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Outcome of High-Risk Stage 3 Neuroblastoma With Myeloablative Therapy and 13-*cis*-Retinoic Acid: A Report From the Children's Oncology Group

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Background. The components of therapy required for patients with INSS Stage 3 neuroblastoma and high-risk features remain controversial. ***Procedure.*** A retrospective cohort design was used to determine if intensive chemoradiotherapy with purged autologous bone marrow rescue (ABMT) and/or 13-*cis*-retinoic acid (13-*cis*-RA) improved outcome for patients with high-risk neuroblastoma that was not metastatic to distant sites. We identified 72 patients with INSS Stage 3 neuroblastoma enrolled between 1991 and 1996 on the Phase 3 CCG-3891 randomized trial. Patients were analyzed on an intent-to-treat basis using a log-rank test. ***Results.*** The 5-year event-free survival (EFS) and overall survival (OS) rates for patients with Stage 3 neuroblastoma were $55 \pm 6\%$ and $59 \pm 6\%$, respectively (n = 72). Patients randomized to ABMT (n = 20) had 5-year EFS of $65 \pm 11\%$ and OS of $65 \pm 11\%$ compared to 41 ± 11 ($P = 0.21$) and $46 \pm 11\%$ ($P = 0.23$) for patients randomized to CC (n = 23), respectively. Patients randomized to 13-*cis*-RA (n = 23) had 5-year EFS of $70 \pm 10\%$ and OS of $78 \pm 9\%$ compared to $63 \pm 12\%$ ($P = 0.67$) and $67 \pm 12\%$ ($P = 0.55$) for those receiving no further therapy (n = 16), respectively. Patients randomized to both ABMT and 13-*cis*-RA (n = 6) had a 5-year EFS of $80 \pm 11\%$ and OS of 100%. ***Conclusion.*** Patients with high-risk Stage 3 neuroblastoma have an overall poor prognosis despite aggressive chemoradiotherapy. Further studies are warranted to determine if myeloablative consolidation followed by 13-*cis*-RA maintenance therapy statistically significantly improves outcome. Pediatr Blood Cancer 2009; 52:44–50. © 2008 Wiley-Liss, Inc.

Key words: hematopoietic stem cell transplant; neuroblastoma

INTRODUCTION

From 1988 to 1996 the Children's Cancer Group (CCG) utilized a risk-adapted treatment strategy incorporating patient age, tumor stage, serum ferritin, tumor histopathology and tumor *MYCN* status to assign eligibility to neuroblastoma treatment regimens [1,2]. The overall survival (OS) was greater than 95% for children with Evans Stage 3 disease whose tumors had favorable biologic characteristics (no *MYCN* amplification, favorable histopathology, serum ferritin of <143 ng/ml) [1]. In contrast, the 4-year event-free survival (EFS) for children with high-risk Stage 3 neuroblastoma, defined as greater than 1 year of age with Stage 3 disease and at least one unfavorable biologic feature (either tumor *MYCN* amplification, unfavorable tumor histopathology or serum ferritin >143 ng/ml) was 54% [1].

Protocol CCG-3891 was a prospective randomized Phase 3 trial designed to assess whether a combination of myeloablative chemotherapy, total-body irradiation and transplantation of purged autologous bone marrow (ABMT) improved EFS when compared to intensive non-myeloablative continuation chemotherapy (CC) in children with high-risk Stage 3 and 4 neuroblastoma. The study design included a second randomization to determine whether treatment with 13-*cis*-retinoic acid (13-*cis*-RA) following either ABMT or CC further improved EFS for children with high-risk neuroblastoma. The initial analysis demonstrated an improvement in 3-year EFS for children with high-risk neuroblastoma treated with ABMT as compared to CC ($34 \pm 4\%$ vs. $22 \pm 4\%$, $P = 0.034$) and for those treated with 13-*cis*-RA compared to no post-consolidation therapy ($46 \pm 6\%$ vs. $29 \pm 5\%$, $P = 0.027$) [2]. The majority of patients treated on CCG-3891 had Stage 4 disease, raising the concern that published data may not accurately delineate whether ABMT and/or 13-*cis*-RA improves the outcome for patients with high-risk Stage 3 neuroblastoma.

We report long-term EFS and OS for children enrolled on CCG-3891 with high-risk Stage 3 neuroblastoma. We further assessed whether ABMT compared to CC and whether subsequent treatment with 13-*cis*-RA compared to no further therapy improved EFS and OS for patients with high-risk Stage 3 neuroblastoma.

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METHODS

Patients

From 1991 to 1996, 539 eligible patients with newly diagnosed high-risk neuroblastoma, including 72 eligible patients with Evans Stage 3, were enrolled on Study CCG-3891. Patients diagnosed with Evans Stage 3 disease with either tumor amplification of *MYCN* oncogene, and/or a serum ferritin level ≥143 ng/ml, and/or tumor unfavorable histopathological findings form the basis for this report. The study was approved by the IRB's of participating institutions and signed informed consent was obtained from participating patients.

Techniques utilized for ferritin measurement, histopathologic verification [3], *MYCN* gene determinations and INSS staging criteria have been previously described [1,4]. We analyzed allelic

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status at chromosome bands 1p36 and 11q23 using described techniques in tumor samples with available matched normal DNA [5]. We use the term "Unb11q LOH" to define a pattern of deletion that affects a region of 11q only, rather than a whole chromosome loss.

Treatment

CCG-3891 therapy has been previously described [2]. All patients received five induction cycles of cisplatin, doxorubicin, etoposide, and cyclophosphamide administered every 28 days followed by surgery and external beam radiotherapy for gross residual disease. Bone marrow harvesting and immunomagnetic purging were done at the neuroblastoma purging center of the CCG prior to the fourth or fifth cycles of induction therapy [2]. The first randomization was performed prior to the third cycle of induction therapy (week 8). The ABMT cohort received a 96-hr continuous infusion of carboplatin (1,000 mg/m^2) and etoposide (640 mg/m^2) beginning 8 days before transplantation; melphalan 140 mg/m^2 7 days before transplantation and 70 mg/m^2 6 days before transplantation; total-body irradiation 333 cGy daily for 3 days before transplantation; infusion of purged autologous bone marrow on day 0 followed by administration of granulocyte macrophage colony stimulating factor. The CC cohorts received three cycles of cisplatin (160 mg/m^2), etoposide (500 mg/m^2), and doxorubicin (40 mg/m^2), administered as a continuous infusion over 96 hr given simultaneously with a bolus ifosfamide (2,500 mg/m^2 on days 0 and 3) and mesna (600 mg/m^2 per dose every 3 hr for five doses), followed by granulocyte colony stimulating factor. After ABMT or CC, patients without disease progression were randomly assigned to receive six cycles of 13-*cis*-RA (160 mg/m^2 per day administered orally in two divided doses for 14 consecutive days every 28 days) or no further therapy. Clinical evaluations were performed at diagnosis, end of induction, ABMT or CC and 13-*cis*-RA therapy. Responses were assessed using the international criteria for response to treatment of neuroblastoma [4].

Statistical Considerations

The trial used a quasi-factorial design with two separate sequential randomizations. Patients with progressive disease before week 8 of induction were ineligible for randomization. Patients with progressive or histologically confirmed disease at the completion of ABMT or CC were ineligible for the second randomization. Patients who were unable to undergo ABMT for medical or psychosocial reasons were non-randomly assigned to CC. If these patients had no disease progression after CC, they were eligible for the second randomization but they were not considered in the first randomization analysis. The overall CCG-3891 study was designed to have 82% power to detect a 20% difference in the EFS rate with 183 high-risk patients randomly assigned to each treatment regimen. Tests of association were performed with Fisher's exact test. Survival curves were constructed by the methods of Kaplan and Meier [6] standard errors (SEs) calculated by the methods of Peto and Peto [7]. Survival comparisons were performed using the log-rank test. The time to event was calculated as the time from study enrollment to the first occurrence of relapse, progressive disease, secondary malignancy or death. Patients without events were censored at the time of last contact. OS time was calculated as the time from study enrollment until the time of death or the time of last contact if the patient was alive. EFS and OS rates are presented as the rate ± SE.

RESULTS

Patients Characteristics

Characteristics of the 72 eligible Evans Stage 3 patients are described in Tables I and II. All patients were classified as INSS Stage 3; INSS designation will be used henceforth. Ploidy data were not collected in this patient cohort. The majority of patients had either adrenal (57%) or other abdominal sites of tumors (18%). Clinical characteristics and response to induction chemotherapy ($P = 0.1704$ for ABMT vs. CC) were not significantly different between the randomly assigned patients. Of the patients non-randomly assigned to receive CC, there was a trend toward younger age (age <18 months) ($P = 0.1249$) and a numerically higher number of patients with improved response to induction chemotherapy ($P = 0.5745$) (Table I).

Randomization

Of the 72 eligible patients, 43 patients accepted random assignment to ABMT (n = 20) or CC (n = 23), 26 patients refused randomization and were non-randomly assigned to CC and three patients were removed from protocol therapy prior to randomization. Of the 43 randomized patients, 12 patients were removed from study prior to receiving assigned therapy due to progressive disease (n = 9) or family preference (n = 3). Following consolidation therapy, 39 of 54 eligible patients were randomized to either no further therapy (n = 16) or 13-*cis*-RA (n = 23), four patients were non-randomly assigned to no further therapy based upon family and/or physician preference, four patients were non-randomly assigned to 13-*cis*-RA due to persistent disease and seven patients declined randomization.

Outcomes

The median follow-up time for patients without an event was 8.2 years. The overall 5-year EFS and OS rates ± SE for patients with Stage 3 disease were 55 ± 6% and 59 ± 6%, respectively as compared to 5-year EFS and OS for patients enrolled onto CCG-3891 with Stage 4 disease of 21 ± 3% ($P < 0.0001$) and 32 ± 3% ($P < 0.0001$), respectively. All deaths were attributed to progressive disease except for one due to infection, one due to toxicity, and one unknown cause. Relapse site was reported in 32 of the 33 patients with relapse/disease progression; 13 (41%) had distant and 19 (59%) had local relapses.

In the intent-to-treat comparison of randomized treatment groups, the 5-year EFS rates for CC patients versus ABMT patients were 41 ± 11% (n = 23) and 65 ± 11% (n = 20), respectively ($P = 0.2141$) (Table III, Fig. 1a). The OS comparison of CC versus ABMT was not statistically significant ($P = 0.2279$) (Table III, Fig. 1b). There was a trend for improved EFS for patients non-randomly assigned to CC versus those randomized to CC ($P = 0.1494$; 5-year EFS: 62 ± 10% (n = 26) vs. 41 ± 11% (n = 23), respectively), suggesting a bias toward more clinically favorable patients in the non-randomly assigned CC arm. *MYCN* status of the tumor was known in 38 of 43 randomized patients. Of the 16 patients with *MYCN* amplified tumors, seven of nine randomized to CC patients relapsed (77%) while four of seven randomized to ABMT patients relapsed (57%) ($P = 0.5962$).

In the "as treated" comparison of the randomized treatment cohort, the 5-year EFS rates for CC patients versus ABMT patients

TABLE I. Characteristics of Patients by Treatment Assignment

	Number of patients				
	First randomization			Second randomization	
Characteristic	ABMT (n = 20)	CC (n = 23)	NRCC (n = 26)	13-*cis*-RA (n = 23)	No 13-*cis*-RA (n = 16)
Age at diagnosis*					
<18 months	2	4	8	3	2
>18 months	18	19	18	20	14
MYCN					
Not amp	10	12	13	8	10
Amp	7	9	7	8	4
Unknown	3	2	6	7	2
Shimada histology					
Fav	2	2	2	3	1
Unfav	15	16	21	17	15
Unknown	3	5	3	3	0
Serum ferritin level					
<143 ng/ml	6	10	15	11	9
>143 ng/ml	14	13	9	11	7
Unknown	0	0	2	1	0
Initial response[#&]					
CR/VGPR	15	10	18	16	10
≤PR	4	9	6	5	4
Unknown	1	4	2	2	2

CC, randomized to chemotherapy only; ABMT, randomized to myeloablative chemotherapy followed by autologous bone marrow transplantation; NRCC, non-randomly assigned to chemotherapy only; 13-*cis*-RA, randomized to 13-*cis*-retinioc acid; No 13-*cis*-RA, randomized to no 13-*cis*-retinioc acid. *$P = 0.1249$ for (ABMT & CC) versus NRCC; [#]$P = 0.1704$ for ABMT versus CC; [&]$P = 0.5745$ for (ABMT & CC) versus NRCC.

were $50 \pm 14\%$ (n = 16) and $73 \pm 12\%$ (n = 15), respectively ($P = 0.2737$). The as treated comparison of 5-year OS rates for CC patients versus ABMT were $54 \pm 14\%$ (n = 16) and $73 \pm 12\%$ (n = 15), respectively ($P = 0.1905$).

Patients randomized to 13-*cis*-RA had 5-year EFS from time of enrollment of $70 \pm 10\%$ (n = 23), while those randomized to no further therapy had 5-year EFS of $63 \pm 12\%$ (n = 16), $P = 0.6684$ (Table III, Fig. 2a). The OS comparison of 13-*cis*-RA versus no 13-*cis*-RA was not statistically significant either ($P = 0.5516$) (Table III, Fig. 2b). Patients randomized to receive both ABMT and 13-*cis*-RA (n = 6) have a 5-year EFS and OS rates of $80 \pm 11\%$ and 100%, respectively. A secondary thyroid malignancy occurring more than 6 years after study enrollment accounted for the single event.

Response data were reported in 64 of 72 patients. Overall, the EFS and OS rates for patients achieving CR/VGPR patients were significantly higher than that of patients who achieved at best a partial response (PR) ($P = 0.0043$ and $P = 0.0081$, respectively) (Table IV). For patients who achieved induction CR/VGPR, there was no statistically significant difference in EFS for the 13-*cis*-RA group in comparison to the no 13-*cis*-RA group ($P = 0.3994$). The 5-year EFS for patients who achieved at best a PR response to induction chemotherapy and received 13-*cis*-RA was $60 \pm 22\%$ (n = 5) as compared to $25 \pm 22\%$ (n = 4) for those who received no further therapy ($P = 0.1239$).

There was no statistically significant relationship between site of relapse (primary vs. metastatic) and response to induction therapy (CR/VGPR vs. PR) ($P = 1.0000$). Radiation therapy administration was documented for 17 of the 19 local relapses, 7 (41%) received radiation therapy to the primary tumor site and 10 (59%) did not ($P = 0.3076$).

Prognostic Factors

Table II shows the proportions of patients and the 5-year EFS and OS rates by patient characteristics. Sample size was insufficient to perform multivariate analyses. Both EFS and OS rates for patients with *MYCN* amplified tumors were significantly lower than that for the patients with *MYCN* non-amplified tumors ($P < 0.0001$). EFS and OS rates were also statistically significantly lower for patients with elevated ferritin levels, 55% of whom also had *MYCN* amplified tumors. No statistically significant differences in the survival rates were identified for age (2-year or 18-month cut-off), Shimada histopathology, primary site, 1p36 and unbalanced 11q LOH, although the ability to detect differences was limited by the small sample size.

The 5-year OS for patients <18 months of age with non-amplified *MYCN* tumors (n = 7) was 100% as compared to a 5-year OS of $74 \pm 9\%$ for patients >18 months with non-amplified *MYCN* tumors (n = 30, $P = 0.1545$), respectively. Two of the seven patients <18 months of age were classified as having unfavorable histopathology; both were non-randomly assigned CC. The 5-year OS for patients >18 months of age with non-amplified *MYCN* tumors and favorable or unknown histopathology (n = 5) was 100% as compared to $70 \pm 11\%$ (9 events) for patients >18 months of age with non-amplified *MYCN* tumors and unfavorable histopathology (n = 25, $P = 0.1959$).

TABLE II. Response and Survival Rates of 72 INSS Stage 3 Patients

Characteristic	N (%)	5-year EFS rate ± SE (%)	*P*-value[#]	5-year OS rate ± SE (%)	*P*-value[#]
Overall	72	55 ± 7		59 ± 7	
Age					
<18 months	14 (19)	57 ± 17	0.9746	57 ± 17	0.7919
≥18 months	58 (81)	54 ± 7		60 ± 7	
MYCN					
Not amp	37 (61)	77 ± 8	<0.0001[a]	79 ± 8	<0.0001[a]
Amp	24 (39)	25 ± 9		29 ± 9	
Unknown	11	45 ± 15		61 ± 17	
Shimada histology					
Fav	6 (10)	83 ± 17	0.2108	83 ± 17	0.2442
Unfav	55 (90)	53 ± 7		58 ± 8	
Not eval/unknown	11				
Ferritin					
<143 ng/ml	32 (46)	78 ± 8	0.0005	81 ± 8	0.0014
>143 ng/ml	38 (54)	38 ± 9		43 ± 9	
Primary site					
Chest	8 (11)	88 ± 15	0.2586	100 ± 0	0.1027
Thoracoabdomen	1 (1)	100 ± 0		100 ± 0	
Adrenal	41 (57)	45 ± 9		49 ± 9	
Celiac	4 (6)	25 ± 22		25 ± 22	
Other abdominal	13 (18)	62 ± 13		67 ± 14	
Pelvis	5 (7)	80 ± 21		80 ± 21	
Unbal 11q LOH					
No loss	15 (65)	53 ± 13	0.7768	60 ± 13	0.7455
LOH	8 (35)	38 ± 17		57 ± 22	
Unknown	49				
1p36 LOH					
No loss	11 (48)	64 ± 15	0.1203	73 ± 14	0.2242
LOH	12 (52)	42 ± 14		55 ± 15	
Unknown	49				
Age, within MYCN not amp, unfav histology					
<18 months	2 (7)	100	0.4000	100	0.4258
≥18 months	25 (93)	70 ± 11		70 ± 11	

[#]*P*-values are from a two-sided log-rank test; [a]Comparison of amplified versus not amplified.

TABLE III. Outcome by Treatment Group

Characteristic	n	%	5-year EFS rate ± SE (%)	*P*-value[#]	5-year OS rate ± SE (%)	*P*-value[#]
Overall	72		55 ± 6		59 ± 6	
Initial treatment						
CC	23	33	41 ± 11	0.2141[b]	46 ± 11	0.2279[b]
ABMT	20	29	65 ± 11		65 ± 11	
NRCC	26	38	62 ± 10		68 ± 9	
Off protocol therapy	18[a]					
Maintenance treatment	54					
13-*cis*-RA	23	49	70 ± 10	0.6684[c]	78 ± 9	0.5516[c]
No 13-*cis*-RA	16	34	63 ± 12		67 ± 12	
NR 13-*cis*-RA	4	9	25 ± 22		25 ± 22	
NR No 13-*cis*-RA	4	9	75 ± 22		75 ± 22	
Off protocol therapy	7					

CC, randomized to chemotherapy only; ABMT, randomized to myeloablative chemotherapy followed by autologous purged bone marrow transplantation; NRCC, non-randomly assigned to chemotherapy only; 13-*cis*-RA, randomized to 13-*cis*-retinioc acid; No 13-*cis*-RA, randomized to no 13-*cis*-retinioc acid; NR 13-*cis*-RA, non-randomly assigned to 13-*cis*-retinioc acid; NR No 13-*cis*-RA, non-randomly assigned to no 13-*cis*-retinioc acid. [#]*P*-values are from a two-sided log-rank test; [a]Patients were removed from protocol therapy for progressive disease or family preference prior to randomization (n = 3) or following randomization (n = 15); [b]Test compares randomized CC versus ABMT; [c]Test compares randomized 13-*cis*-RA versus No 13-*cis*-RA.

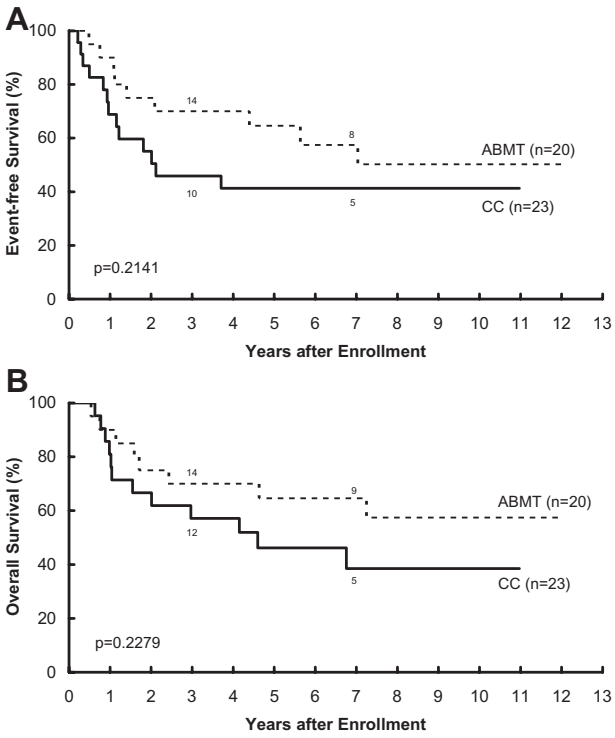

Fig. 1. Kaplan–Meier survival curves from time of study enrollment for patients who accepted the first randomization, by treatment group (intent-to-treat): transplantation (ABMT, n = 20) versus continuation chemotherapy (CC, n = 23). **A**: Event-free survival ($P = 0.2141$). **B**: Overall survival ($P = 0.2279$).

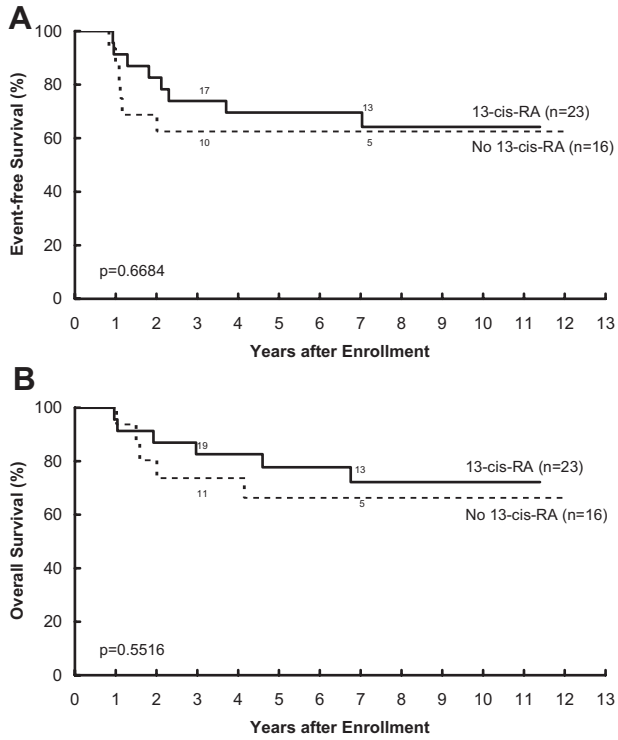

Fig. 2. Kaplan–Meier survival curves from time of study enrollment for patients who accepted the second randomization, by treatment group (intent-to-treat): 13-*cis*-RA (n = 23) versus no 13-*cis*-RA (n = 16). **A**: Event-free survival ($P = 0.6684$). **B**: Overall survival ($P = 0.5516$).

TABLE IV. Survival Rates by Response to Induction Chemotherapy: Overall and Within Treatment Group

Characteristic	n	5-year EFS rate ± SE	*P*-value[#]	5-year OS rate ± SE	*P*-value[#]
Overall					
CR/VGPR	45	66 ± 8%	0.0043	70 ± 8%	0.0081
≤PR	19	26 ± 11%		30 ± 13%	
CC patients					
CR/VGPR	10	56 ± 19%	0.1029	65 ± 19%	0.1562
≤PR	9	22 ± 14%		25 ± 13%	
Unknown	4				
ABMT patients					
CR/VGPR	15	73 ± 12%	0.1728	73 ± 12%	0.1221
≤PR	4	25 ± 22%		25 ± 22%	
Unknown	1				
NRCC patients					
CR/VGPR	18	67 ± 12%	0.1625	72 ± 12%	0.3503
≤PR	6	33 ± 27%		40 ± 31%	
Unknown	2				
13-*cis*-RA patients					
CR/VGPR	16	69 ± 12%	0.9280	81 ± 11%	0.6589
≤PR	5	60 ± 22%		60 ± 19%	
No 13-*cis*-RA patients					
CR/VGPR	10	80 ± 16%	0.0195	79 ± 16%	0.1025
≤PR	4	25 ± 22%		38 ± 30%	
NR 13-*cis*-RA patients					
CR/VGPR	3	2 events	NA	2 died	NA
≤PR	1	1 event		1 died	

CC, randomized to chemotherapy only; ABMT, randomized to myeloablative chemotherapy followed by autologous bone marrow transplantation; NRCC, non-randomly assigned to chemotherapy only; 13-*cis*-RA, randomized to 13-*cis*-retinioc acid; No 13-*cis*-RA, randomized to no 13-*cis*-retinioc acid; NR 13-*cis*-RA, non-randomly assigned to 13-*cis*-retinioc acid. [#]*P*-values are from a two-sided log-rank test.

DISCUSSION

Stage 3 neuroblastoma is a heterogenous group of tumors with significant variability in outcome. Our data confirm an overall poor outcome for Stage 3 disease and high-risk features. The small numbers of Stage 3 patients enrolled onto the CCG-3891 study limit our ability to detect a statistically significant difference between treatment groups. However, the data support further investigation to better delineate the efficacy of myeloablative consolidation therapy for patients with high-risk Stage 3 neuroblastoma.

The presence of *MYCN* gene amplification within neuroblastoma is a powerful predictor of outcome independent of anatomic stage, age, and tumor histopathology [8,9]. A statistically worse EFS and OS exist for patients with Stage 3 *MYCN* amplified neuroblastoma ($P < 0.0001$). The small numbers of randomized patients with Stage 3 *MYCN* amplified neuroblastoma limit our ability to identify a statistically significant improvement following ABMT. Yet both the CCG-3891 and the German Society of Pediatric Oncology and Hematology NB97 trials suggest a benefit of ABMT for patients with MYCN amplified neuroblastoma [2,10]. These data and the overall poor prognosis for *MYCN* amplified Stage 3 neuroblastoma support the use of ABMT consolidation for patients with Stage 3 *MYCN* amplified neuroblastoma.

Patients with poor response to induction chemotherapy are more likely to have minimal residual disease despite ABMT, as evidenced by their higher relapse rate. In contrast, the subgroup of patients with poor response to induction chemotherapy who subsequently received 13-*cis*-RA ($n = 5$) had an excellent survival. Small patient numbers limit statistical significance but suggest that 13-*cis*-RA may be beneficial in targeting minimal residual disease that remains after myeloablative therapy. The previously reported analysis for all patients (Stages 3 and 4 combined) enrolled onto CCG-3891 further supports this hypothesis [2].

Neuroblastoma histopathologic classification has prognostic value across all disease stages, independent of *MYCN* gene amplification [3,11]. The small sample size of our trial limits the ability to detect a statistically significant prognostic value of histology. We observed an OS of only 70% for patients who are >18 months of age at diagnosis of Stage 3 neuroblastoma without *MYCN* gene amplification, but with unfavorable histopathology. This is notably less than the survival of 85–90% previously reported for patients with Stage 3 neuroblastoma >2 years of age with favorable biologic features including favorable histology [1]. In contrast, patients <18 months of age with MYCN non-amplified Stage 3 tumors treated on CCG-3891 have an excellent prognosis ($n = 7$, OS 100%), similar to the excellent prognosis demonstrated for patients up to 18 months of age diagnosed with biologically favorable Stage 4 neuroblastoma [12,13]. These data suggest that reduction in therapeutic dose intensity and eliminating myeloablative therapy for patients <18 months of age with Stage 3, MYCN non-amplified neuroblastoma, regardless of histopathology should be considered.

Despite its prognostic value, MYCN amplification is only present in 30% of neuroblastoma tumors leaving a significant proportion of high-risk patients that cannot be uniquely identified using genomic evaluation. The loss of heterozygozity (LOH) at chromosomes 1p and 11q has prognostic significance independent of MYCN amplification [5,14–16], Unfortunately, due to the limited tumor genetic data available, we remain unable to demonstrate a prognostic significance of these genetic factors for Stage 3 disease. The overall poor EFS in Stage 3 patient with high-risk features supports the need for continued prospective evaluation of these genetic factors.

There remains a need to develop new therapies to improve survival for Stage 3 neuroblastoma with MYCN amplification, unfavorable histopathology in those >18 months of age or with poor response to initial therapy. Our data together with the previously published CCG-3891 data provide support for the use of aggressive consolidation therapy including ABMT for this subset of the high-risk Stage 3 patients. The occurrence of metastatic relapse in Stage 3 neuroblastoma confirms the presence of minimal residual disease and provides rationale for the use of 13-*cis*-RA following consolidation ABMT. We were not able to demonstrate a statistically significant improvement in survival for those Stage 3 patients randomized to receive 13-*cis*-RA, perhaps due to the small numbers of Stage 3 patients who participated in the 13-*cis*-RA randomization. Further investigations are planned to test novel inductions regimens that incorporate non-cross resistant chemotherapeutic or biologically targeted agents, uniform use of radiotherapy to the primary tumor site and novel treatment of minimal residual disease.

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