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ORIGINAL ARTICLE

Extended induction chemotherapy does not improve the outcome for high-risk neuroblastoma patients: results of the randomized open-label GPOH trial NB2004-HR

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Background: Long-term survival of high-risk neuroblastoma patients is still below 50% despite intensive multimodal treatment. This trial aimed to address whether the addition of two topotecan-containing chemotherapy courses compared to standard induction therapy improves event-free survival (EFS) of these patients.

Patients and methods: An open-label, multicenter, prospective randomized controlled trial was carried out at 58 hospitals in Germany and Switzerland. Patients aged 1—21 years with stage 4 neuroblastoma and patients aged 6 months to 21 years with *MYCN*-amplified tumors were eligible. The primary endpoint was EFS. Patients were randomly assigned to standard induction therapy with six chemotherapy courses or to experimental induction chemotherapy starting with two additional courses of topotecan, cyclophosphamide, and etoposide followed by standard induction chemotherapy (eight courses in total). After induction chemotherapy, all patients received high-dose chemotherapy with autologous hematopoietic stem cell rescue and isotretinoin for consolidation. Radiotherapy was applied to patients with active tumors at the end of induction chemotherapy.

Results: Of 536 patients enrolled in the trial, 422 were randomly assigned to the control arm ($n = 211$) and the experimental arm ($n = 211$); the median follow-up time was 3.32 years (interquartile range 1.65—5.92). At data lock, the 3-year EFS of experimental and control patients was 34% and 32% [95% confidence Interval (CI) 28% to 40% and 26% to 38%; $P = 0.258$], respectively. Similarly, the 3-year overall survival of the patients did not differ [54% and 48% (95% CI 46% to 62% and 40% to 56%), respectively; $P = 0.558$]. The response to induction chemotherapy was not different between the arms. The median number of non-fatal toxicities per patient was higher in the experimental group while the median number of toxicities per chemotherapy course was not different.

Conclusion: While the burden for the patients was increased by prolonging the induction chemotherapy and the toxicity, the addition of two topotecan-containing chemotherapy courses did not improve the EFS of high-risk neuroblastoma patients and thus cannot be recommended.

Clinical Trials.gov number: NCT number 03042429.

Key words: high-risk neuroblastoma, randomized clinical trial, induction chemotherapy, topotecan

INTRODUCTION

While long-term survival of high-risk neuroblastoma patients has improved over recent decades, it is still below 50% to date.[1—3] Thus, there is still an urgent need for more efficacious treatment strategies for these patients. Currently, first-line treatment consists of multimodal therapeutic regimens including multi-agent induction chemotherapy, resection,

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and radiotherapy of the primary tumor,[4,5] high-dose chemotherapy with autologous hematopoietic stem cell rescue,[6–8] and post-consolidation treatment with oral isotretinoin and/or immunotherapy, for example.[9–11] The cytostatic agents carboplatin/cisplatin, cyclophosphamide/ifosfamide, doxorubicin, etoposide, and vincristine are considered key drugs for neuroblastoma and have been applied in various combinations, doses, and timings.[3,12] Almost all these drugs had been selected based on in-vitro efficacy[13] whereas randomized studies on drug efficacy have been exceptions. Even the length of the induction chemotherapy to achieve long-lasting responses has remained uncertain.[14–16]

Multiple clinical observations suggest that topotecan-containing regimens are efficacious in recurrent neuroblastoma and may thus be prime candidates for first-line therapy. Treatment with high-dose cyclophosphamide, topotecan, and vincristine achieved responses in 52% (13/25) and 19% (11/58) of recurrent or refractory neuroblastoma, respectively.[17] In our experience, 61% partial and complete responses were observed in 31 relapsed patients and 72% in 11 untreated patients using topotecan, cyclophosphamide, and etoposide.[18] A randomized phase II trial showed the superiority of topotecan and cyclophosphamide compared with topotecan alone in terms of progression-free survival.[19] In an International Society of Paediatric Oncology Europe Neuroblastoma (SIOPEN) trial, topotecan, vincristine, and doxorubicin improved the response rate of high-risk neuroblastoma patients after COJEC induction enabling patients to proceed to high-dose therapy.[20] A Children's Oncology Group (COG) study demonstrated that two courses of pharmacokinetically guided topotecan with cyclophosphamide followed by four courses of multi-agent chemotherapy achieved tumor response in 84% of 26 newly diagnosed neuroblastoma patients.[21] Based on these findings and our previous observations,[18] we aimed to assess in a large multicenter randomized trial whether the addition of two topotecan-containing chemotherapy cycles improves the event-free survival (EFS) of newly diagnosed high-risk neuroblastoma patients.

METHODS

Study design

The NB2004-HR trial was an open-label, multicenter, prospective randomized controlled phase III trial carried out at 58 sites in Germany and Switzerland. It was approved by the national regulatory authorities and by the ethical committee of the University of Cologne and is registered at Clinical Trials.gov (NCT number 03042429). The inclusion criteria were (i) diagnosis of neuroblastoma according to the International Neuroblastoma Staging System (INSS) criteria,[22] (ii) high-risk neuroblastoma as defined by either stage 4 and age $\geq$1–21 years regardless of the *MYCN* status, or stage 1–3/4S with *MYCN* amplification and age $\geq$6 months to 21 years, and (iii) informed consent of the guardians and—if appropriate for age and mental development—also of the patient. Exclusion criteria were (i) participation in other clinical trials that might interfere with the interventions or outcome assessment of the NB2004-HR trial, (ii) pregnancy or lactation, insufficient contraception for girls of childbearing age (Pearl index exceeds 1%), (iii) any concomitant non-protocol anticancer therapy, and (iv) incomplete initial staging.

Central diagnostic review

Histology, *MYCN* assessment, and bone marrow cytology and immunocytology were centrally reviewed. Radiological tumor images were centrally reviewed on request for difficult cases (for details see supplementary information 1, available at *Annals of Oncology* online).

Randomization

Before any treatment, patients meeting the inclusion criteria were randomly assigned to either the standard induction treatment or the experimental treatment with two additional topotecan-containing courses. The randomization was done blockwise and stratified according to lactate dehydrogenase (LDH), stage, age, and *MYCN* into four groups (see supplementary information 2, available at *Annals of Oncology* online).

Treatment

The standard induction chemotherapy consisted of six alternating courses, N5 (vindesine, cisplatin, etoposide) and N6 (vincristine, dacarbacine, ifosfamide, doxorubicin). In the experimental arm, patients started treatment with two courses of N8 consisting of topotecan (1.0 mg/m^2 a day for 7 days as continuous infusion over 168 h), cyclophosphamide (100 mg/m^2 a day on days 1–7 i.v. over 1 h), and etoposide (100 mg/m^2 a day on days 8–10 i.v. over 1 h). After these two courses, six courses of N5 and N6 followed (for the schematic see supplementary Figure S1, available at *Annals of Oncology* online).

Post-induction therapy consisted of high-dose chemotherapy with autologous blood stem cell re-infusion,[8] primary tumor resection, radiotherapy to meta-iodobenzylguanidine (mIBG)-avid residuals (percutaneous irradiation to the primary tumor[5]), ^{131}I-mIBG-therapy to primary and metastatic lesions,[23] and oral isotretinoin (for therapeutic details see supplementary information 3, available at *Annals of Oncology* online).

Endpoints

The primary endpoint was EFS. Key secondary endpoints were overall survival (OS), response to induction chemotherapy (after two courses and before high-dose chemotherapy), the impact of induction chemotherapy on the extent of surgical resectability, the need for percutaneous and/or radionuclide irradiation, and toxicity during induction chemotherapy. Adverse events were monitored continuously, graded according to the WHO and CTCAEv3 criteria (issued 12 December 2003; http://ctep.cancer.gov/forms. see version: CTCAEv3), and analyzed solely for the as-treated cohort.

Statistical analysis

EFS was calculated from diagnosis to the first event which is defined as progression, recurrence, secondary malignant disease, or death for any reason or until the last follow-up for patients without events. OS was calculated from diagnosis to death for any reason or until last follow-up. The median follow-up was calculated using the inverse Kaplan–Meier estimate.

The confirmatory null hypothesis was that EFS of children in the experimental arm does not differ from EFS in the standard arm and was analyzed by two-sided log-rank tests at a significance level of 5% within the intention-to-treat population (ITT) using a three-step adaptive group sequential design based on the inverse normal method.[24] The characteristics were defined according to Pampallona and Tsiatis[25] with the option for stopping for futility, $\alpha = 5\%$, equally-weighted stages, shape parameter $\Delta = 0$, and power of 80% if the true hazard ratio (HR) (standard versus experimental) is 1.443. This HR corresponds to a 3-year EFS of 45% in the standard arm and an assumed 3-year EFS of 57.5% in the experimental arm. Analyses were intended to be carried out after 85, 171, and 257 events pooled over both arms. At the second interim analysis, a data-dependent sample size recalculation was carried out according to the conditional power principle resulting in a final analysis based on 286 events.

Analyses of secondary endpoints and *post hoc* analyses for the as-treated per protocol (AT) and treated-as-randomized (TAR) cohorts (Figure 1) are exploratory and *P* values are given as descriptive measures to detect meaningful effects. Definitions of the ITT, AT, and TAR cohorts are given in the supplementary information 4 (available at *Annals of Oncology* online).

IBM SPSS statistical package version 25 (IBM, Ehningen, Germany) was used for the statistical analysis. Pearson's χ^2 test and Fisher's exact test were used to compare the proportions of two nominal variables, while medians were compared nonparametrically by two-sided Mann-Whitney U-tests. The frequency of grade 3 and 4 toxicities across all induction chemotherapy courses was compared between treatment arms using the generalized estimation equation (GEE) approach with logit link function and AR(1) working correlation structure to account for clustering due to ordered multiple observations per patients. The log-rank test was applied to compare the survival distribution between independent groups and univariable Cox's proportional hazards regression analysis was used to calculate HR. For multivariable Cox's regression analyses, the covariates were fitted into a stepwise model selection process (forward and backward with Bayesian information criterion to select the better fitting model). The likelihood ratio test *P* value for inclusion was ≤ 0.05 and for exclusion was >0.10. The data lock for this analysis was 1 September 2018.

RESULTS

Between 12 October 2004, and 31 December 2016, 536 patients were enrolled and eligible for random allocation. Of these, 111 patients (21%) were not randomly allocated to cohorts for several reasons (Figure 1). Of the 425 patients who were randomly assigned (79%), two ultimately did not meet the inclusion criteria and one withdrew consent for randomization thus resulting in 422 randomized patients for the final analysis. Of these, 211 were allocated to the standard arm and 211 to the experimental arm (Table 1). The patient cohorts of the two arms were not different with respect to age, stage, genomic *MYCN* status, LDH levels (Table 1), sex, primary or metastatic sites, and tumor markers (supplementary Table S1, available at *Annals of Oncology* online).

The median follow-up time was 3.32 years [interquartile range (IQR) 1.65–5.92]. At the time of the data lock, 140/211 patients in the experimental arm and 146/211 patients in the standard arm had events. The corresponding 3-year EFS did not differ between the arms (log-rank $P = 0.258$) and was 34% (95% CI 28% to 40%) in the experimental arm and 32% (95% CI 26% to 38%) in the standard arm (Figure 2). Similarly, no difference was observed for overall survival ($P = 0.558$) with a 3-year OS of 54% (95% CI 46% to 62%) in the experimental arm and 48% (95% CI 40% to 56%) in the standard arm (Figure 2).

In a separate comparison of the AT and TAR cohorts, neither of the compared arms showed differences regarding EFS or OS (see supplementary Figure S2 and Table S2, available at *Annals of Oncology* online). Analyses of major clinical subgroups, defined by the stratifying risk factors LDH, *MYCN* amplification, stage, age, or by response to induction chemotherapy, also revealed no difference in EFS and OS between the experimental and the standard arm (see Table 2 for ITT and supplementary Table S3, available at *Annals of Oncology* online, for AT and TAR). In addition, neither the early response rates [complete response (CR), very good partial response (VGPR), partial response (PR), stable disease (SD), mixed response (MR), progression (PROG)] assessed after the first two courses of induction chemotherapy nor those at the completion of induction chemotherapy were different between the groups (Table 1). Moreover, the degree of surgical resection in the experimental arm was not improved compared to the standard arm (Table 1). There were also no differences in the fraction of patients treated with external beam irradiation or ^{131}I-mIBG therapy as a compensatory measure to treat tumor residuals after completion of induction chemotherapy (Table 1).

Univariable analyses of potential risk factors demonstrated an unfavorable prognostic impact only for elevated LDH levels at diagnosis, liver metastasis, lung or pleural metastasis, and poor response (PR/SD/MR) to induction chemotherapy on EFS (see supplementary Table S4, available at *Annals of Oncology* online). In multivariable analysis (see supplementary Table S5, available at *Annals of Oncology* online), the randomization result and the treatment arm had no influence on EFS or OS in the analyzed cohorts (ITT, AT, TAR). LDH, the involvement of rare metastatic sites (liver, lung/pleura), and tumor response before high-dose treatment (HDT) remained independent

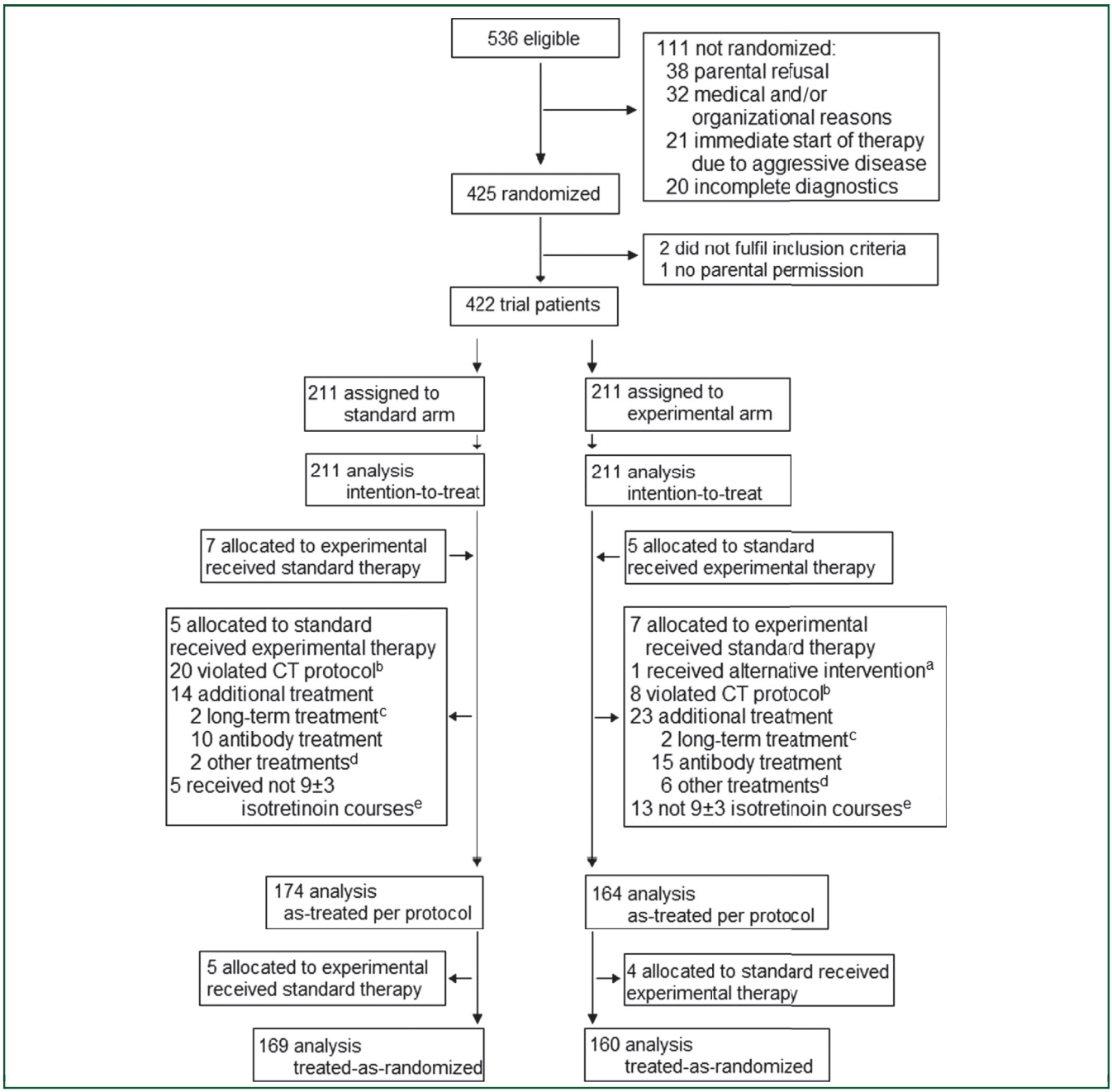

Figure 1. Trial profile (CONSORT diagram).

[a] Received alternative treatment in another country after diagnosis.

[b] Protocol violation: more or fewer than 6 ± 1 courses of induction chemotherapy in the control or more or fewer than 8 ± 1 in the experimental arm.

[c] Additional oral cyclophosphamide while waiting for high-dose chemotherapy.

[d] Dendritic cell vaccine ($n = 1$), oral bisphosphonate ($n = 2$), interleukin 2 ($n = 2$), ALK inhibitor ($n = 3$).

[e] More or fewer than 9 ± 3 courses of isotretinoin.

ALK, anaplastic lymphoma kinase.

prognostic markers in most models. Furthermore, *MYCN* amplification was an independent prognostic marker for poor OS but not for EFS (see supplementary Table S5, available at *Annals of Oncology* online).

Non-fatal toxicities grade 3 and 4 were more frequently observed in the experimental arm with two additional courses of chemotherapy ($P < 0.001$, AT cohorts; Table 3) but the median number of toxicities per chemotherapy course did not differ. Grade 3/4 thrombocytopenia was more often observed in the experimental arm while oral mucositis occurred less frequently although a statistical significance was not reached ($P = 0.07$). Patients receiving experimental N8 courses experienced grade 3 and 4 anemia, leuco/neutropenia, and thrombocytopenia more often compared with patients receiving N5 courses (first course) and more anemia and thrombocytopenia but less oral mucositis than patients receiving N6 cycles (second course; see supplementary Tables S6 and S7, available at *Annals of Oncology* online). Seven patients of the experimental arm experienced second neoplasms but none of the standard

Table 1. Patients characteristics and treatments (ITT population)

	Standard (N5/N6) *n* (%)	Experimental (N8 + N5/N6) *n* (%)	*P value*
Age at diagnosis (years)	211	211	0.427[a]
<1	2 (1)	3 (1)	
≥1−1.5	16 (8)	25 (12)	
≥1.5−5	153 (73)	149 (71)	
≥5−10	33 (16)	23 (11)	
≥10−15	5 (2)	8 (4)	
≥15	2 (1)	3 (1)	
Median (95% CI)	3.2 (2.8−3.6)	3.0 (2.7−3.3)	0.352[b]
Disease stage (INSS)	211	211	0.837[a]
Stage 1,2,3	15 (7)	12 (6)	
Stages 4S or 4 <1 year	2 (1)	2 (1)	
Stage 4 ≥1 year	194 (92)	197 (93)	
MYCN assessment	210	207	
MYCN amplification	87	87	0.695[a]
Stages 1,2,3	15 (17)	12 (14)	
Stage 4S	1 (1)	1 (1)	
Stage 4 <1 year	1 (1)	0 (0)	
Stage 4 ≥1 year	70 (81)	74 (85)	
Time from randomization to 1st chemotherapy	*n* = 211	*n* = 211	
Median days (95% CI)	6 (5−7)	6 (6−7)	0.803[b]
Lengths of induction chemotherapy	*n* = 211	*n* = 211	
Median days (95% CI)[d]	134 (130−138)	195 (191−201)	<0.001[b]
Response status after 2 courses	172	185	0.133[a]
Complete response	0 (0)	1 (1)	
Very good partial response	6 (4)	6 (3)	
Partial response	152 (88)	147 (80)	
Stable disease	12 (7)	27 (15)	
Mixed response	2 (1)	4 (2)	
Progression/death before third course	3 (1)	5 (2)	0.724[c]
Response status before HDT	196	193	0.837[a]
Complete response	53 (27)	56 (29)	
Very good partial response	30 (15)	29 (15)	
Partial response	92 (47)	85 (44)	
Stable disease	2 (1)	1 (1)	
Mixed response	1 (1)	0 (0)	
Progression/death	18 (9)	22 (11)	
HDT	211	211	0.473[c]
Given	186 (88)	180 (86)	
Not given	25 (12)	31 (14)	
Surgery	211	211	0.884[a]
Complete resection	92 (44)	84 (40)	
Incomplete resection	83 (39)	86 (41)	
Biopsy only	16 (8)	17 (8)	
No resection/biopsy	20 (9)	24 (11)	
Radiotherapy (external beam)	211	211	
Not given	184 (87)	184 (87)	
Given	27 (13)	27 (13)	1.000[c]
^{131}I-mIBG therapy	211	211	
Not given	150 (71)	153 (73)	
Given	61 (29)	58 (28)	0.829[c]

Biopsy, only biopsy material obtained, no tumor resection; CI, confidence interval; complete, macroscopic complete resection with or without microscopic residuals (best operation); HDT, high-dose treatment; ^{131}I-mIBG, ^{131}I-meta-iodobenzylguanidine therapy; incomplete, macroscopic incomplete resection (best operation); INSS, International Neuroblastoma Staging System; ITT, intention to treat; MNA, *MYCN* amplification; *n*, number of patients; VMA/HVA, vanillylmandelic acid and/or homovanillic acid in urine.

[a] χ^2 test.

[b] Median test.

[c] Fisher's exact test.

[d] Length of induction chemotherapy is calculated from the first day of the first course to the first day of the last course before HDT.

therapy arm (n.s.). The diagnoses were acute myeloid leukemia ($n = 3$), renal cell carcinoma ($n = 2$), Ewing's sarcoma, and B-non-Hodgkin's lymphoma. The time interval between the two diagnoses had a range of 12−125 months. Two toxic deaths resulting from induction chemotherapy were observed in each of the treatment arms (1.2% of the AT cohorts; see supplementary Tables S8, available at *Annals of Oncology* online). No death was caused by surgical therapy. High-dose-therapy was associated with 2.9% (standard arm) and 3.7% (experimental arm) of toxic deaths.

DISCUSSION

Our study demonstrates that intensification of induction therapy in high-risk neuroblastoma with the addition of

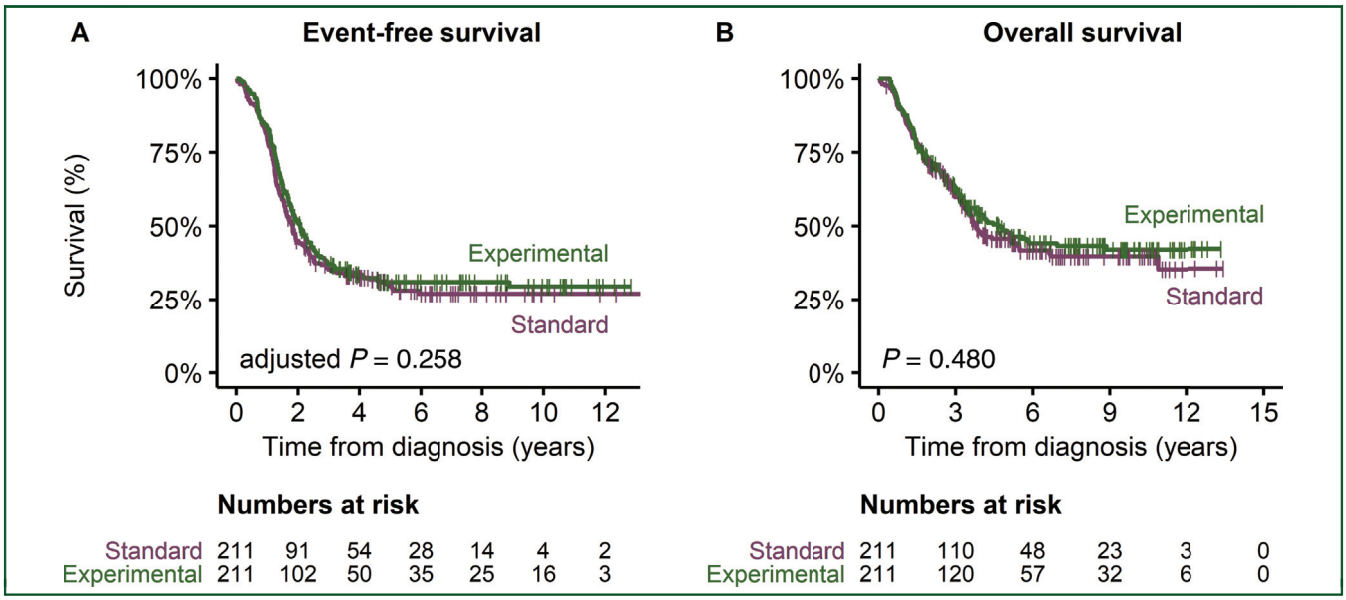

Figure 2. **Event-free survival (A) and overall survival (B) of the intention-to-treat cohort by treatment arm.**

two chemotherapy courses containing topotecan, cyclophosphamide, and etoposide to the NB2004-HR standard chemotherapy did not improve patient EFS and OS but increased the total number of toxic side-effects.

A major strength of the study is its design as a randomized, prospective, multicenter trial. Ninety-nine percent of all high-risk patients diagnosed in Germany during the trial period and known to the National Children's Cancer Tumor Registry participated in the trial; thus selection bias for the registration of the patients can be largely excluded. The stratifying risk factors chosen for randomization (LDH, stage, age, *MYCN*) were equally distributed over the treatment arms. The central review of tumor histology, *MYCN* copy number status, bone marrow cytology, immunocytology, ambiguous mIBG scintigraphy, and ambiguous radiodiagnostic imaging represent other strengths of the NB2004-HR trial.

Several limitations of the study need to be highlighted. The study was powered to detect differences between the treatment arms but not between the subgroups. Therefore, subgroup analyses should be regarded as descriptive only. Of the 536 patients eligible for randomization, 21% were not randomized. Although each individual reason may be acceptable, the sum is high and may result in a bias.

The trial was designed to increase the 3-year EFS from 45% (NB97, HDT arm) to 57.5% but this was not achieved. In contrast, 3-year EFS was 34% (95% CI 28% to 40%) for the experimental arm and 32% (95% CI 26% to 38%) for the standard arm (for details on the outcome differences

Table 2. **Five-year event-free survival and 5-year overall survival of the intention-to-treat subcohorts by treatment group**

Subcohort	Exp./st.	5-year event-free survival, (95% CI)			*P* log rank[b]	5-year overall survival, (95% CI)			*P* log rank
	n/n	Experimental	Standard	HR (95% CI) Cont. vs Ex.		Experimental	Standard	HR (95% CI)	
All	211/211	0.29 (0.21–0.37)	0.25 (0.17–0.33)	1.09 (0.87–1.38)	0.457	0.39 (0.31–0.47)	0.36 (0.28–0.44)	1.08 (0.82–1.42)	0.582
Elevated LDH	202/201	0.25 (0.19–0.31)	0.23 (0.17–0.29)	1.04 (0.82–1.31)	0.766	0.36 (0.28–0.44)	0.33 (0.25–0.41)	1.05 (0.80–1.38)	0.746
Normal LDH	9/10	NA	NA	NA	NA	NA	NA	NA	NA
MYCN amplification	87/87	0.31 (0.21–0.41)	0.21 (0.11–0.31)	1.22 (0.85–1.75)	0.281	0.44 (0.32–0.56)	0.26 (0.14–0.38)	1.32 (0.87–1.99)	0.170
No *MYCN* amplification	120/123	0.25 (0.15–0.35)	0.24 (0.14–0.34)	1.02 (0.75–1.39)	0.912	0.35 (0.23–0.47)	0.39 (0.27–0.51)	0.94 (0.65–1.36)	0.726
Stage 4 and age >1 year	197/194	0.26 (0.20–0.32)	0.21 (0.13–0.29)	1.07 (0.84–1.36)	0.584	0.37 (0.29–0.45)	0.33 (0.25–0.41)	1.05 (0.79–1.39)	0.737
Stage 1,2,3,4S or stage 4 age <1 year	14/17	0.52 (0.24–0.80)	0.44 (0.20–0.68)	1.62 (0.57–4.55)	0.363	0.54 (0.26–0.82)	0.44 (0.20–0.68)	1.57 (0.56–4.41)	0.396
CR/VGPR before HDT[a]	85/82	0.37 (0.25–0.49)	0.42 (0.30–0.54)	0.82 (0.55–1.23)	0.822	0.56 (0.44–0.68)	0.56 (0.44–0.68)	0.94 (0.59–1.52)	0.813
PR/MR/SD before HDT[a]	86/95	0.34 (0.24–0.44)	0.24 (0.14–0.34)	1.28 (0.90–1.83)	0.170	0.47 (0.35–0.59)	0.45 (0.35–0.55)	1.12 (0.74–1.70)	0.601

CI, confidence interval; CR, complete response; exp., experimental arm; HDT, high-dose chemotherapy with autologous blood stem cell transplantation; LDH, lactate dehydrogenase (elevation defined as >400 U/L <1 year, >300 U/L 1–17 years, >200 U/L >17 years); MR, mixed response; *MYCN*, oncogene *MYCN*; NA, not applicable; PR, partial response; SD, stable disease; St., standard arm; VGPR, very good partial response.

[a] Five years measured from the time of response evaluation.

[b] Observations were censored at the end of the 5-year period.

Table 3. Non-fatal grade 3 and grade 4 toxicities after all induction chemotherapy courses per treatment arm (as-treated per protocol cohort)

Toxicity	Standard arm ($N = 174$)			Experimental arm ($N = 164$)			P (3 and 4 comb.)
	Ass'd	Grade 3	Grade 4	Ass'd	Grade 3	Grade 4	
		n (%)	*n* (%)		*n* (%)	*n* (%)	
General condition	959	87 (9)	10 (1)	1182	68 (6)	26 (2)	0.157[a]
Blood count							
Hemoglobin	992	486 (49)	331 (33)	1250	604 (48)	424 (34)	0.759[a]
White blood count	1004	196 (20)	721 (72)	1255	276 (22)	873 (70)	0.547[a]
Granulocytes	866	89 (10)	703 (81)	1108	137 (12)	892 (81)	0.399[a]
Platelets	1000	165 (17)	624 (62)	1253	185 (15)	884 (71)	0.008[a]
Infections							
Infection	992	82 (8)	4 (0.4)	1251	101 (8)	7 (1)	0.989[a]
Fever	1000	15 (2)	0 (0)	1248	18 (1)	0 (0)	0.927[a]
Oral mucositis	992	28 (3)	26 (3)	1249	25 (2)	18 (1)	0.073[a]
Diarrhea	991	25 (3)	12 (1)	1244	35 (3)	20 (2)	0.474[a]
Creatinine abnormal	999	2 (0.2)	0 (0)	1246	3 (0.2)	0 (0)	NA
Bilirubin abnormal	968	11 (1)	4 (0.4)	1228	9 (1)	2 (0.2)	NA
SGOT/SGPT abnormal	985	83 (8)	5 (1)	1239	107 (9)	2 (0.2)	0.400[a]
Abnormal LV-SF	599	0 (0)	0 (0)	739	2 (0.3)	1 (0.1)	0.836[a]
Ototoxicity	549	20 (4)	2 (0.4)	671	29 (4)	9 (1)	NA
Peripheral neurotoxicity	977	1 (0.1)	1 (0.1)	1240	2 (0.2)	1 (0.1)	0.261[a]
Constipation	989	4 (0.4)	2 (0.2)	1240	2 (0.2)	2 (0.2)	NA
All toxicities (*n*)	1006	1295	2444	1259	1603	3161	
Median number of toxicities per patient *n* (95% CI)		173 patients 22 (22–23)			163 patients 31 (29–33)		<0.001[b]
Median number of toxicities per course *n* (95% CI)		173 patients 4 (4–5)			163 patients 4 (4–5)		0.531[b]
Median number of courses per patient *n* (95% CI)		174 patients 6 (6–7)			164 patients 8 (8-8)		<0.001[b]
Secondary malignancy		174 patients 0 (0)			164 patients 7 (4)		NA
Growth retardation		174 patients 10 (6)			164 patients 9 (6)		1.000[a]
Spine deformities		174 patients 4 (2)			164 patients 2 (1)		0.686[a]

Ass'd, number of courses assessed; CI, confidence interval; comb., combined; LV-SF, left ventricular shortening fraction; NA, not applicable, *P*, *P* value comparing grade 3 and 4 combined.

[a] Generalized estimation equation (GEE).

[b] Median test.

between the trials NB97 and NB2004-HR see supplementary information 5, available at *Annals of Oncology* online).

The most appropriate length and dose intensity of induction chemotherapy for high-risk neuroblastoma have remained controversial in the literature. A meta-analysis of 44 trials[26] indicated a correlation between increased drug dose intensity and improved response and survival but this finding was not confirmed later when bone marrow was used as a measure of metastatic disease.[27] In a study of the SIOPEN group, initial failure to achieve a complete metastatic response to rapid COJEC was overcome in half of the patients by adding two cycles of topotecan, vincristine, and doxorubicin.[20] The reduction from seven to five chemotherapy courses was not associated with changes in the response rate of approximately 80%, as shown by a comparison of two subsequent studies in a single institution.[16] In our study, the proportion of responses before HDT did not differ between the arms and the poor responding subgroup did not benefit from longer induction chemotherapy.

Moreover, neither the surgical resectability nor the proportion of patients who needed radiotherapy (to compensate for surgical irresectability) was diminished by the addition of two chemotherapy cycles. Thus the clinical benefit of topotecan-containing courses observed in refractory or recurrent disease[17–20] was not validated for newly diagnosed high-risk neuroblastoma in our trial and within the context of therapy given. The authors cannot offer a good explanation for the surprising result.

In conclusion, the addition of two courses of chemotherapy, which had been established in patients with relapsed neuroblastoma previously, was not beneficial in newly diagnosed high-risk neuroblastoma patients in our study. The quality of the response after two courses and before high-dose therapy with autologous blood stem cell transplantation, the grade of resectability of the primary tumor, the frequency of irradiation needed for active tumor residuals at the end of induction chemotherapy, and both EFS and OS were not improved. By contrast, the patients had a longer duration of induction chemotherapy and suffered from more grade 3 and 4 toxicities. Our data, therefore, strongly suggest that extended induction chemotherapy with topotecan, cyclophosphamide, and etoposide cannot be recommended for high-risk neuroblastoma patients.

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DISCLOSURE

The authors have declared no conflicts of interests.

REFERENCES

1. Pinto NR, Applebaum MA, Volchenboum SL, et al. Advances in risk classification and treatment strategies for neuroblastoma. *J Clin Oncol*. 2015;33:3008—3017.
2. Morgenstern DA, London WB, Stephens D, et al. Prognostic significance of pattern and burden of metastatic disease in patients with stage 4 neuroblastoma: a study from the International Neuroblastoma Risk Group database. *Eur J Cancer*. 2016;65:1—10.
3. Berthold F, Spix C, Kaatsch P, Lampert F. Incidence, survival, and treatment of localized and metastatic neuroblastoma in Germany 1979-2015. *Paediatr Drugs*. 2017;19:577—593.
4. Arumugam S, Manning-Cork NJ, Gains JE, et al. The evidence for external beam radiotherapy in high-risk neuroblastoma of childhood: a systematic review. *Clin Oncol (R Coll Radiol)*. 2019;31:182—190.
5. Simon T, Hero B, Bongartz R, et al. Intensified external-beam radiation therapy improves the outcome of stage 4 neuroblastoma in children >1 year with residual local disease. *Strahlenther Onkol*. 2006;182:389—394.
6. Pritchard J, Cotterill SJ, Germond SM, et al. High dose melphalan in the treatment of advanced neuroblastoma: results of a randomised trial (ENSG-1) by the European Neuroblastoma Study Group. *Pediatr Blood Cancer*. 2005;44:348—357.
7. Matthay KK, Reynolds CP, Seeger RC, et al. Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a children's oncology group study. *J Clin Oncol*. 2009;27:1007—1013.
8. Berthold F, Ernst A, Hero B, et al. Long-term outcomes of the GPOH NB97 trial for children with high-risk neuroblastoma comparing high-dose chemotherapy with autologous stem cell transplantation and oral chemotherapy as consolidation. *Br J Cancer*. 2018;119:282—290.
9. Yu AL, Gilman AL, Ozkaynak MF, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med*. 2010;363:1324—1334.
10. Ladenstein R, Potschger U, Pearson ADJ, et al. Busulfan and melphalan versus carboplatin, etoposide, and melphalan as high-dose chemotherapy for high-risk neuroblastoma (HR-NBL1/SIOPEN): an international, randomised, multi-arm, open-label, phase 3 trial. *Lancet Oncol*. 2017;18:500—514.
11. Moroz V, Machin D, Faldum A, et al. Changes over three decades in outcome and the prognostic influence of age-at-diagnosis in young patients with neuroblastoma: a report from the International Neuroblastoma Risk Group Project. *Eur J Cancer*. 2011;47:561—571.
12. Berlanga P, Canete A, Castel V. Advances in emerging drugs for the treatment of neuroblastoma. *Expert Opin Emerg Drugs*. 2017;22:63—75.
13. Berthold F, Hero B. Neuroblastoma: current drug therapy recommendations as part of the total treatment approach. *Drugs*. 2000;59:1261—1277.
14. Pearson AD, Pinkerton CR, Lewis IJ, et al. High-dose rapid and standard induction chemotherapy for patients aged over 1 year with stage 4 neuroblastoma: a randomised trial. *Lancet Oncol*. 2008;9:247—256.
15. Moreno L, Vaidya SJ, Schrey D, et al. Long-term analysis of children with metastatic neuroblastoma treated in the ENSG5 randomised clinical trial. *Pediatr Blood Cancer*. 2019;66:e27565.
16. Kushner BH, Kramer K, LaQuaglia MP, et al. Reduction from seven to five cycles of intensive induction chemotherapy in children with high-risk neuroblastoma. *J Clin Oncol*. 2004;22:4888—4892.
17. Kushner BH, Kramer K, Modak S, et al. Differential impact of high-dose cyclophosphamide, topotecan, and vincristine in clinical subsets of patients with chemoresistant neuroblastoma. *Cancer*. 2010;116:3054—3060.
18. Simon T, Langler A, Harnischmacher U, et al. Topotecan, cyclophosphamide, and etoposide (TCE) in the treatment of high-risk neuroblastoma. Results of a phase-II trial. *J Cancer Res Clin Oncol*. 2007;133:653—661.
19. London WB, Frantz CN, Campbell LA, et al. Phase II randomized comparison of topotecan plus cyclophosphamide versus topotecan alone in children with recurrent or refractory neuroblastoma: a Children's Oncology Group study. *J Clin Oncol*. 2010;28:3808—3815.
20. Amoroso L, Erminio G, Makin G, et al. Topotecan-vincristine-doxorubicin in stage 4 high-risk neuroblastoma patients failing to achieve a complete metastatic response to rapid COJEC: a SIOPEN study. *Cancer Res Treat*. 2018;50:148—155.
21. Park JR, Scott JR, Stewart CF, et al. Pilot induction regimen incorporating pharmacokinetically guided topotecan for treatment of newly diagnosed high-risk neuroblastoma: a Children's Oncology Group study. *J Clin Oncol*. 2011;29:4351—4357.
22. Brodeur GM, Pritchard J, Berthold F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *J Clin Oncol*. 1993;11:1466—1477.
23. Schmidt M, Simon T, Hero B, et al. Is there a benefit of 131 I-MIBG therapy in the treatment of children with stage 4 neuroblastoma? A retrospective evaluation of the German neuroblastoma trial NB97 and implications for the German neuroblastoma trial NB2004. *Nuklearmedizin*. 2006;45:145—151. quiz N139-140.
24. Wassmer G. Planning and analyzing adaptive group sequential survival trials. *Biom J*. 2006;48:714—729.
25. Pampallona S, Tsiatis AA. Group sequential designs for one-sided and 2-sided hypothesis-testing with provision for early stopping in favor of the null hypothesis. *J Stat Plan Inference*. 1994;42:19—35.
26. Cheung NV, 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.
27. Pinkerton CR, Blanc Vincent MP, Bergeron C, et al. Induction chemotherapy in metastatic neuroblastoma—does dose influence response? A critical review of published data standards, options and recommendations (SOR) project of the National Federation of French Cancer Centres (FNCLCC). *Eur J Cancer*. 2000;36:1808—1815.