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STRIKING DICHOTOMY IN OUTCOME OF *MYCN*-AMPLIFIED NEUROBLASTOMA IN THE CONTEMPORARY ERA

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Abstract

Background—We exploited a large database to investigate the outcome of high-risk neuroblastoma (HR-NB) in the contemporary era.

Methods—We studied all HR-NB patients <12 years old treated during induction at our hospital in 2000–2011, including 118 patients with *MYCN*-amplified(+) disease, and 127 patients >18 months old with *MYCN*-non-amplified(−) stage 4.

Results—Complete/very good partial response (CR/VGPR) to induction correlated with significantly superior event-free (EFS) ($p<0.001$) and overall survival (OS) ($p<0.001$) compared to partial response or less (≤PR). *MYCN*(+) and *MYCN*(−) patients had similar rates of CR/VGPR to induction ($p=0.366$); *MYCN*(+) and *MYCN*(−) patients in CR/VGPR had similar EFS ($p=0.346$) and OS ($p=0.542$). In contrast, only *MYCN*(+) patients had progressive disease (PD) as response to induction ($p<0.001$), and early death from PD (<366 days post-diagnosis) was significantly more common ($p<0.001$) with *MYCN*(+) disease. Overall, among patients with ≤PR, *MYCN*(+) patients had significantly inferior EFS ($p<0.001$) and OS ($p<0.001$) compared to *MYCN*(−) patients, which accounted for the significantly worse EFS ($p=0.008$) and OS ($p=0.002$) of the entire *MYCN*(+) cohort versus *MYCN*(−) cohort.

Conclusions—*MYCN*(−) HR-NB patients display a broad, continuous spectrum as regards response and outcome, whereas *MYCN*(+) patients have either an excellent response to induction associated with good long-term outcome, or early PD with poor outcome. This extreme dichotomy in the clinical course of *MYCN*(+) patients points to underlying biological differences with *MYCN*(+) NB, the elucidation of which may have far-reaching implications, including improved risk classification at diagnosis and identification of targets for treatment.

Keywords

neuroblastoma; contemporary therapy; *MYCN*; induction

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INTRODUCTION

Population-based data[1–3] and group studies through the 1990s[4–11] showed 5-year event-free survival (EFS) and overall survival (OS) rates of 17–37% for high-risk neuroblastoma (HR-NB), with little difference between EFS and OS (Table 1A). In large studies extending beyond 2000, 5-year EFS and OS rates improved to 35–64% (Table 1B).[12–15] The results may represent overestimations of survival given that subsequent analyses revealed a more limited definition of HR-NB. Thus, these studies included: a) patients 12–18 months old with *MYCN*-non-amplified(−) stage 4 which is now recognized as intermediate-risk disease;[16,17] and b) toddlers with *MYCN*(−) stage 3 with unfavorable histology, which may be cured by limited therapy.[18] Indeed, a 1999–2004 group study focusing on a well-recognized high-risk group – i.e., *MYCN*-amplified(+) NB in infants – yielded 2-year EFS/OS of 29%/30%,[19] and an international experience covering 1997–2002 for patients >18 months with *MYCN*(+) stage 4 revealed EFS 18%.[17]

Better results have been expected with the recent adoption of therapies, many developed in the 1990s, promising more effective induction (dose-dense or dose-intensive chemotherapy),[8,20,21] consolidation (13-*cis*-retinoic acid,[10] local radiotherapy,[22] anti-G_{D2} monoclonal antibody [MoAb][23,24]), and salvage (^{131}I-metaiodobenzylguanidine [MIBG] therapy,[25] topoisomerase 1 inhibitors[26–28]).

We used the large Memorial Sloan-Kettering Cancer Center (MSKCC) database to investigate the outcome of HR-NB in the contemporary era, i.e., since 2000. We took into account *MYCN* amplification, since large studies differ whether this finding is[4,6,10,12] or is not[5,8,13,14] prognostic with HR-NB (Table 1). We also assessed response to induction vis-à-vis EFS and OS, given differences in past studies whether a good response does[8,10,12,19] or does not[4,6,14] impact survival of HR-NB patients (Table 1). The data revealed a striking dichotomy in the clinical course of patients with *MYCN*(+) disease, a scenario not seen with *MYCN*(−) HR-NB.

PATIENTS AND METHODS

Study subjects were identified from the NB patient registry of MSKCC. This source listed 1185 patients in the period 2000–2011. The current study was limited to the 247 patients who were <12 years old when diagnosed with HR-NB and were treated during induction at MSKCC from diagnosis or after starting induction elsewhere. Excluded were patients who came for consultation alone or only surgery. The definition of HR-NB conformed with current criteria: *MYCN*(+) stage 2, 3, 4S, or 4 disease of any age, or *MYCN*(−) stage 4 diagnosed at age >18 months.[16,17] *MYCN* amplification was defined by international criteria using fluorescence *in situ* hybridization.[29] In accordance with rules of the MSKCC institutional review board, informed written consents for evaluations and treatments were obtained from guardians, and, for this exploratory study via a retrospective review, a waiver was obtained for examination and analysis of patient records.

Disease status was defined by widely accepted international response criteria,[6,8,11,14,15,19,30] including ^{123}I-MIBG findings: complete remission (CR), no evidence of NB in soft tissue,

bones, or bone marrow (BM), and urine catecholamines normal; very good partial remission (VGPR), primary mass reduced by ≥90%, no evidence of distant disease in soft tissue, bones, or BM, including negative ^{123}I-MIBG scan, and urine catecholamines normal; partial response (PR), >50% decrease in measurable soft tissue disease and the number of metastatic skeletal lesions in ^{123}I-MIBG scan, and ≤1 positive BM site; mixed response (MR) , >50% decrease of any lesion with <50% decrease in any other, and ^{123}I-MIBG scan improved by <50% decrease in number of (+) sites; no response (NR), <50% decrease but <25% increase in any existing lesion, unchanged MIBG findings; and progressive disease (PD), new, or >25% increase in an existing, lesion.

A complete evaluation of disease status comprised computed tomography or magnetic resonance imaging, ^{123}I-MIBG scan, urine catecholamine levels, and BM histology (aspirates and biopsies from bilateral posterior iliac crests, and aspirates±biopsies from bilateral anterior iliac crests). BM and imaging studies were read by MSKCC specialists outside the Department of Pediatrics unaware of treatment or patient status. A complete evaluation was performed at the end of induction. Patients who achieved CR/VGPR underwent a complete evaluation at least every three months for an additional two years, and then ^{123}I-MIBG scan ± other staging studies every three months for three more years. Patients who achieved only PR or less (≤PR) with induction underwent a complete evaluation every 1–3 months while on therapy; if they achieved CR/VGPR, a complete evaluation was repeated every three months for three more years.

The software SPSS, version 11.0 (SPSS Inc, Chicago, IL), was used for the survival statistical analyses, calculating from the first day of induction chemotherapy. Survival curves were generated according to the Kaplan-Meier method, with point estimates including ±SE, and compared using the two-sided log-rank test. EFS continued through the date of PD, toxic death, or secondary cancer. OS was defined through the date of death from any cause. Two-tailed chi squared test was used for comparisons of response rates to induction therapy.

RESULTS

Patient characteristics

The total series of patients included 118 *MYCN*(+) patients (1 stage 2, 13 stage 3, 1 stage 4S, 103 stage 4), 127 *MYCN*(−) patients with stage 4 diagnosed at age >18 months, and two patients with unknown *MYCN* status. The latter two patients were excluded from further analysis, leaving a total of 245 patients for study (Table 2). Thirty-one patients were infants, i.e., <18 months old, and all had *MYCN*(+) disease. *MYCN*(+) and *MYCN*(−) patients received the same upfront and salvage therapies. Thus, the initial (1st-line) induction regimens were all for HR-NB: 223 (91%) patients received Children's Oncology Group[15,21] (or similar MSKCC[20]) regimens, and 22 (9%) received other group-wide[8,9] or single-institutional programs. Consolidation of CR/VGPR included anti-G_{D2} MoAb ± autologous stem-cell transplantation (ASCT).[24] Initial 2nd-line treatments for refractory or progressive disease with induction included high-dose conventional chemotherapy[31–34] or moderate-dose regimens[28,35] using agents with known anti-NB activity. Relapse was also treated uniformly, including intra-thecal radioimmunotherapy for central nervous system relapse,[36]

high-dose conventional chemotherapy[31–34] for disseminated or soft-tissue relapse, and moderate-dose chemotherapy[28,35] plus radiotherapy for focal skeletal relapse. Patients achieving 2nd CR/VGPR received MoAb.

Responses to induction (Table 2)

CR/VGPR was achieved with 1st-line induction treatment in 127/245 (52%, 95% confidence interval [CI] 45–58%) patients, including 68/118 (58%, 95% CI 48–67%) *MYCN*(+) patients and 59/127 (46%, 95% CI 38–56%) *MYCN*(−) patients (p=0.366). PR rates were also similar between the *MYCN*(+) cohort (11%, 95% CI 6–18%) and the *MYCN*(−) cohort (17%, 95% CI 11–25%) (p=0.2). In contrast, a PD response to induction was limited to *MYCN*(+) disease, occurring in 24/118 (20%, 95% CI 13–29%) *MYCN*(+) patients, compared to 0/127 *MYCN*(−) children (p<0.001).

EFS and OS correlated with *MYCN*

The EFS and OS rates at 3/5/7 years of the entire study cohort of 245 patients were 44/37/35% and 67/57/53%, respectively. The *MYCN*(+) patients (n=118) had significantly inferior EFS and OS compared to *MYCN*(−) patients (n=127) (Table 3; Figure 1). Thus, at 3/5/7 years, the EFS rates were 37/32/32% versus 49/41/38% (p=0.008), and the OS rates were 56/48/46% versus 77/66/60% (p=0.002).

Early treatment failure was significantly more common with *MYCN*(+) disease: PD at <366 days from diagnosis (including PD response to induction) emerged in 50/118 (42%, 95% CI 33–52%) *MYCN*(+) patients, compared to 17/127 (13%, 95% CI 8–21%) *MYCN*(−) patients (p<0.001), and death from PD at <366 days from diagnosis occurred in 26/118 (22%, 95% CI 15–31%) *MYCN*(+) patients versus 6/127 (5%, 95% CI 2–10%) *MYCN*(−) patients (p<0.001) (Table 2). Early death of patients who achieved CR/VGPR with 1st-line induction was rare: this occurred in 1/26 *MYCN*(+) patients consolidated with ASCT+MoAb and 1/40 consolidated with MoAb, and in 0/24 *MYCN*(−) patients consolidated with ASCT+MoAb and 1/34 consolidated with MoAb.

Among the *MYCN*(+) patients, infants (n=31) and children (n=87) had overlapping outcomes (Table 3). Thus, at 3/5/7 years, the EFS rates were 34/34/34% compared to 38/31/31% (p=0.828), and the OS rates were 55/55/55% compared to 56/44/42% (p=0.664).

Correlation of EFS and OS with response to induction

EFS and OS of the patients who achieved CR/VGPR with initial induction therapy (n=127) were significantly better than those of the patients who had ≤PR (n=118) (Table 3; Figure 2). Thus, at 3/5/7 years, the EFS rates were 58/51/50% versus 28/21/19% (p<0.001), and the OS rates were 80/74/70% versus 53/38/35% (p<0.001).

Among the patients who achieved CR/VGPR, EFS and OS of the *MYCN*(+) patients (n=68) and the *MYCN*(−) patients (n=59) were similar (Table 2; Figure 3). Thus, at 3/5/7 years, the EFS rates were 54/48/48% versus 63/54/52% (p=0.346), and the OS rates were 75/68/68% versus 86/80/72% (p=0.542). These results show that the long-term OS rates of *MYCN*(+)

patients and *MYCN*(−) patients in CR/VGPR post-induction were ~20% greater than their respective long-term EFS rates.

Among the patients with a ≤PR to induction, EFS and OS of the *MYCN*(+) patients (n=50) were significantly inferior to those of the *MYCN*(−) patients (n=68) (Table 3; Figure 4). Thus, at 3/5/7 years, the EFS rates were 14/9/9% versus 37/30/26% ($p<0.001$), and the OS rates were 29/18/14% versus 70/53/50% ($p<0.001$). These results show that the long-term EFS and OS of these *MYCN*(+) patients were similar, whereas the long-term EFS and OS of the *MYCN*(−) patients differed by ~20–30%.

DISCUSSION

MYCN(−) HR-NB patients display a broad, continuous spectrum as regards response and survival, whereas *MYCN*(+) patients have either an excellent response to induction associated with good long-term outcome, or early PD with poor outcome. This extreme dichotomy in the clinical scenarios of *MYCN*(+) patients may result from underlying distinctive genetic features. The elucidation of these divergent biological profiles may have far-reaching implications, including improved risk classification at diagnosis and identification of targets for treatment. Unfortunately, there are currently no known biological findings predictive at diagnosis of outcome for *MYCN*(+) patients, as *MYCN*(+) NBs are virtually all associated with certain biomarkers (e.g., chromosome 17q gain and chromosome 1p deletion) and not with others (e.g., chromosome 11q deletion and *ATRX* gene mutations).

The *MYCN*(+) and *MYCN*(−) cohorts received the same induction, consolidative, and salvage treatments (Table 2); this uniformity lent validity to the comparisons of outcome. A key finding was PD response to 1st-line induction with *MYCN*(+) disease, before consolidation could start. As regards widely used post-induction treatments, among patients who achieved CR/VGPR with 1st-line induction, early death was rare, occurring in 2/66 *MYCN*(+) patients consolidated with ASCT+MoAb (n=26) or MoAb (n=40), and in 1/58 *MYCN*(−) patients consolidated with ASCT+MoAb (n=24) or MoAb (n=34). Initial treatment of refractory disease or PD involved chemotherapy with well-recognized anti-NB activity, rather than investigative agents.

MYCN(+) and *MYCN*(−) patients have equivalent CR/VGPR rates with induction ($p=0.366$), and *MYCN* is not prognostic for patients in CR/VGPR at completion of induction. Thus, *MYCN*(+) and *MYCN*(−) patients who achieve CR/VGPR with induction have similar long-term EFS and OS (Table 3; Figure 3). In sharp contrast, PD response to induction is limited to *MYCN*(+) patients ($p<0.001$), and early PD is significantly associated with *MYCN*(+) disease ($p<0.001$). Early treatment failure not only adversely affects EFS but contributes to a dismal OS since PD of HR-NB during, or soon after completion of, induction responds poorly to salvage therapy,[31] and short time from diagnosis to PD is a significant adverse factor for OS.[37–41]Indeed, *MYCN*(+) patients are significantly more likely than *MYCN*(−) patients to die early from PD ($p<0.001$), i.e., <366 days from diagnosis. Overall, among patients with ≤PR to induction, *MYCN*(+) disease is associated with a significantly inferior EFS ($p<0.001$) and OS ($p<0.001$); these results account for the significantly worse long-term

EFS (p=0.008) and OS (p=0.002) of the entire *MYCN*(+) cohort compared to the entire *MYCN*(−) cohort (Table 3; Figure 1). Age had no prognostic impact with *MYCN*(+) disease: infants and children with this chromosomal aberration had similar long-term EFS and OS (Table 3).

The PD findings, combined with the significantly worse EFS/OS of *MYCN*(+) compared to *MYCN*(−) patients in ≤PR post-induction, suggest that a major response to upfront therapy is crucial for a good outcome of *MYCN*(+) patients and less so for *MYCN*(−) HR-NB patients. This point is supported by the absence of continued decline in EFS of *MYCN*(+) patients over the long term, whereas EFS rates of *MYCN*(−) patients decrease steadily beyond 3 years (Table 3; Figure 1). The findings reflect a paucity of late relapses among *MYCN*(+) compared to *MYCN*(−) HR-NB patients.

The paramount prognostic importance of initial response for *MYCN*(+) patients, but not for *MYCN*(−) HR-NB patients, is also illustrated by the relation between EFS and OS rates within subsets of patients. Thus, the *MYCN*(+) patients with ≤PR to induction have long-term EFS and OS rates that approximate each other (Table 3; Figure 4). This similarity between EFS and OS is consistent with a rapid demise of these patients after PD/relapse and is attributable to persistence of underlying chemo-resistance as well as ineffective second-line therapy (see below). In contrast, the *MYCN*(+) patients who achieve CR/VGPR with induction have long-term OS ~20% higher than EFS, evidence of prolonged survival post-relapse attributable to continued chemosensitivity (Table 3; Figure 3). This difference between outcomes of *MYCN*(+) patients in ≤PR versus those in CR/VGPR is not seen with *MYCN*(−) HR-NB: indeed, all *MYCN*(−) HR-NB patients - those in CR/VGPR, as well as those in ≤PR following induction - have long-term OS rates that, like the *MYCN*(+) patients in CR/VGPR post-induction, are ~20% higher than EFS rates (Table 3; Figures 3 and 4).

These large differences between long-term EFS and OS in the 2000–2011 period among all *MYCN*(−) HR-NB patients and the *MYCN*(+) patients in CR/VGPR, though not those in ≤PR post-induction, were not seen in the 1990s (Table 1).[4–11] In that decade, EFS and OS were closely related, which supported the view that PD/relapse of HR-NB was synonymous with a subsequent rapid demise from PD or toxicity of salvage therapy. Exceptions to this scenario led to the initial reports of long-term survival of children with HR-NB despite persistence or relapse of disease.[38,42] This phenomenon of chronic NB was much less common in *MYCN*(+) than *MYCN*(−) patients.[37–42]An underlying biologic factor for chronicity may be mutations of the *ATRX* gene which were recently found to be significantly associated with *MYCN*(−) HR-NB in older patients,[43] in whom NB is often characterized by an indolent and prolonged, though ultimately lethal, course.[44]

Consistent with a low likelihood of prolonged survival after *MYCN*(+) PD/relapse, EFS and OS were poor and virtually identical in the few reports offering details about *MYCN*(+) patients. Thus, in a 1999–2004 multicenter study of *MYCN*(+) infants, EFS/OS rates at only 2 years were 29%/30% for all stages (2-year OS was 20% for stage 4);[19] reviews of a 1990–2002 international experience found *MYCN*(+) stage 4 and 4S patients <18 months old to have 5-year EFS/OS rates of 28%/34%[45] and *MYCN*(+) stage 4 patients >18 months old to

have 5-year EFS/OS rates of <25%;[16] and a 1991–1996 group-wide study of *MYCN*(+) stage 3 (all ages) yielded 5-year EFS/OS rates of 25%/27%.[11]

One reason why the above clinical scenario typical in the 1990s - i.e., death soon after PD/relapse - may no longer hold true is the availability of novel, relatively non-toxic (i.e., second-line) salvage therapies that lack cross-resistance with induction. Examples include chemotherapy regimens (e.g., irinotecan-temozolomide[28]), investigative agents (e.g., fenretinide, ABT-751, crizotinib),[46] and targeted radiotherapy (^{131}I-MIBG,[25] ^{131}I-monoclonal antibodies[36]). Another factor contributing to prolonged survival may be improved disease surveillance. In the 1990s, MIBG scintigraphy often used ^{131}I and was not regularly performed (Table 2);[5,8,10] subsequently, ^{123}I-MIBG became widely adopted and proved to be superior in detecting NB, especially relapse that is asymptomatic (and presumably has lower tumor burden) which may be more amenable to control than bulky metastatic relapse.[47] Unfortunately, the welcome prospect that the recent advances in therapy and surveillance might, after relapse, lead to a chronic course with long-term survival plus good quality of life, or even cure, is not relevant to patients with early PD – a subset of patients significantly associated with *MYCN*(+) NB ($p<0.001$; Table 3).

In conclusion, results from the 1970s through the contemporary era indicate that better upfront, consolidative, and salvage therapies have improved survival of patients with HR-NB.[1–3,12–15,17,23,24] Yet *MYCN*(+) NB differs significantly from *MYCN*(−) HR-NB as regards a) PD response to induction, and b) extreme differences in outcome, i.e., early death from disease or excellent PFS and OS. The two sharply divergent clinical scenarios of *MYCN*(+) patients merit investigation at the molecular/genetic level, analogous to other studies into the underlying biology of NB,[43,46] to identify markers predictive at diagnosis of good response/good outcome versus poor response/early demise, and to expand the availability of targets for therapy.

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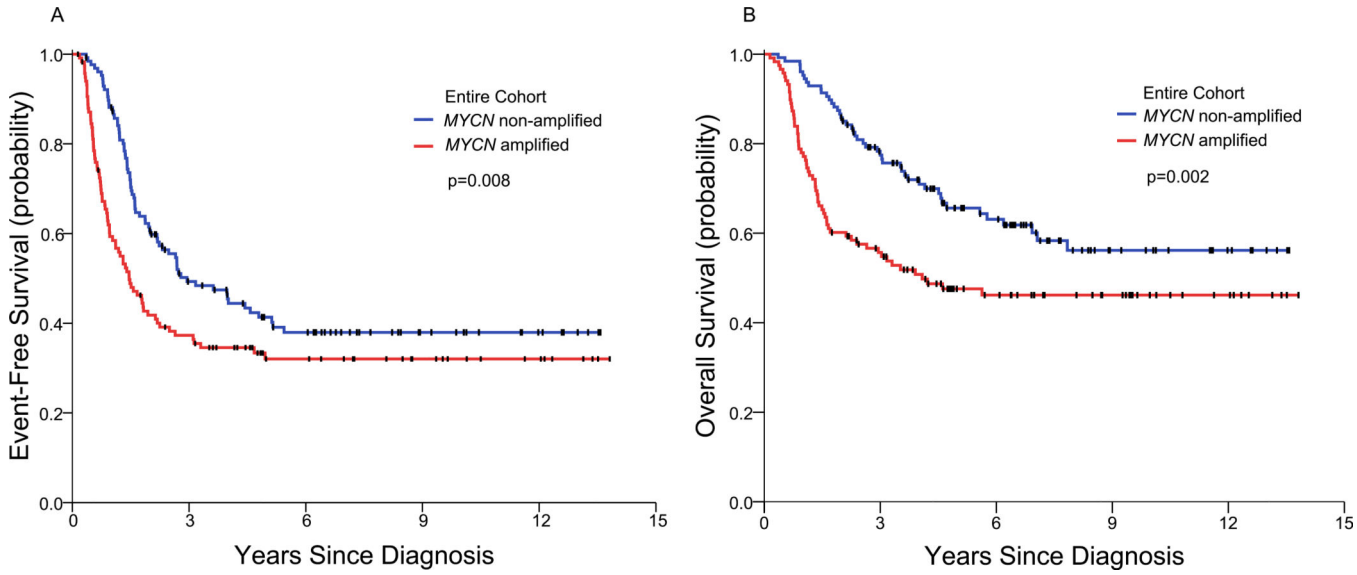

Figure 1.

Event-free survival (A) and overall survival (B) of all *MYCN*(+) patients and all *MYCN*(−) patients.

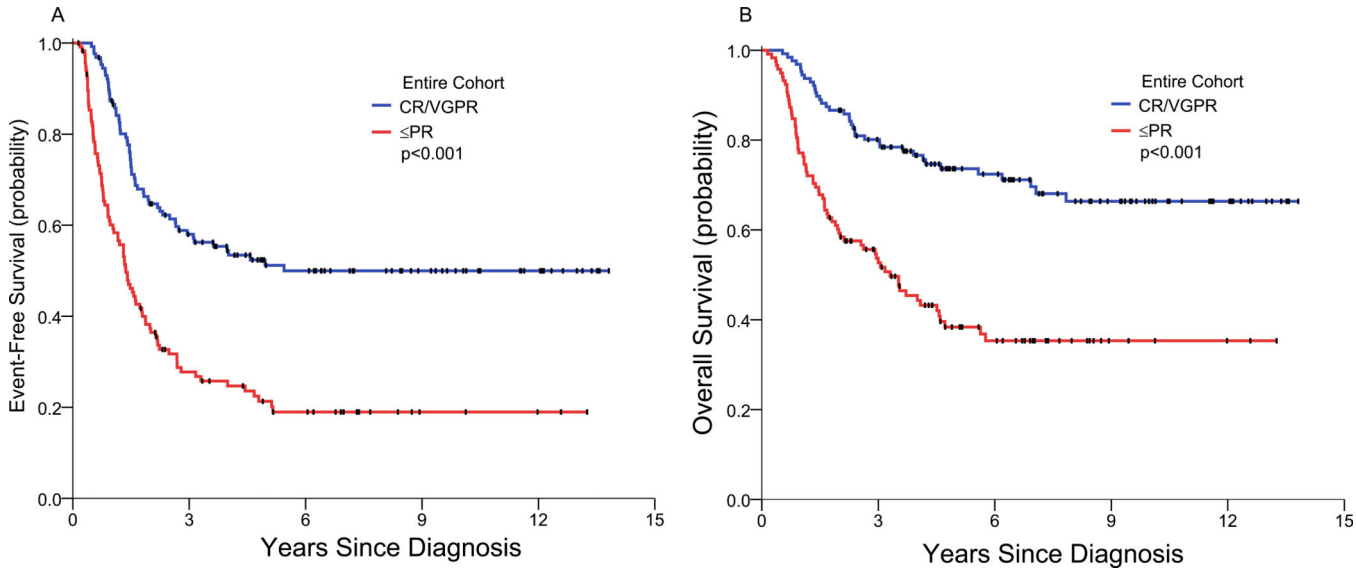

Figure 2.

Event-free survival (A) and overall survival (B) of all patients who achieved complete/very good partial remission compared to all patients who had partial remission or less.

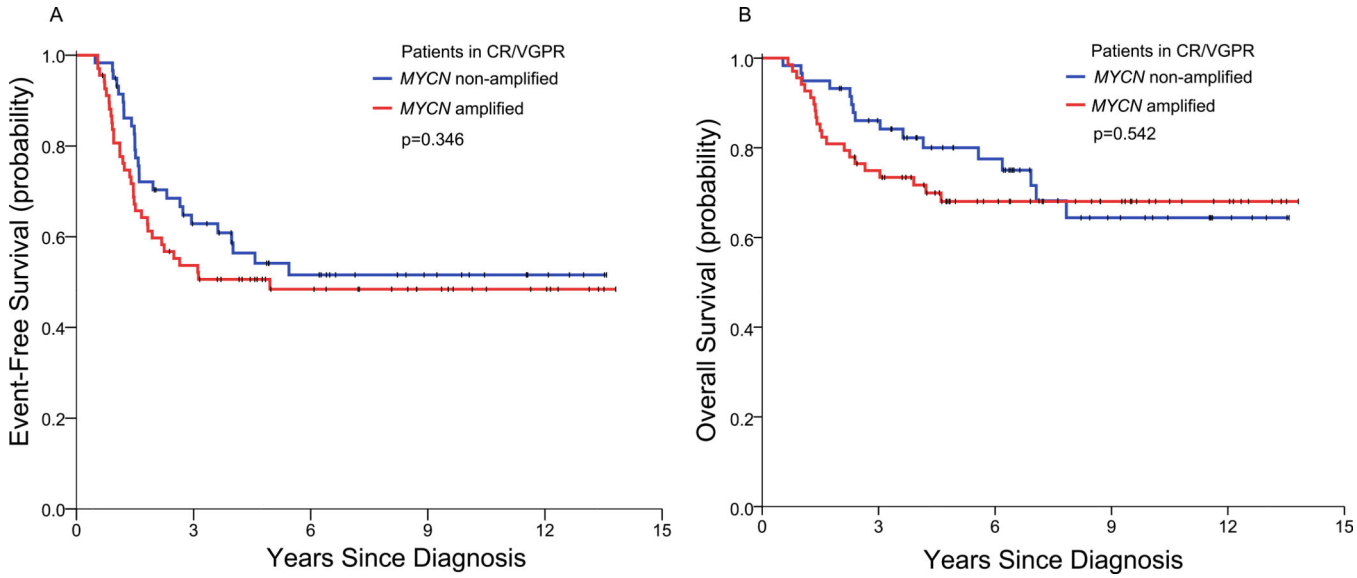

Figure 3.

Event-free survival (A) and overall survival (B) of the *MYCN*(+) patients and the *MYCN*(−) patients who achieved complete/very good partial remission.

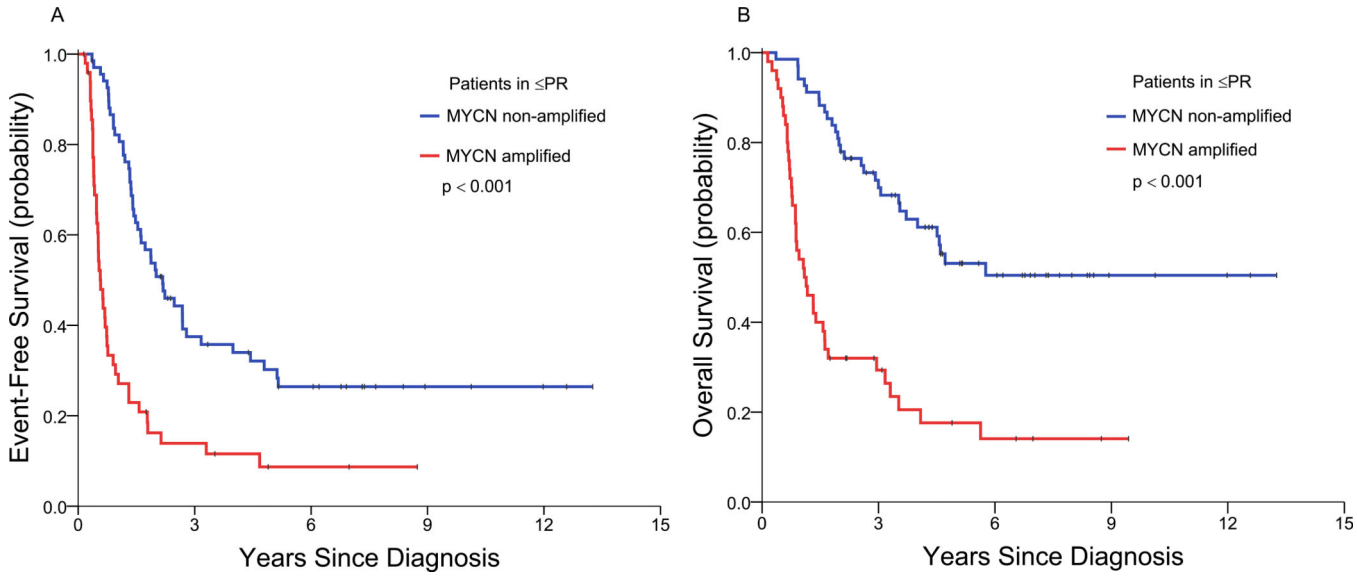

Figure 4.

Event-free survival (A) and overall survival (B) of the *MYCN*(+) patients and the *MYCN*(−) patients who achieved partial remission or less.

Table 1

Studies of high-risk neuroblastoma with long-term follow-up

References (years of study)[a]	No. of pts	Stages and age of study population	Prognostic impact of response/ *MYCN*(+)	Staging studies at diagnosis/ follow-up	Local RT	CRA	Anti-GD2 immuno-therapy	Event-free survival	Overall survival
1A. Studies in the 1990s									
Zage[9] (1993–95)	142	4, >1 yr 2 or 3, *MYCN*(+), >1 yr	…/…	…/post-protocol every 3–6m, x2 yr	yes	no	no	23% (7 yr)	28% (7 yr)
Matthay[10] (1991–96)	539	4, >1 yr 4, <1 yr, *MYCN*(+) 3, *MYCN*(+) 3, UH and/or ferritin ≥143 ng/ml	yes/yes	MIBG optional/ at end of protocol	no	ran-domised	no	26% (5 yr) stage 4: 21% (5 yr) stage 3: 55% (5 yr)	36% (5 yr) 32% (5 yr) 59%(5 yr)[b]
Berthold[7] (1990–97)	335	4, >1 yr	…/…	…/…	no	no	no	27% (5 yr)	33% (5 yr)
DeBernardi[6] (1992–97)	159	4, >1 yr	none/yes	…/…	no	no	no	17% (5 yr)	28% (5 yr)
Kaneko[5] (1991–98)	221	4, >1 yr	…/none	MIBG optional/ …	no	no	no	34% (5 yr)	37% (5 yr)
Castel[4] (1992–98)	83	4, >1 yr 4, <1 yr, *MYCN*(+) 3, *MYCN*(+)	none/yes	…/…	no	no	no	33% (4 yr) …	33% (4 yr) 20% (5 yr)
Pearson[8] (1990–99)	262	4, >1 yr	yes/none	MIBG optional/ “local preference”	no	no	no	28% (3 yr) 24% (5 yr) 23% (10 yr)	… 26% (5 yr) 24%(10yr)
1B. Studies completed in the 2000s									
George[13] (1994–2002)	97	4, >1 yr 3, 4, 4S, <1 yr, *MYCN*(+) 3, >1 yr, UH	…/none	MIBG variable/ …	yes	yes	no	55% (3 yr) 47% (5 yr) 46% (7 yr)	72% (3 yr) 60% (5 yr) 53% (7 yr)

References (years of study)[a]	No. of pts	Stages and age of study population	Prognostic impact of response/ *MYCN*(+)	Staging studies at diagnosis/ follow-up	Local RT	CRA	Anti-GD2 immuno-therapy	Event-free survival	Overall survival
Berthold[12]	295	4, >1 yr	yes/yes	…/…	24 pts	39 pts	160 pts	39% (3 yr)	58% (3 yr)
(1997–2002)		all stages *MYCN*(+)						35% (5 yr)	45% (5 yr)
Canete[19]	35	2, 3, 4, 4S,	yes/…	MIBG if available/	yes	yes	no	29% (2 yr)	30% (2 yr)
(1999–2004)		<1 yr, *MYCN*(+)		…					stage 4: 20% (2 yr)
Sung[14]	52	4, >1 yr	none/none	MIBG included/	35 pts	yes	no	62% (5 yr)	64% (5 yr)
(1997–2005)				…					
Kreissman[15]	495	4, >1 yr	…/…	…/…	yes	yes	78 pts	38% (5 yr)	50% (5 yr)
(2001–2006)		3, 4, 4S, <1 yr, *MYCN*(+)							
		3, >1 yr, UH							

Abbreviations: CRA, 13-*cis*-retinoic acid; *MYCN*(+), *MYCN*-amplified; pts, patients; UH, unfavorable histology; RT, radiotherapy

… Indicates not reported or described.

[a] Listed chronologically by year of study's completion.

[b] 5-yr EFS/OS rates for *MYCN*(+) stage 3 were 25%/27% and for *MYCN*(−) stage 3 were 77%/79%.[11]

Table 2

Patient characteristics

	***MYCN*-amplified (n=118)**	***MYCN*-non-amplified (n=127)**	**Entire cohort (n=245)**
Male:Female (ratio)	61:57 (1.07)	47:53 (0.89)	108:110 (0.98)
Age at diagnosis (years)			
Range	0.12–10.97	1.54–11.82	0.12–11.82
Median	2.00	3.65	3.02
Induction regimens			
A3973/N8	89 (75%)	95 (75%)	184 (75%)
ANBL0532	20 (17%)	19 (15%)	39 (16%)
Other	9 (8%)	13 (10%)	22 (9%)
Responses to induction			
CR/VGPR	68 (58%)	59 (46%)	127 (52%)
PR	13 (11%)	22 (17%)	35 (14%)
MR/NR	13 (11%)	46 (36%)	59 (24%)
PD	24[a] (20%)	0	24 (10%)
Early PD (<366 days)[b]	50 (42%)	17 (13%)	67 (27%)
Early death from PD (<366 days)	26 (22%)	6 (5%)	32 (13%)
Non-neuroblastoma events	4[c] (3%)	6[d] (5%)	10 (4%)
Consolidation of 1st CR/VGPR			
MoAb	40/68 (59%)	34/59 (58%)	74/127 (58%)
ASCT + MoAb	26/68 (38%)	24/59 (41%)	50/127 (39%)
No ASCT or MoAb	2/68 (3%)	1/59 (2%)	3/127 (2%)
Initial treatment for ≤PR			
High-dose chemotherapy[e]	37/48[f] (77%)	52/68 (76%)	89/116 (77%)
ASCT + MoAb	5/48[f] (10%)	5/68 (7%)	10/116 (9%)

ASCT, autologous stem-cell transplantation; MoAb, immunotherapy with anti-G_{D2} monoclonal antibody

[a] Includes one stage 4S patient and three stage 3 patients

[b] Includes patients with PD response to induction and patients who relapsed <366 days from diagnosis.

[c] Two deaths during induction (tumor lysis syndrome, pulmonary failure), one transplant-related toxic death, and one secondary myelodysplastic syndrome (detected at 42 months and successfully treated)

[d] Two transplant-related toxic deaths, two secondary leukemias (diagnosed at 28 and 58 months and successfully treated), one death from hemolytic-uremic syndrome during induction, and one late death in an accident without prior relapse

[e] Previously reported alkylator-based conventional chemotherapy regimens.[31–34]

[f] Not including 2 patients who died of toxicity during 1st-line induction.

Table 3

Event-free survival (EFS) and overall survival (OS)

Subsets of patients	EFS rates (%) 3/5/7 years	p-value	OS rates (%) 3/5/7 years	p-value
All patients (n=245)	44/37/35		67/57/53	
MYCN(+) (n=118) vs. *MYCN*(−) (n=127)	37/32/32 vs. 49/41/38	0.008	56/48/46 vs. 77/66/60	0.002
CR/VGPR (n=127) vs. ≤PR (n=118)	58/51/50 vs. 28/21/19	<0.001	80/74/70 vs. 53/38/35	<0.001
All *MYCN*(+) patients (n=118)				
infants (n=31) vs. children (n=87)	34/34/34 vs. 39/32/32	0.824	55/55/55 vs. 56/45/43	0.671
Patients in CR/VGPR (n=127)				
MYCN(+) (n=68) vs. *MYCN*(−) (n=59)	54/48/48 vs. 63/54/52	0.346	75/68/68 vs. 86/80/72	0.542
Patients in ≤PR (n=118)				
MYCN(+) (n=50) vs. *MYCN*(−) (n=68)	14/9/9 vs. 37/30/26	<0.001	29/18/14 vs. 70/53/50	<0.001