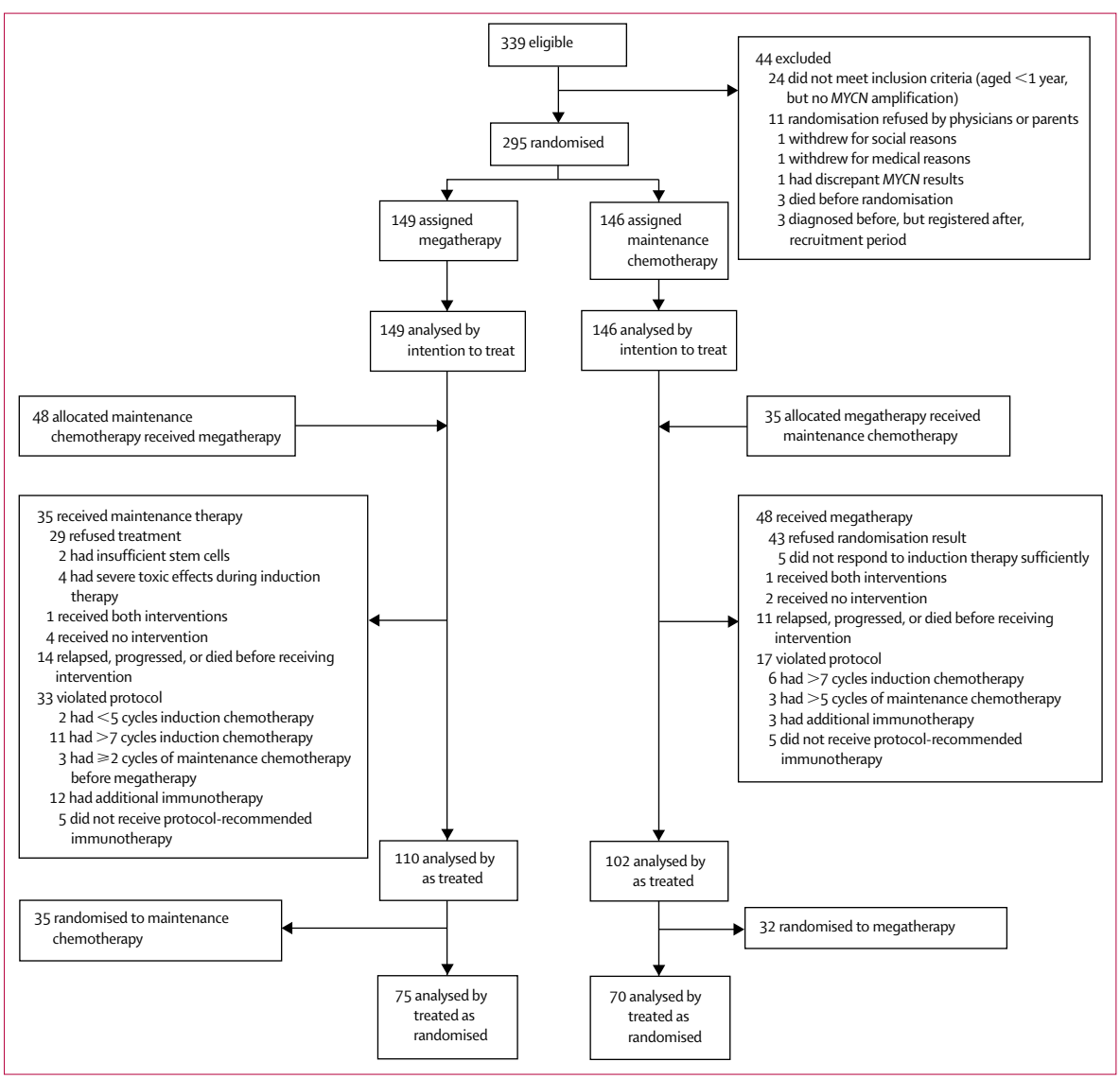

Figure 1: **Trial profile**

patients older than 1 year or as *MYCN*-amplified tumours in patients with stage 1, 2, 3, or 4S disease or with stage 4 disease and aged younger than 1 year. *MYCN* status was ascertained in one or two of the three central laboratories (M Schwab, Heidelberg: Southern blot;[18] H Christiansen, Marburg: PCR;[19] and R Spitz, Cologne: fluorescence in situ hybridisation[20]). *MYCN* status was defined with blinding to other patient characteristics. Definitions and cut-offs have been defined previously[18–20] (eg, >4 fold increase in *MYCN* copy number in relation to the number of copies of chromosome 2 on FISH) and met the guidelines of the International Society for Pediatric Oncology Europe Neuroblastoma Pathology, Biology and Bone Marrow Group.[21] The congruity of results from two methods for *MYCN* status was 100% (n=173).

Randomisation

Randomisation was done centrally at the Institute for Medical Biostatistics, Epidemiology and Informatics, University of Mainz, Germany, by use of a computer-generated sequence with a block size of eight. Those who had a role in randomisation also had a consulting role in statistical analysis. Randomisation was done a median 39 days (range 7–224) after diagnosis. The stratifying criteria were *MYCN* alone (amplified *vs* not amplified *vs* unknown), concentration of serum lactate dehydrogenase at diagnosis (raised *vs* not raised *vs* unknown), and age (1 to <2 years *vs* ⩾2 years at diagnosis). Stage was not a stratification factor within the *MYCN*-amplified group. Physicians, parents, and patients were not blinded as to the results of randomisation.

295 patients were randomised (figure 1), 249 of whom had stage 4 disease and were older than 1 year of age and 46 of whom had *MYCN* amplification (two with stage 1 disease, six with stage 2, 19 with stage 3, five with stage 4S, and 14 <1 year with stage 4). We chose to include patients with stage 1 disease who had *MYCN* amplification because this stage is consistent with microscopic residual tumour, because of the proven aggressive biology of the disease in all other stages if *MYCN* is amplified, and because of the rarity of *MYCN*-amplified stage 1 tumours (one patient in each group). The subgroup of patients aged 12–18 months with stage 4 disease, which is thought to have a more favourable outcome, was equally distributed between groups (eight assigned megatherapy, 11 assigned maintenance chemotherapy).

Treatment

The 212 patients in the as-treated analysis received induction chemotherapy in accordance with the design of the NB97 trial,[22] which consisted of alternating N5 and N6 cycles (figure 2). Cycle N5 consisted of 40 mg/m² cisplatin a day and 100 mg/m² etoposide a day, both given as continuous infusion over 96 h on days 1–4, and 3 mg/m² vindesine given intravenously over 1 h on day 1. Cycle N6 consisted of 1·5 mg/m² vincristine a day given intravenously over 1 h on days 1 and 8, 200 mg/m² dacarbazine a day given intravenously over 1 h on days 1–5, 1·5 g/m² ifosfamide a day given as continuous infusion over 120 h on days 1–5, and 30 mg/m² doxorubicin a day given intravenously over 4 h on days 6 and 7. In a pilot setting, three patients received cycles with slightly higher doses of etoposide (125 mg/m² a day given as continuous infusion over 96 h on days 1–4) and a different infusion time for doxorubicin (48 h continuous infusion instead of two infusions of 4 h each). The timing for surgery varied during induction chemotherapy, and took place at diagnosis or after two, four, or six cycles of chemotherapy. A second and third operation was encouraged if feasible and if without increased risk for the patient.

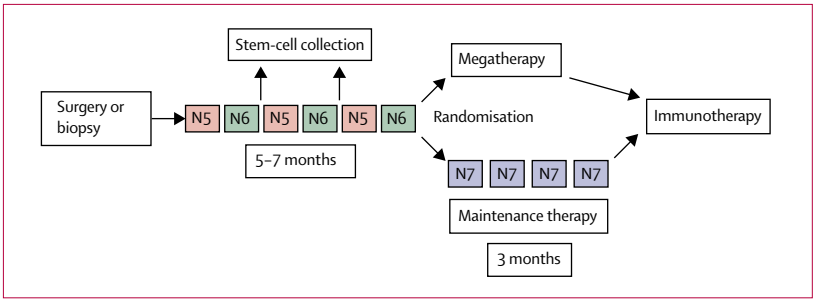

Figure 2: **Flow chart of treatment**

Stem-cell collection was recommended as soon as the bone marrow was clear of residual neuroblastoma cells (ie, no or minimum residual disease on four-site cytological and immunological analyses with antiGD2) and was done after two to four cycles of chemotherapy. The stem cells were harvested from autologous peripheral blood by cytapheresis in the 110 patients in the as-treated group who had megatherapy, and selection of CD34-positive cells[23] as a purging procedure was done in most autologous harvests (documented in 96, unknown in 13 patients, and not done in one patient). 1×10^6 to 2×10^6 CD34-positive cells per kg were recommended for reinfusion.

The myeloablative regimen consisted of 45 mg/m² melphalan a day given intravenously over 30 min on days 8–5 before transplantation, 40 mg/kg etoposide a day given intravenously over 4 h on day 4 before transplantation, and 500 mg/m² carboplatin a day given intravenously over 1 h on days 4–2 before transplantation. Some dose and drug adjustments were made in six patients because of hearing loss: three received cyclophosphamide instead of carboplatin and three received no carboplatin. In nine children, melphalan was combined with busulfan.[11] Patients with clear uptake of 123-iodine metaiodobenzylguanidine (mIBG) in metastatic lesions at the end of

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	Megatherapy (n=149)	Maintenance chemotherapy (n=146)
Stage		
1	1 (1%)	1 (1%)
2	2 (1%)	4 (3%)
3	8 (5%)	11 (8%)
4S	4 (3%)	1 (1%)
4	134 (90%)	129 (88%)
Age (years)		
<1 year	12 (8%)	10 (7%)
>1 year	137 (92%)	136 (93%)
***MYCN* amplification**		
Yes	63 (42%)	51 (35%)
No	83 (56%)	93 (64%)
Unknown	3 (2%)	2 (1%)
Serum lactate dehydrogenase		
Raised	131 (88%)	131 (90%)
Not raised	15 (10%)	14 (10%)
Unknown	3 (2%)	1 (1%)
Bone metastases		
Present	97 (65%)	82 (56%)
Absent	52 (35%)	64 (44%)
Response before randomisation		
Complete remission or very good partial remission	82 (55%)	81 (55%)
Partial remission	44 (30%)	43 (29%)
Stable disease or mixed remission	4 (3%)	9 (6%)
Progression or death	14 (9%)	11 (8%)
Unknown	1 (1%)	0
Not applicable	4 (3%)	2 (1%)

Data might not add to 100% because of rounding.

Table 1: **Baseline characteristics**

induction chemotherapy (ie, those without complete response) received mIBG therapy during myeloablative treatment: 26 of 110 patients received therapeutic mIBG (median 11·0 GBq [range 2·0–18·8]) calculated for a maximum whole-body irradiation of 2·0 Gy. 12 patients with contrast medium or mIBG uptake in the primary tumour at the end of induction chemotherapy had 36–40 Gy local radiotherapy to the primary residual tumour.

Patients in the maintenance-chemotherapy group received four N7 cycles of 150 mg/m^2 oral cyclophosphamide a day on days 1–8 and 50 mg/m^2 oral mesna given three times a day on days 1–8 as a uroprotective agent. 12 of the 102 patients given maintenance chemotherapy received 36–40 Gy percutaneous radiotherapy to the primary tumour and two had therapeutic mIBG.

Immunotherapy for 1 year was started 4–6 weeks after the end of the maintenance chemotherapy or after recovery of the haemopoiesis after megatherapy. Treatment consisted of six infusion cycles (one cycle every 2 months) of 20 mg/m^2 ch14.18 (chimeric monoclonal antibody against GD2) a day given intravenously over 8–12 h on days 1–5 of every cycle. 75 patients given megatherapy and 71 given maintenance chemotherapy received ch14.18. Drugs given concomitantly to control pain (eg, morphine, paracetamol, and dipyrone) and allergic reactions (eg, clemastine, dimetindene, and prednisone) are described in detail elsewhere.[24] After November, 2002, instead of the antibody, patients received 160 mg/m^2 oral retinoic acid a day on days 1–14, followed by a break for 14 days, for 6 months. After 3 months' break, retinoic acid was resumed for another 3 months. 26 of 110 patients given megatherapy and nine of 102 given maintenance chemotherapy had retinoic-acid post-consolidation therapy.

Toxic effects were assessed by use of WHO criteria. Response to treatment was assessed by use of the International Neuroblastoma Remission Criteria.[17]

Definition of patient groups

Intention- to-treat analysis

The intention-to-treat group comprised all 295 patients randomised, irrespective of whether they received the intended treatment (figure 1).

As-treated analysis

This group of 212 patients was defined by the treatment received independent of assigned group, and by adherence to the protocol—regarded sufficient if the patient received five, six (recommended), or seven cycles of induction chemotherapy and if either of the allocated treatments had started. 25 patients who relapsed, progressed, or died before receiving allocated treatment, six patients who did not receive either allocated treatment, and two patients who had both interventions, were excluded from this analysis (figure 1). Previous treatments that were allowed were: a short prephase chemotherapy before treatment started (3–5 days' cyclophosphamide with or without vincristine to reduce heavy initial tumour load in six patients, 2–4 weeks' previous treatment for Wilms' tumour in five patients, and 19 days' treatment for acute lymphoblastic leukaemia in one patient); other deviations from the protocol allowed were one additional N7 cycle before autologous stem-cell transplantation (n=5), or five N7 cycles instead of the recommended four during maintenance chemotherapy (n=7); a change in the order of therapy (n=3); and an additional 4 g/m^2 cyclophosphamide or 2 g/m^2 etoposide during induction therapy for stem-cell collection (n=3).

Treated-as-randomised analysis

This group consisted of 145 children who received the randomised treatment assigned to them and were analysed according to the guidelines for the as-treated group above.

Statistical analysis

This study had a censoring date of Nov 1, 2004. Life-table estimates were calculated from the time of diagnosis by use of the Kaplan-Meier method, and

differences were assessed with the log-rank test. Univariate Cox regression analysis was used to calculate hazard ratios (HR) and 95% CI. Event-free survival was defined as time until disease progression or relapse, a second neoplastic disease, or death from any cause, whichever occurred first, or until the last examination. Overall survival was defined as death from any cause or until the last examination if the patient survived. The primary endpoint was event-free survival; the secondary endpoints were overall survival and the number of treatment-related deaths up to the censoring date. For multivariate analysis, we applied Cox's proportional hazards regression model based on event-free survival and overall survival. Three covariates, treatment group (maintenance chemotherapy *vs* megatherapy as reference), *MYCN* (amplified *vs* non-amplified as reference), and concentration of lactate dehydrogenase (raised *vs* not raised for age as reference) were fitted into a stepwise-backward selection and a stepwise-forward selection. The likelihood-ratio test p value for inclusion was $<0\cdot05$ and for exclusion $>0\cdot10$.

	n (megatherapy/ maintenance chemotherapy)	3-year event-free survival, % (95% CI)				3-year overall survival, % (95% CI)			
		Megatherapy	Maintenance chemotherapy	HR (95% CI)	p	Megatherapy	Maintenance chemotherapy	HR (95% CI)	p
Intention to treat									
All	149/146	47% (38–55)	31% (23–39)	1·404 (1·048–1·881)	0·0221	62% (54–70)	53% (45–62)	1·329 (0·958–1·843)	0·0875
CR/VGPR before randomisation	82/81	57% (46–68)	35% (24–45)	1·754 (1·149–2·676)	0·0083	73% (63–83)	59% (49–70)	1·629 (1·009–2·628)	0·0436
PR/MR/SD before randomisation	48/52	45% (31–60)	30% (17–44)	1·403 (0·852–2·307)	0·1809	62% (48–77)	48% (33–63)	1·558 (0·872–2·784)	0·1311
Raised LDH at diagnosis	131/131	44% (35–52)	29% (21–37)	1·385 (1·021–1·879)	0·0357	59% (50–68)	49% (40–57)	1·302 (0·929–1·825)	0·1247
Normal LDH at diagnosis	15/14	67% (39–94)	50% (24–76)	1·864 (0·545–6·371)	0·3132	92% (76–>100)	93% (79–>100)	3·072 (0·519–18·191)	0·1985
MYCN amplification	63/51	40% (28–53)	22% (9–34)	1·522 (0·968–2·392)	0·0669	49% (36–62)	36% (22–51)	1·410 (0·865–2·296)	0·1658
No *MYCN* amplification	83/93	50% (39–62)	35% (25–45)	1·444 (0·976–2·136)	0·0643	72% (62–82)	62% (52–72)	1·347 (0·859–2·113)	0·1921
Stage 4 and age >1 year	128/121	45% (35–53)	30% (21–38)	1·354 (0·992–1·848)	0·0549	60% (51–69)	54% (45–63)	1.266 (0·894–1·792)	0·1820
MYCN amplification with stage 1, 2, 3, or 4S or with stage 4 age <1 year	21/25	60% (38–82)	40% (19–61)	1·725 (0·717–4·149)	0·2183	75% (56–94)	47% (23–70)	1·845 (0·671–5·076)	0·2287
ch14.18 treatment	79/81	60% (49–71)	37% (27–48)	1·813 (1·178–2·792)	0·0061	75% (65–85)	62% (52–73)	1·746 (1·076–2·834)	0·0221
Retinoic acid treatment	24/15	38% (4–72)	49% (19–78)	0·983 (0·366–2·640)	0·9732	59% (29–90)	32% (<0–80)	0·918 (0·267–3·156)	0·8918
As treated									
All	110/102	53% (43–63)	30% (21–39)	1·892 (1·321–2·710)	0·0004	66% (56–75)	52% (42–62)	1·683 (1·131–2·503)	0·0094
CR/VGPR before randomisation	73/69	60% (48–72)	35% (24–46)	2·061 (1·294–3·281)	0·0019	74% (64–85)	58% (46–70)	2·028 (1·193–3·449)	0·0076
PR/MR/SD before randomisation	37/32	40% (24–57)	18% (4–33)	1·611 (0·902–2·876)	0·1038	51% (34–68)	35% (16–54)	1·350 (0·723–2·523)	0·3443
Raised LDH at diagnosis	97/94	49% (38–60)	30% (21–39)	1·719 (1·185–2·494)	0·0039	62% (51–72)	48% (37–58)	1·614 (1·075–2·422)	0·0196
Normal LDH at diagnosis	11/7	89% (68–>100)	29% (<0–62)	..	0·0046*	100%	100%	..	0·4335*
MYCN amplification	48/39	47% (31–62)	21% (8–33)	2·062 (1·216–3·497)	0·0061	54% (37–70)	35% (19–50)	1·933 (1·092–3·421)	0·0212
No *MYCN* amplification	61/60	60% (46–73)	34% (21–46)	2·064 (1·249–3·409)	0·0039	75% (64–87)	62% (50–75)	1·640 (0·930–2·892)	0·0841
Stage 4 and age >1 year	95/87	51% (40–62)	29% (19–39)	1·712 (1·174–2·498)	0·0047	64% (54–74)	52% (41–63)	1·565 (1·032–2·371)	0·0333
MYCN amplification with stage 1, 2, 3, or 4S or with stage 4 age <1 year	15/15	69% (38–100)	33% (9–57)	4·456 (1·223–16·240)	0·0132	80% (53–>100)	49% (21–76)	3·748 (0·791–17·765)	0·0744
ch14.18 treatment	75/71	58% (47–70)	39% (28–51)	1·686 (1·068–2·662)	0·0233	71% (60–81)	69% (58–79)	1·402 (0·841–2·337)	0·1925
Retinoic acid treatment	26/9	51% (19–84)	25% (<0–56)	2·724 (0·913–8·123)	0·0611	68% (42–94)	0%	1·690 (0·444–6·429)	0·4369
Treated as randomised									
All	75/70	56% (44–68)	28% (17–38)	2·206 (1·427–3·412)	0·0003	72% (61–83)	52% (40–63)	2·067 (1·259–3·392)	0·0033
CR/VGPR before randomisation	54/50	63% (49–77)	30% (17–43)	2·526 (1·472–4·335)	0·0005	77% (65–89)	54% (40–68)	2·529 (1·340–4·773)	0·0030
PR/MR/SD before randomisation	21/20	40% (18–62)	20% (1–39)	1·653 (0·779–3·507)	0·1863	60% (38–82)	40% (14–66)	1·780 (0·754–4·200)	0·1827
Raised LDH at diagnosis	66/65	54% (41–67)	30% (19–41)	1·999 (1·266–3·156)	0·0024	69% (57–81)	48% (35–60)	2·010 (1·203–3·360)	0·0065
Normal LDH at diagnosis	7/4	80% (45–>100)	0%	..	0·0011*	100%	100%	..	0·2220*
MYCN amplification	35/28	50% (32–68)	18% (4–32)	2·430 (1·307–4·519)	0·0038	61% (43–79)	34% (16–52)	2·454 (1·227–4·908)	0·0087
No *MYCN* amplification	40/41	61% (45–78)	33% (18–47)	2·403 (1·296–4·455)	0·0041	80% (67–94)	63% (47–78)	1·873 (0·919–3·819)	0·0790
Stage 4 and age >1 year	67/58	55% (42–68)	28% (16–40)	1·956 (1·233–3·102)	0·0037	70% (58–82)	54% (41–67)	1·843 (1·096–3·102)	0·0193
MYCN amplification with stage 1, 2, 3, or 4S or with stage 4 age <1 year	8/12	69% (32–>100)	25% (1–50)	5·386 (1·144–25·360)	0·0179	83% (54–>100)	37% (6–66)	5·944 (0·728–48·547)	0·0590
ch14.18 treatment	54/52	61% (48–75)	34% (21–47)	2·158 (1·263–3·688)	0·0040	74% (62–86)	65% (52–78)	1·842 (1·003–3·382)	0·0452
Retinoic acid treatment	17/4	45% (5–84)	33% (<0–87)	2·183 (0·419–11·376)	0·3420	80% (60–>100)	0%	1·310 (0·135–12·711)	0·8153

CR=complete remission. VGPR=very good partial remission. PR=partial remission. MR=mixed remission. SD=stable disease. LDH=lactate dehydrogenase. *HR and 95% CI not meaningful because of small number of patients.

Table 2: **3-year event-free survival and 3-year overall survival by treatment group**

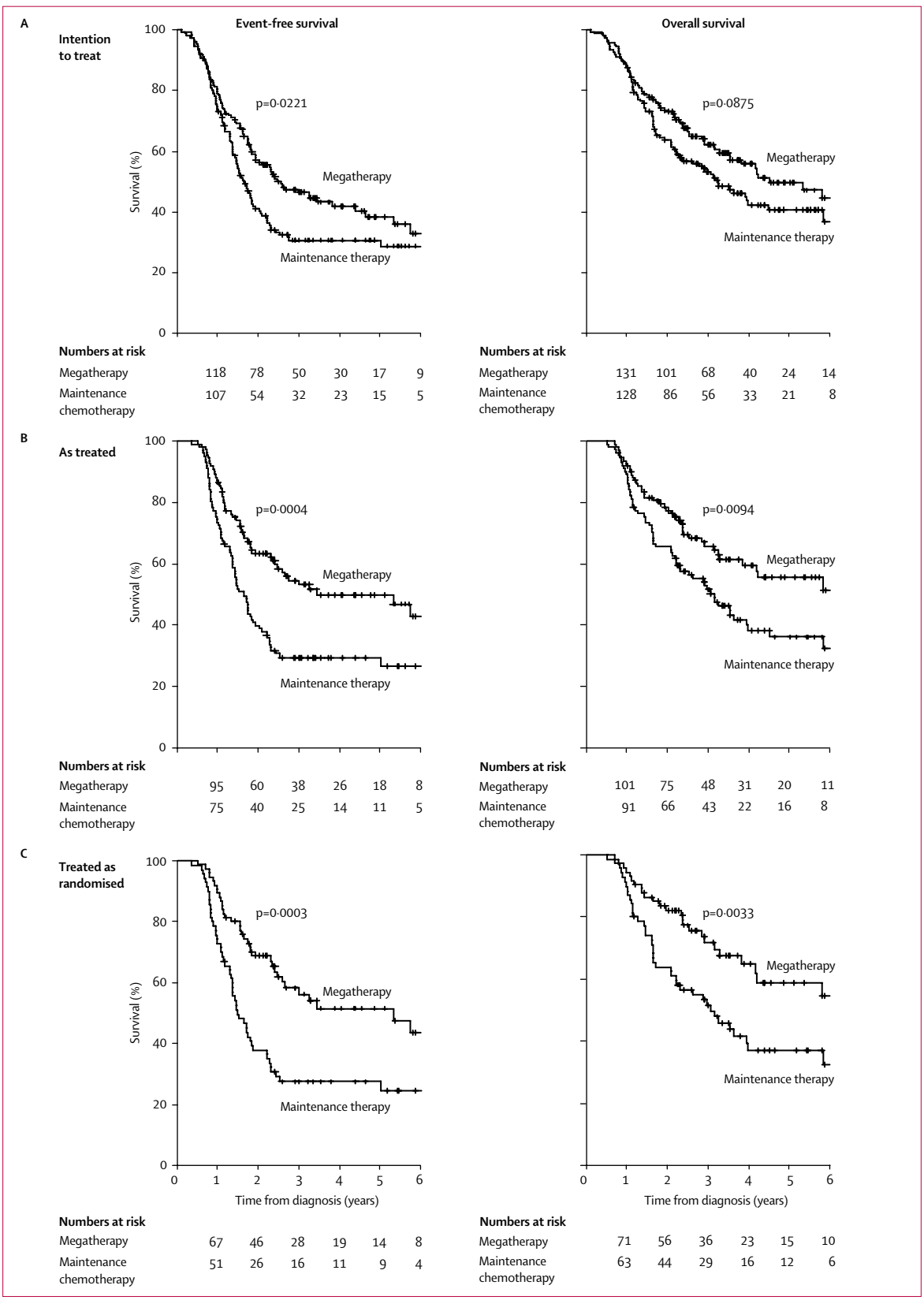

Figure 3: **Kaplan-Meier estimates for patients by treatment group**

Role of the funding source
The sponsors of the study had no role in the study design; in the collection, analysis, or interpretation of data; or in the writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Of 339 consecutively registered high-risk patients, 295 were randomised. 24 patients aged younger than 1 year with stage 4 disease who did not have *MYCN* amplification were excluded from the study but were given maintenance chemotherapy (figure 1).

Table 1 summarises baseline characteristics of randomised patients with regard to the well known risk factors. 95 (64%) of 149 patients randomly assigned megatherapy were treated accordingly, as were 84 (58%) of 146 patients randomly assigned maintenance chemotherapy. The main reasons for choosing the opposite intervention were refusal by the parents in combination with some opinions of the local physicians, whereas medical reasons were a minor cause (figure 1).

The 3-year event-free survival of all 295 patients was 39% [95% CI 33–45] and the 3-year overall survival was 58% [95% CI 52–64]; 5-year event-free survival was 35% [95% CI 29–41] and 5-year overall survival was 45% [95% CI 39–52]. The median follow-up in the patients alive at the censoring date was 3·57 years (range 1·01–7·02).

Table 2 shows 3-year event-free survival and 3-year overall survival in the intention-to-treat, as-treated, and treated-as-randomised groups. In the intention-to-treat analysis, megatherapy with autologous stem-cell transplantation increased event-free-survival overall (figure 3A), and for subgroups of patients who had a complete remission or very good partial remission to induction chemotherapy, those with raised serum concentration of lactate dehydrogenase, and those receiving antibody treatment (table 2). By contrast, subgroups in the intention-to-treat group who had: partial remission, mixed remission, or stable disease; treatment with retinoic acid; *MYCN* amplification with stage 1, 2, 3, or 4S disease or with stage 4 disease aged younger than 1 year; *MYCN* amplification; no *MYCN* amplification; or normal concentrations of serum lactate dehydrogenase did not differ between groups in 3-year event-free survival.

Multivariate Cox's regression model confirmed the prognostic role of treatment group (HR 1·49 [95% CI 1·10–2·01], p=0·009), *MYCN* status (1·52 [1·12–2·06], p=0·008), and concentration of lactate dehydrogenase (1·82 [1·002–3·29], p=0·033) for event-free survival and for overall survival (treatment group: 1·42 [1·02–1·98], p=0·040; *MYCN*: 1·70 [1·21–2·39], p=0·003; lactate dehydrogenase: 3·13 [1·37–7·15, p=0·001).

As-treated analysis showed that patients who actually received megatherapy had increased event-free survival and overall survival compared with those given maintenance therapy (table 2, figure 3B). The difference between groups in event-free survival remained for subgroups with raised concentrations of serum lactate dehydrogenase, amplified *MYCN*, no *MYCN* amplification, antibody treatment, a complete remission or very good partial remission, those with stage 4 disease aged older than 1 year, and those with MYCN amplification with stage 1, 2, 3, or 4S disease or with stage 4 disease and aged younger than 1 year. The difference between groups in overall survival remained for subgroups with raised concentrations of serum lactate dehydrogenase, amplified *MYCN*, a complete remission or very good partial remission, and those with stage 4 disease aged older than 1 year (table 2).

In the as-treated group, patients given autologous stem-cell transplantation who had a complete remission or very good partial remission had increased overall survival compared with those who received transplantation who had a partial remission, mixed remission, or stable disease (table 2; HR 1·979 [1·071–3·655], p=0·0263), but did not have increased event-free survival (1·707 [0·979–2·975], p=0.0563). Patients in the as-treated group who received maintenance chemotherapy who had a complete remission or very good partial remission did not have increased survival compared with those who had maintenance chemotherapy who achieved a partial remission, mixed remission, or stable disease (event-free survival: HR 1·293 [0·792–2·122], p=0·2999; overall survival: 1·313 [0·759–2·272], p=0·3276).

A separate comparison of the as-treated group including patients who were excluded for protocol violation (n=50) showed that results were much the same for these 262 children. 3-year event-free survival was 53% (95% CI 44–62) and overall survival was 68% (95% CI 60–77) for the megatherapy group, and 3-year event-free survival 30% (95% CI 22–38) and 3-year overall survival 53% (95% CI 44–63) for the maintenance-chemotherapy group (log-rank p for event-free survival 0·0002 and for overall survival 0·0023).

In the treated-as-randomised group, patients given megatherapy had increased 3-year event-free survival and 3-year overall survival compared with those given maintenance chemotherapy (table 2, figure 3C). Subgroups in the megatherapy group with complete remission or very good partial remission, raised concentrations of serum lactate dehydrogenase, non-amplified *MYCN*, amplified *MYCN*, those with stage 4 disease and older than 1 year, and those given antibody treatment had increased 3-year event-free survival and 3-year overall survival compared with those in these subgroups who received maintenance chemotherapy (table 2).

During induction chemotherapy, treatment-related toxic effects did not differ between groups. Data for toxic effects were available for 594 (38%) of 1557 N5 and N6 chemotherapy cycles. Grade 3–4 bone-marrow toxic effects were recorded for 264 (95%) of 279 induction cycles in the megatherapy group and for 301 (96%) of 315 induction cycles in the maintenance-chemotherapy group. Patients given megatherapy had grade 2 febrile neutropenia in 92 (33%) of 279 induction cycles and grade 3–4 in 21 (8%) induction cycles. Patients given maintenance chemotherapy had grade 2 febrile neutropenia in 110 (35%) of 315 induction cycles and grade 3–4 in 24 (8%) induction cycles. Mucositis was noted for ten (4%) of 279 induction cycles in the megatherapy group and in 16 (5%) of 315 induction cycles in the maintenance-chemotherapy group.

Most patients completed maintenance chemotherapy in an outpatient setting, during which grade 2–4 bone-marrow toxic effects occurred in 75 (52%) of 143 documented cycles and febrile neutropenia in 12 (8%) of 143 cycles. All 110 patients given megatherapy had bone-marrow aplasia, and 85 (77%) had septic neutropenic fever (ie, grade 2–4). No severe post-transplantation viral infections with Epstein-Barr virus or cytomegalovirus reactivation, which have been described in the context of post-transplantation lymphoproliferative disease,[25] were recorded.

Nine (3%) of the 295 randomised patients died from treatment-related complications. Two patients assigned maintenance therapy died from therapy-related complications during induction treatment. Five patients given megatherapy died from acute complications related to megatherapy (two randomly assigned megatherapy and three randomly assigned maintenance chemotherapy), one patient died from acute myeloblastic leukaemia after megatherapy, and one patient died from second acute lymphoblastic leukaemia after maintenance therapy. No patients given maintenance therapy died from acute treatment-related toxic effects.

Because of improved event-free survival and overall survival in the megatherapy group (log-rank and Wilcoxon test $p<0·05$), randomisation was stopped on Nov 1, 2002, by the trial board, and the maintenance-chemotherapy group was closed. Until the statistical significance for event-free survival and overall survival was reached, the trial committee was blinded to treatment groups. Parents, patients, and physicians were not informed of any interim results, and the refusal by parents to accept the assigned treatment after randomisation could therefore not have been affected by previous knowledge of the treatment results.

Immunotherapy with ch14.18 after megatherapy with autologous stem-cell transplantation or maintenance chemotherapy was given from the beginning of the trial until Nov 1, 2002, and was then stopped because the data did not show a positive effect on event-free-survival and overall survival compared with findings from earlier trials.[23] After that date, patients who completed intervention treatment were given retinoic acid instead (n=35, as-treated analysis). This subgroup had the shortest follow-up, and differences between megatherapy and maintenance chemotherapy has not yet reached significance. However, because the number of patients given immunotherapy and retinoic acid was equally distributed between groups, an effect of these interventions on the megatherapy group is unlikely.

In the intention-to-treat analysis, 24 (16%) of 149 patients in the megatherapy group had mIBG treatment, as did 24 (16%) of 146 in the maintenance-chemotherapy group. When these patients were excluded from the intention-to-treat analysis, 3-year event-free survival was 43% (95% CI 34–52) for patients assigned megatherapy and 28% for those assigned maintenance chemotherapy (19–36; $p=0·0282$). In the as-treated analysis, two (2%) of 102 patients in the maintenance-chemotherapy group and 26 (24%) of 110 patients in the megatherapy group received mIBG therapy. No difference in outcome was noted for the 26 patients who received mIBG before autologous stem-cell transplantation and the 84 who were not given mIBG (3-year event-free survival: HR 1·710 [0·831–3·515], log-rank $p=0·1401$; 3-year overall survival: HR 1·506 [0·695–3·266], log-rank $p=0·2961$). Further subset analyses showed that mIBG therapy alone did not contribute to the better outcome of the megatherapy group. Two of 12 patients in the megatherapy group and six of 12 patients in the maintenance-chemotherapy group given local radiotherapy had local primary recurrence, as did 62 (33%) of 188 patients who did not have local radiotherapy.

Discussion

We have shown that myeloablative chemotherapy with autologous stem-cell rescue increases event-free and overall survival for children with high-risk neuroblastoma compared with 3-month maintenance chemotherapy with oral cyclophosphamide. Our study contradicts findings from a retrospective analysis,[16] in which treatment was assigned on the basis of local physicians' preference, that showed a similar outcome for 40 patients given megatherapy with autologous stem-cell transplantation (5-year survival 36% [SD 11]) and for 92 patients given maintenance chemotherapy with oral cyclophosphamide, melphalan, etoposide, and intravenous vincristine for 1 year (37% [7]). Because 13 patients in the maintenance group developed secondary leukaemia, we decided to avoid the use of oral etoposide, melphalan, and vincristine in our treatment design, and treatment duration was shortened from 12 cycles (1 year) to four cycles (3 months). To date, in our study, one patient in each group has developed secondary leukaemia.

Event-free survival was increased in the megatherapy group for the intention-to-treat, as-treated, and treated-as-randomised analyses. Only data for overall survival in the intention-to-treat analysis was not significant (p=0·0875, figure 3A). We regard the intention-to-treat analysis as the most robust because it takes into account deviations from the assigned treatment. Moreover, we expect that our results reported here will endure over time: our survival data continued to stabilise from the closure of the maintenance-chemotherapy group (Nov 1, 2002) until the censoring date (Nov 1, 2004).

Further analyses in the intention-to-treat, as-treated, and treated-as-randomised analyses showed that some subgroups defined according to commonly accepted risk factors benefited from megatherapy. Patients with *MYCN* amplification with stage 1, 2, 3, or 4S disease or with stage 4 disease and aged younger than 1 year did not differ between treatment groups in intention-to-treat analysis, but did show increased event-free survival with megatherapy in the as-treated and treated-as-randomised analyses. We therefore think that megatherapy could be appropriate for this group of patients.

Compliance with the randomisation result for both groups was surprisingly low. However, we regard the intention-to-treat analysis as valid, despite these refusals, even though they could have resulted in underestimation of the difference between treatments. Moreover, the similar numbers of refusals in the two groups suggests that effect-size estimates in the as-treated and treated-as-randomised analyses are valid.

Local radiotherapy to the primary tumour site can prevent local relapse in patients with high-risk neuroblastoma.[26] Because 12 patients in each group were given local radiotherapy, our results suggest that such treatment did not modify the effect of megatherapy or maintenance chemotherapy. By contrast, because the use of mIBG differed between groups, we cannot exclude a contribution of mIBG therapy to the improved outcome of patients in the megatherapy group.

The effect of the therapeutic intervention on the outcome of patients depends on the design of the conditioning myeloablative regimen, on the chemotherapy under comparison with the intervention, and on the curative level achieved with any preceding treatment (eg, induction chemotherapy, surgery, or radiotherapy). Although autologous stem-cell transplantation has been in use for more than 20 years,[1] we are aware of only two randomised trials that have been reported. The European Neuroblastoma Study Group-1 trial[7] randomly assigned 48 patients with stage 4 neuroblastoma, who were older than 1 year of age, and who had achieved a complete remission or good partial remission after six to ten courses of vincristine, cisplatin, etoposide, and cyclophosphamide during induction chemotherapy to 180 mg/m^2 melphalan for myeloablation (n=24) or to no further treatment (n=24). The stem-cell source was unpurged bone marrow. 5-year event-free survival was 38% for the melphalan group and 17% for the no-treatment group (p=0·01); 5-year overall survival was 46% and 21%, respectively (p=0·03). A US trial[6] randomly allocated 189 patients to transplantation and 190 to continuation chemotherapy with cisplatin, etoposide, doxorubicin, and ifosfamide. 3-year event-free survival was 34% (SD 4) for patients allocated transplantation and 22% (4) for those allocated continuation chemotherapy (p=0·034); groups did not differ in 3-year overall survival (43% [4] *vs* 44% [4], respectively, p=0·87). In the as-treated category,[6] 3-year event-free survival for 129 patients who received transplantation was 43% (6) compared with 27% (5) for those who received continuation chemotherapy. By contrast, overall survival did not differ between groups in the as-randomised and as-treated analyses. In this US trial, 379 (70%) of 539 eligible patients were randomised. The conditioning regimen consisted of the same drugs as used in our protocol, but with different doses and schedules (640 mg/m^2 etoposide and 1000 g/m^2 carboplatin given over 96 h, and 140 mg/m^2 melphalan given intravenously), and included total body radiotherapy at 10 Gy. The stem-cell source was immunomagnetically purged bone marrow. Other differences between the US trial and ours included the induction chemotherapy regimen, the use of radiotherapy for gross residual disease, and the structure of the continuation-chemotherapy regimen. Despite differences in study design, our results are consistent with those for event-free survival from this US trial.

In conclusion, we provide further evidence that patients with high-risk neuroblastoma might benefit from myeloablative megatherapy with autologous stem-cell transplantation. The increased short-term burden associated with this treatment (ie, increased toxic effects and protracted hospital stay) and the additional cost seem justified by data showing a significantly better outcome than that achieved with maintenance chemotherapy.

Contributors

The individual contribution of all authors resulted from being an active part of the trial committee and discussing extensively the study design, the collection and interpretation of data, and from treatment experiences obtained in a major participating institution. B Hero and T Simon were trial coordinators and did statistical analyses. F Berthold was the principal investigator of the trial.

Conflict of interest

We declare no conflicts of interest.

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