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Comprehensive evaluation of context dependence of the prognostic impact of *MYCN* amplification in neuroblastoma: A report from the International Neuroblastoma Risk Group (INRG) project

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Abstract

Background: *MYCN* amplification (*MYCN*-A) is an established adverse prognostic factor in neuroblastoma. The extent to which the prognostic impact of *MYCN*-A depends on other factors has not been fully characterized.

Patients and methods: Using the International Neuroblastoma Risk Group database, we constructed Cox models of overall survival (OS) to obtain hazard ratios of the effect of *MYCN*-A within subgroups defined by other prognostic factors. Cox models assessed the degree to which the prognostic impact of *MYCN*-A was modulated by each other covariate. We used absolute

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

hazard ratio (HR) differences to construct classification trees to identify subgroups with greatest differential prognostic effect of MYCN-A.

Results: In a cohort of 6223 patients with known *MYCN* status, the OS hazard ratio associated with MYCN-A was 6.3 (95% confidence interval 5.7-7.0, $P < .001$). Age at diagnosis conferred the largest HR absolute difference for MYCN-A between subgroups (HR absolute difference 16.6; HRs for MYCN-A of 19.6 for <18 months, 3.0 for ≥18 months). MYCN-A remained significantly prognostic of OS after controlling for other factors, abrogating their prognostic strength. Patients whose outcome was *most* impacted by *MYCN* status were those who were <18 months, had high mitosis karyohexis index (MKI) and low ferritin.

Conclusion: The prognostic strength of MYCN-A varies depending on which patient subgroup defined by other neuroblastoma risk factors is examined, with greatest strength in patients with otherwise favorable features. MYCN-A has little effect within some subgroups, aiding clinical decision-making if *MYCN* status cannot be assessed. Subgroups where MYCN-A has large effect may be prioritized for agents targeting Myc family proteins.

Keywords

amplification; context; MYCN; neuroblastoma; prognosis

1 | INTRODUCTION

The classification and treatment approach to neuroblastoma, the most common extracranial solid tumor of childhood, is complicated by the clinical heterogeneity of the disease. *MYCN* status, determined at the time of diagnosis, is an important adverse prognostic factor.¹ It has been over three decades since the historic discoveries linking amplification of the oncogene *MYCN* with rapid tumor progression,²⁻⁴ resulting in *MYCN* status as a critical prognostic factor that remains a cornerstone of current risk classification systems.⁵

Previous work from our group identified associations between other known features of neuroblastoma and differential rates of *MYCN* amplification (MYCN-A). MYCN-A demonstrates complex and differential associations with many other prognostic factors.^{6,7} Few studies have examined the prognostic context of these associations in specific subpopulations of neuroblastoma, demonstrating the presence of MYCN-A appears to have a greater adverse prognostic impact in patients with otherwise favorable features (eg, younger age and lower stage).⁸⁻¹¹ In contrast, in older patients with higher stage disease, the prognostic impact of MYCN-A has been more modest or even undetectable.^{5,12} For example, one study found that *MYCN* status did not significantly impact overall survival (OS) in older patients with stage 4 disease.¹³ Additionally, two recent studies did not demonstrate a prognostic effect of MYCN-A on patients with high-risk disease.^{14,15}

A comprehensive investigation into the context dependence of *MYCN* status is needed to provide clinicians a more nuanced understanding of its prognostic impact in the setting of other clinical, biological, and treatment factors. Further, as treatment strategies have evolved, it is unclear whether the prognostic impact of *MYCN* status has evolved as well. In this study, we utilized the International Neuroblastoma Risk Group (INRG) database to perform

a comprehensive evaluation of the variation of the prognostic impact of MYCN-A. This analysis, while primarily serving to provide improved understanding of MYCN status as a prognostic factor in neuroblastoma, may also aid our understanding of the important interactions between MYCN status and other clinical, biological, and treatment factors. Discerning differences in the degree to which MYCN-A adversely impacts prognosis may enable providers to more accurately weigh MYCN status when assessing an individual patient's risk of treatment failure.

2 | METHODS

2.1 | Patients

Patients diagnosed with neuroblastoma between 1990 and 2016 were selected from the INRG database and were eligible for the analysis if they had known outcome and MYCN status (coded as amplified vs nonamplified). There were no other inclusion or exclusion criteria for this analysis.

2.2 | Covariates

MYCN status was the primary predictor variable of interest for this analysis. MYCN status was dichotomized as amplified (MYCN-A) versus nonamplified (MYCN-NA). MYCN status was determined according to local standards as previously described.⁶

Clinical factors of interest, at the time of diagnosis, included sex, age, International Neuroblastoma Staging System (INSS) stage (dichotomized as stage 4 vs all other stages including 4S),¹⁶ primary site, presence of bone marrow metastases, presence of bone metastases, lactate dehydrogenase (LDH) level (dichotomized using updated cut point as previously¹⁷), ferritin level (dichotomized using updated cut point as previously¹⁷), and year of diagnosis (dichotomized around the year 1999 when the addition of high-dose therapy with autologous stem cell rescue became routine). Biological covariates of interest included ploidy (hyperdiploidy = any DNA index >1.0), 1p loss of heterozygosity (LOH), 11q aberration (unbalanced LOH),¹⁸ presence of any segmental chromosomal aberration (SCA) (either 1p LOH and/or 11q LOH), International Neuroblastoma Pathology Classification histologic classification,¹⁹ diagnostic category (neuroblastoma and nodular ganglioneuroblastoma vs all others), MKI, and grade of differentiation.

OS was the sole clinical outcome of interest. OS time was calculated from the time from diagnosis to death, with surviving patients censored at time of last follow-up.

2.3 | Statistical analyses

The INRG cohort of 14501 patients who met eligibility for this analysis was randomly and equally divided into a Test cohort and a Validation cohort. Except where noted, analyses were performed first in the Test cohort and then confirmed in the Validation cohort.

Point estimates of OS were calculated using the methods of Kaplan and Meier,²⁰ with standard errors according to the methods of Greenwood.²¹

We used absolute hazard ratio differences to construct classification trees to identify subgroups with the strongest and weakest prognostic effect of MYCN-A.

Starting from the overall Test cohort, we used a series of univariate Cox models of OS to calculate the HR for *MYCN* within each of the subgroups for each factor. For example, the HR for *MYCN* was calculated within patients <18 months of age at diagnosis, and also within ≥18 months. Then we calculated the absolute difference in the HR (HRdiff) by subtracting the HR of the group with more favorable outcome from the HR of the group with less favorable outcome, for example, $HRdiff = (MYCNHR \text{ for age } \geq 18 \text{ months}) - (MYCNHR \text{ for age } < 18 \text{ months})$. The factor with the largest HRdiff was selected to form the first split/branch of the classification tree. This procedure was repeated within each branch until the sample size became too small (subgroup <5), $HRdiff < 1$, or until there were no further subgroups in which *MYCN* was significant. Data from the Validation cohort were then placed into categories according to the tree created from the Test cohort to determine if there was evidence to support the split/branch decisions made based on the Test cohort.

In addition, we used bivariate Cox models to assess the extent to which each covariate impacted the HR for death associated with MYCN-A. We also used Cox models that included an interaction term of [*MYCN* status] × [covariate] to formally assess for modification of the prognostic impact of MYCN-A by each covariate.

3 | RESULTS

3.1 | Patient characteristics

In the Test cohort ($n = 7251$), MYCN-A was observed in 928 cases (12.8%), MYCN-NA in 5295 cases (73.0%), and *MYCN* status was unknown in 1028 cases (14.2%). Cases with unknown *MYCN* status were excluded from further analysis. Details of the clinical and biological features for the Test and Validation cohorts are shown in Tables 1 and S1, respectively. Features of patients universally considered high risk (patients with metastatic disease diagnosed ≥18 months of age) for the Test and Validation cohorts are shown in Table S2.

3.2 | Prognostic impact of MYCN amplification varies by context

In the overall Test cohort, the hazard ratio for death for patients with MYCN-A was 6.3 (95% confidence interval 5.7-7.0, $P < .001$) compared to the reference group of MYCN-NA. MYCN-A remained prognostic within each clinical or biological subgroup, though the prognostic impact was not uniform across subgroups (Table 2). MYCN-A generally had the strongest adverse prognostic impact in otherwise more favorable subgroups of patients (eg, younger age or lower stage), while the prognostic impact was generally weaker among unfavorable subgroups of patients (eg, older age or INSS stage 4).

Age at diagnosis was the factor with the largest HRdiff (16.6; 19.6 [<18 months] vs 3.0 [≥ 18 months]) (Table 2). Thoracic primary site was the factor with the smallest HRdiff (0.5; 6.1 [thoracic] vs 5.6 [other sites]). These analyses were repeated in the Validation cohort and similar patterns were observed (Table S3).

We repeated this analysis focused exclusively on the subset of patients in the Test cohort with high-risk disease (Table 3). In this subgroup, MYCN-A was again significantly prognostic in each clinical or biological subgroup, though HRs were somewhat attenuated compared to the overall Test cohort. The HRdiffs were likewise attenuated compared to analyses in the overall Test cohort. These analyses were repeated in the Validation cohort and similar patterns were observed (Table S4).

Within patients with high-risk disease, we assessed the influence of treatment on the prognostic impact of MYCN-A. We observed a differential prognostic impact of MYCN-A in patients treated with (HR = 1.5 for MYCN-A vs MYCN-NA) and without (HR = 4.1 for MYCN-A vs MYCN-NA) high-dose chemotherapy (HRdiff = 2.6). Too few patients had received GD2-directed therapy for meaningful analysis ($n = 50$).

3.3 | Effect modification of the prognostic impact of MYCN-A by covariates

Compared to the univariate HR for MYCN-A of 6.3, HRs for MYCN-A were lower after controlling separately for each other factor, except in the case of 11q, controlling for which increased the MYCN-A hazard ratio to 7.3 (Table 4). The covariates with the greatest attenuation of the prognostic effect of MYCN-A included LDH (MYCN-A HR = 3.1 after controlling for LDH), stage (HR = 3.2), and SCA (HR = 3.3). Similar results were seen in the Validation cohort (Table S5). Among patients with high-risk disease in the Test cohort, the HR for *MYCN* was already attenuated (univariate hazard ratio of 1.6) and did not vary appreciably after controlling for each other factor (Table S6).

In Cox models of interaction of *MYCN* with a covariate, each other covariate had a significant interaction with *MYCN* status, with the exception of thoracic primary site (Table 5). The effects of these interactions appeared to be bidirectional, with differential prognostic impact of *MYCN* status depending on the covariate subgroup (as also seen in Table 2) and differential prognostic impact of the covariate depending on *MYCN* status. HRs for the covariates were generally attenuated in the context of MYCN-A, with some prognostic factors losing statistical significance altogether. A similar pattern was observed in the Validation cohort (Table S7).

3.4 | Classification trees define subgroups with differential prognostic effect of MYCN-A

In a classification tree based on HRdiff (Figure 1), age had the largest differential impact on OS given *MYCN* status (HRdiff = 16.6), and age subgroups formed the first branch. Within patients <18 months, the second branch was MKI (HRdiff = 61.4). Within patients ≥18 months, the second branch was grade (HRdiff = 8.6). Third and fourth branch points are further depicted in Figure 1 and revealed that patients with all of the following features had outcomes most impacted by *MYCN* status: <18 months, high MKI, and low ferritin (Figure S1A). In contrast, patients with all of the following features had outcomes least impacted by *MYCN* status: ≥18 months, undifferentiated grade, stage 4, high ferritin (Figure S1B). Application of data from patients from the Validation cohort to these same subgroups yielded similar findings (Figure S2).

4 | DISCUSSION

In this comprehensive analysis of the prognostic impact of MYCN-A, we provide a refined understanding of *MYCN* status as a prognostic factor in neuroblastoma. While MYCN-A remained a significant prognostic factor after controlling for other clinical and biological factors, we found the prognostic value of MYCN-A to be highly context-dependent, with specific clinical and biological factors serving to augment or diminish its prognostic impact. We were able to identify groups with survival most and least impacted by *MYCN* status. This work complements prior work assessing the interaction between other prognostic factors and the incidence of MYCN-A. While these previous studies show MYCN-A has differential associations with other prognostic factors,^{5–12} the current analysis provides a deeper understanding of the prognostic value of MYCN-A that may guide clinicians as they assess an individual patient's prognosis.

The feature demonstrating the greatest absolute difference in OS relative to MYCN-A was age. The difference in the *MYCN*HRs for OS for patients <18 months compared to ≥18 months was 16.6, with a strong negative adverse prognostic effect of MYCN-A in young patients. This finding is supported by previous work demonstrating patients <12 months with MYCN-A have a 2-year OS of ~30%.²² Initial studies into this age-dependent MYCN-A effect, in which age was dichotomized at 12 months, hypothesized a transient differentiation period for neuroblasts.¹² More recent data demonstrate a nonlinear relationship, as MYCN-A incidence peaks between 15 and 20 months of age.⁶ The available data from this and previous studies highlight the complex interplay between age, overall prognosis, prognostic impact of MYCN-A, and incidence of MYCN-A all taking place in this transition period between infancy and toddlerhood.

Our classification tree examined the impact of *MYCN* status in subgroups, identifying specific subgroups for which MYCN-A is most impactful. As MYCN-A tumors are inherently higher risk tumors, most patients with MYCN-A are subject to the intense and prolonged standard of care treatment for high-risk neuroblastoma. For a subset of patients with otherwise lower risk features, our study yields evidence of the disproportionately large negative prognostic impact of MYCN-A in this setting, reinforcing the need for *MYCN* testing in nearly all neuroblastoma cases. Furthermore, of those patients who relapse, MYCN-A has been identified as an independent risk factor for worse postrelapse survival,²³ underscoring the need to abrogate the effects of MYCN-A upfront. As *MYCN*-directed therapies enter clinical trials, our novel description of the relative impact of MYCN-A in specific subgroups may nominate ideal patient candidates for these trials.

All of the HRs for *MYCN* were attenuated in the high-risk population compared with the overall cohort, yet *MYCN* still remained prognostic (Table 3). These findings demonstrate that MYCN-A can still have a negative prognostic impact even among patients already deemed to be high risk. Among patients with high-risk disease, the prognostic impact of *MYCN* status varied depending on whether patients received high-dose chemotherapy with stem cell rescue, with a greater negative impact of MYCN-A for patients who did not receive this therapy. While we are unable to fully explain this result, we postulate two potential explanations. First, it is possible that the group of patients who did not undergo

transplant may have had otherwise more favorable features, consistent with our findings that *MYCN* has an outsized prognostic impact in more favorable groups. Second, it is possible that the diminished impact of *MYCN*-A in patients treated with high-dose chemotherapy may reflect a role for increased chemotherapy dose intensity in overcoming the adverse prognostic impact of *MYCN*-A.

Moving forward, as newer agents intended to target Myc family proteins are developed, our results suggest potential subgroups of particular interest for these approaches. Examples of potential strategies to target *MYCN* and downstream dependencies include bromodomain inhibition, dual HDAC/PI3K inhibition, MDM2 inhibition, and aurora A kinase inhibition.^{24–28} Whether patients with *MYCN*-A as their sole unfavorable prognostic factor will be more likely to benefit from these strategies will require further study.

Although this study was undertaken using the largest cohort available, the strength of our cohort size may be mitigated by different INRG member groups using divergent, though validated, techniques to identify *MYCN* status and also different treatment strategies.^{10,29,30} Given the size of the cohort, we were not able to centrally confirm *MYCN* status. We note as well that *MYCN* status was not missing at random, with patients missing *MYCN* status more likely to have unfavorable features and to be diagnosed prior to 1999 (Table 1). In completing our classification tree, given the novelty of this method, we made an a priori (but arbitrary) decision to not proceed with a branch if subgroup sample size was <5 subjects. As such, a larger sample size may have been able to provide evidence for additional splits. Our analysis does not account for different treatment approaches, including observation for some patients. However, our subanalysis focused on patients with high-risk disease mitigates this limitation as nearly all such patients would have been treated with intensive therapy.

In summary, this study represents a novel approach in demonstrating the context dependence of the prognostic impact of *MYCN*-A. Though it remains significant in all settings, we demonstrate the degree of impact is not uniform, but greatly dependent on the context of clinical and biological features. The differences in the degree to which *MYCN*-A adversely impacts prognosis can enable providers to more accurately weigh *MYCN* status when assessing an individual patient's risk of treatment failure. Moreover, we have identified subgroups for which *MYCN* status has little impact, which may have implications in settings in which *MYCN* status cannot be assessed. Finally, as new agents targeting Myc family proteins mature, our results indicate specific subgroups of patients in which it would be most crucial to abrogate the effects of *MYCN*-A, thus identifying the best patient candidates for this class of new therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

HR	hazard ratio
INRG	International Neuroblastoma Risk Group
INSS	International Neuroblastoma Staging System
LDH	lactate dehydrogenase
LOH	loss of heterozygosity
MKI	mitosis karrhyohexis index
MYCN-A	<i>MYCN</i> amplification
OS	overall survival
SCA	segmental chromosomal aberration

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FIGURE 1.

Classification tree of the Test cohort that defines subgroups with greatest differential effect of *MYCN* status on hazard for death. Each node has a minimum *MYCN*-A or *MYCN*-NA subgroup sample size = 5, *MYCN* statistically significant for overall survival, and an absolute HR difference > 1

TABLE 1

Characteristics of the Test cohort according to *MYCN* status

Variable	All patients n (%) (n = 7251)	MYCN-A n (%) (n = 928)	MYCN-NA n (%) (n = 5295)	MYCN unknown ^a n (%) (n = 1028)
Male	2535 (53)	371 (56)	1895 (52)	269 (56)
Female	2241 (47)	296 (44)	1732 (48)	213 (44)
(Unknown)	2475	261	1668	546
Age ≥ 18 months	3290 (45)	587 (63)	2109 (40)	594 (58)
Age < 18 months	3961 (55)	341 (37)	3186 (60)	434 (42)
INSS stage 4	2567 (37)	667 (73)	1401 (27)	499 (53)
All other stages	4455 (63)	245 (27)	3765 (73)	445 (47)
(Unknown)	229	16	129	84
Adrenal primary	2777 (43)	460 (57)	1927 (40)	390 (48)
Other primary sites	3640 (57)	343 (43)	2868 (60)	429 (52)
(Unknown)	834	125	500	209
Thoracic primary	1233 (19)	39 (5)	1041 (22)	153 (18)
Other primary sites	5198 (81)	765 (95)	3755 (78)	678 (82)
(Unknown)	820	124	499	197
Bone metastasis	1024 (29)	247 (49)	524 (22)	253 (40)
No bone metastasis	2547 (71)	257 (51)	1912 (78)	378 (60)
(Unknown)	3680	424	2859	397
Bone marrow metastasis	1347 (38)	315 (62)	728 (30)	304 (48)
No bone marrow metastasis	2229 (62)	190 (38)	1706 (70)	333 (52)
(Unknown)	3675	423	2861	391
High LDH ≥ 1400 U/L	588 (16)	278 (66)	215 (8)	95 (15)
Low LDH < 1400 U/L	3024 (84)	146 (34)	2351 (92)	527 (85)
(Unknown)	3639	504	2729	406

Variable	All patients n (%) (n = 7251)	MYCN-A n (%) (n = 928)	MYCN-NA n (%) (n = 5295)	MYCN unknown ^a n (%) (n = 1028)
High ferritin ≥ 30 ng/mL	2055 (58)	298 (78)	1433 (55)	324 (54)
Low ferritin <30 ng/mL	1507 (42)	85 (22)	1150 (45)	272 (46)
(Unknown)	3689	545	2712	432
Diploid	1350 (31)	300 (54)	1001 (28)	49 (39)
Hyperdiploid	2937 (69)	253 (46)	2608 (72)	76 (61)
(Unknown)	2964	375	1686	903
LOH/aberration at 1p	373 (19)	174 (73)	183 (11)	16 (28)
No 1p LOH/aberration	1596 (81)	66 (27)	1488 (89)	42 (72)
(Unknown)	5282	688	3624	970
Aberration at 11q	217 (15)	16 (10)	199 (16)	2 (12)
No 11q Aberration	1184 (85)	138 (90)	1032 (84)	14 (88)
(Unknown)	5850	774	4064	1012
Any SCA	539 (36)	176 (83)	346 (27)	17 (68)
No SCA	963 (64)	36 (17)	919 (73)	8 (32)
(Unknown)	5749	716	4030	1003
Unfavorable histology	1630 (36)	477 (89)	1018 (28)	135 (45)
Favorable histology	2882 (64)	61 (11)	2655 (72)	166 (55)
(Unknown)	2739	390	1622	727
High MKI	491 (15)	261 (59)	185 (7)	45 (19)
Low/intermediate MKI	2874 (85)	181 (41)	2506 (93)	187 (81)
(Unknown)	3886	486	2604	796
Undiff/poorly differentiated	3035 (85)	477 (98)	2316 (82)	242 (89)
Differentiating	536 (15)	12 (2)	493 (18)	31 (11)
(Unknown)	3680	439	2486	755
Neuroblastoma and GNB, nodular	3955 (91)	521 (99)	3029 (90)	405 (89)
Ganglioneuroblastoma; intermixed, maturing subtype, well differentiated	397 (9)	6 (1)	342 (10)	49 (11)

Variable	All patients <i>n</i> (%) (<i>n</i> = 7251)	MYCN-A <i>n</i> (%) (<i>n</i> = 928)	MYCN-NA <i>n</i> (%) (<i>n</i> = 5295)	MYCN unknown ^a <i>n</i> (%) (<i>n</i> = 1028)
(Unknown)	2899	401	1924	574
Diagnosed <1999	3224 (44)	415 (45)	2107 (40)	702 (68)
Diagnosed 1999	4027 (56)	513 (55)	3188 (60)	326 (32)

Abbreviations: SCA, segmental chromosomal aberration of either 1p and/or 11q; Undiff, undifferentiated.

^aTwo-sided chi-squared test identified a significantly higher proportion of patients with “MYCN unknown” than “MYCN known” ($P < .05$) for: age 18 months; INSS stage 4; presence of bone metastasis; presence of bone marrow metastasis; presence of any SCA; unfavorable histology; and diagnosed <1999.

TABLE 2

Differential effects of *MYCN* amplification on overall survival (OS) according to other clinical and biological variables for Test cohort

Variable	5-year OS for MYCN-A (%)	5-year OS for MYCN-NA (%)	OS hazard ratio (95% CI)	P-value	Hazard ratio absolute difference α
Overall	37 $\pm$ 1.7	85 $\pm$ 0.5	6.3 (5.7, 7.0)	<0.0001	NA
Sex					
Male	37 $\pm$ 3	84 $\pm$ 1	6.0 (5.0, 7.2)	<0.0001	1.3
Female	37 $\pm$ 3	87 $\pm$ 1	7.3 (6.0, 8.9)	<0.0001	
Age					
18 months	34 $\pm$ 2	67 $\pm$ 1	3.0 (2.7, 3.4)	<0.0001	16.6
<18 months	41 $\pm$ 3	96 $\pm$ 0.4	19.6 (15.6, 24.5)	<0.0001	
INSS stage					
Stage 4	30 $\pm$ 2	58 $\pm$ 1	2.5 (2.2, 2.8)	<0.0001	9.9
All other stages	56 $\pm$ 3	96 $\pm$ 0.4	12.4 (9.7, 15.8)	<0.0001	
Primary site adrenal					
Adrenal	36 $\pm$ 2	80 $\pm$ 1	5.0 (4.3, 5.8)	<0.0001	2.0
Other sites	44 $\pm$ 3	89 $\pm$ 1	7.0 (5.9, 8.4)	<0.0001	
Primary site thoracic					
Thoracic	61 $\pm$ 8	92 $\pm$ 1	6.1 (3.5, 10.5)	<0.0001	0.5
Other sites	38 $\pm$ 2	83 $\pm$ 1	5.6 (4.9, 6.3)	<0.0001	
Bone metastasis					
Present	28 $\pm$ 3	49 $\pm$ 2	2.1 (1.7, 2.5)	<0.0001	4.8
Absent	44 $\pm$ 3	89 $\pm$ 1	6.9 (5.6, 8.5)	<0.0001	
Bone marrow metastasis					
Present	29 $\pm$ 3	53 $\pm$ 2	2.3 (1.9, 2.7)	<0.0001	5.9
Absent	49 $\pm$ 4	92 $\pm$ 1	8.2 (6.3, 10.7)	<0.0001	
LDH					
1400 U/L	29 $\pm$ 3	52 $\pm$ 4	1.9 (1.5, 2.4)	<0.0001	3.6
<1400 U/L	47 $\pm$ 4	86 $\pm$ 1	5.5 (4.3, 7.1)	<0.0001	
Ferritin					
30 ng/mL	34 $\pm$ 3	78 $\pm$ 1	4.7 (3.9, 5.6)	<0.0001	6.6
<30 ng/mL	43 $\pm$ 6	92 $\pm$ 1	11.3 (7.9, 16.2)	<0.0001	
Ploidy					
Diploid	38 $\pm$ 3	77 $\pm$ 2	4.2 (3.5, 5.2)	<0.0001	4.3
Hyperdiploid	42 $\pm$ 3	90 $\pm$ 1	8.5 (6.9, 10.4)	<0.0001	
Ip aberration					
Present	39 $\pm$ 4	71 $\pm$ 4	3.0 (2.1, 4.2)	<0.0001	3.6

Variable	5-year OS for MYCN-A (%)	5-year OS for MYCN-NA (%)	OS hazard ratio (95% CI)	P-value	Hazard ratio absolute difference ^a
	44 ± 7	87 ± 1	6.6 (4.5, 9.5)	<0.0001	
11q aberration					
Absent	43 ± 16	63 ± 4	2.3 (1.1, 4.9)	0.03	8.0
Present	41 ± 5	91 ± 1	10.3 (7.4, 14.2)	<0.0001	
SCA					
Any	39 ± 4	67 ± 3	2.7 (2.1, 3.6)	<0.0001	8.0
No	47 ± 10	92 ± 1	10.7 (6.1, 18.7)	<0.0001	
INPC histology					
Unfavorable	38 ± 2	66 ± 2	2.6 (2.2, 3.0)	<0.0001	13.2
Favorable	62 ± 6	97 ± 0.4	15.8 (9.9, 25.3)	<0.0001	
MKI					
High	38 ± 3	75 ± 3	3.7 (2.6, 5.1)	<0.0001	3.4
Low / Intermediate	43 ± 4	88 ± 1	7.1 (5.6, 9.0)	<0.0001	
Grade of differentiation					
Undiff./poorly differentiated	39 ± 2	85 ± 1	5.9 (5.0, 6.9)	<0.0001	9.5
Differentiating	48 ± 15	95 ± 1	15.4 (6.2, 38.5)	<0.0001	
Diagnostic category					
Neuroblastoma and GNBL, nodular	39 ± 2	86 ± 1	6.4 (5.5, 7.4)	<0.0001	2.6
Other ^b	83 ± 15	97 ± 1	9.0 (1.1, 75.3)	0.0417	
Year of diagnosis					
<1999	30 ± 2	82 ± 1	6.0 (5.1, 6.9)	<0.0001	0.5
1999	43 ± 2	87 ± 1	6.5 (5.8, 7.3)	<0.0001	

Note. Hazard ratios reflect outcome for patients with *MYCN* amplified (MYCN-A) tumors relative to patients with *MYCN* non-amplified (MYCN-NA) tumors (reference level).

Abbreviations: CI, confidence interval; GNBL, ganglioneuroblastoma; INPC, International Neuroblastoma Pathology Classification; SCA, segmental chromosome aberration; Undiff., undifferentiated.

^aThe reference level for calculation of the hazard ratios is *MYCN* not amplified. The HR absolute difference (HRdiff) is calculated by subtracting the HR for the prognostic factor subgroup that has the better outcome from the subgroup with the worse outcome.

^bGanglioneuroblastoma; intermixed, maturing subtype, well differentiated.

TABLE 3

Differential effects of *MYCN* amplification on overall survival (OS) according to other clinical, biological, and treatment variables for high-risk patients in the **Test cohort**

Variable	MYCN-A n	MYCN-NA n	5-year OS for MYCN-A (%)	5-year OS for MYCN-NA (%)	OS hazard ratio (95% CI)	P-value	Hazard ratio absolute difference ^a
Overall	443	880	29 ± 2.3	40 ± 1.8	1.6 (1.4, 1.8)	<0.0001	NA
Sex							
Male	189	375	33 ± 3.6	44 ± 2.7	1.6 (1.3, 2.0)	<0.0001	0.2
Female	150	254	28 ± 3.9	44 ± 3.3	1.8 (1.4, 2.3)	<0.0001	
Age							
<18 months	N/A	N/A	N/A	N/A	N/A	N/A	N/A
18 months	443	880	29 ± 2.3	40 ± 1.8	1.6 (1.4, 1.9)	<0.0001	
INSS stage							
Stage 4	443	880	29 ± 2.3	40 ± 1.8	1.6 (1.4, 1.9)	<0.0001	N/A
All other stages	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Primary site adrenal							
Adrenal	227	382	27 ± 3.1	35 ± 2.6	1.5 (1.2, 1.8)	<0.0001	0
Other sites	151	359	34 ± 4.1	44 ± 2.8	1.5 (1.1, 1.9)	0.0024	
Primary site thoracic							
Thoracic	18	77	41 ± 13.1	43 ± 6.0	1.1 (0.5, 2.2)	0.8228	0.4
Other sites	360	665	30 ± 2.5	39 ± 2.1	1.5 (1.3, 1.8)	<0.0001	
Bone metastasis							
Present	158	366	24 ± 3.5	33 ± 2.6	1.6 (1.3, 2.0)	<0.0001	0
Absent	98	174	31 ± 4.8	42 ± 3.9	1.6 (1.2, 2.2)	0.0024	
Bone marrow metastasis							
Present	209	437	24 ± 3.0	32 ± 2.3	1.6 (1.3, 1.9)	<0.0001	0.1
Absent	49	107	35 ± 7.0	54 ± 5.0	1.7 (1.1, 2.6)	0.023	
LDH							
1400 U/L	137	88	21 ± 3.6	23 ± 4.9	1.3 (0.9, 1.7)	0.13	0.6
<1400 U/L	48	303	19 ± 6.3	36 ± 3.0	1.9 (1.3, 2.7)	0.0009	
Ferritin							
30 ng/mL	135	313	18 ± 3.4	33 ± 2.8	1.8 (1.5, 2.3)	<0.0001	1.1
<30 ng/mL	24	62	21 ± 9.5	45 ± 7.0	2.9 (1.6, 5.4)	0.0006	
Ploidy							
Diploid	148	230	31 ± 4.0	39 ± 3.5	1.6 (1.3, 2.1)	0.0003	0.2

Variable	MYCN-A n	MYCN-NA n	5-year OS for MYCN-A (%)	5-year OS for MYCN-NA (%)	OS hazard ratio (95% CI)	P-value	Hazard ratio absolute difference ^a
Hyperdiploid	119	286	32 ± 4.5	40 ± 3.1	1.4 (1.1, 1.8)	0.0137	
1p aberration							
Present	86	57	28 ± 5.1	36 ± 7.2	1.6 (1.1, 2.5)	0.0252	0
Absent	30	185	26 ± 9.3	35 ± 3.9	1.6 (0.99, 2.6)	0.057	
11q aberration							
Present	10	87	23 ± 18.5	34 ± 6.2	1.6 (0.7, 3.8)	0.2640	0.1
Absent	75	90	34 ± 6.0	43 ± 5.8	1.5 (1.0, 2.3)	0.0425	
SCA							
Any	87	124	28 ± 5.1	33 ± 5.0	1.6 (1.1, 2.2)	0.0097	0.1
No	18	73	36 ± 14.4	47 ± 6.5	1.7 (0.8, 3.5)	0.1823	
Histology							
Unfavorable	254	476	34 ± 3.2	44 ± 2.5	1.5 (1.3, 1.9)	<0.0001	2.9
Favorable	4	33	25 ± 21.7	64 ± 9.1	4.4 (1.2, 16.4)	0.0288	
MKI							
High	108	66	36 ± 4.9	50 ± 6.4	1.6 (1.1, 2.5)	0.0219	0.1
Low/intermediate	102	308	32 ± 4.9	41 ± 3.1	1.5 (1.1, 2.0)	0.0038	
Grade of differentiation							
Undiff./poorly differentiated	236	387	34 ± 3.3	40 ± 2.7	1.4 (1.1, 1.7)	0.0042	0.1
Differentiating	2	32	50 ± 35.4	63 ± 9.3	1.3 (0.2, 10.5)	0.077	
High-dose chemotherapy							
Yes	192	458	30 ± 3	36 ± 2	1.5 (1.2, 1.8)	0.0003	2.6
No	36	94	13 ± 6	57 ± 5	4.1 (2.6, 6.6)	<0.0001	
Year of diagnosis							
<1999	258	464	35 ± 3	44 ± 3	1.5 (1.2, 1.9)	<0.0001	0.3
1999	185	416	21 ± 3	37 ± 2	1.8 (1.5, 2.2)	<0.0001	

Note. Hazard ratios reflect outcome for patients with MYCN-amplified tumors (MYCN-A) relative to patients with MYCN non-amplified (MYCN-NA) tumors (reference level).

Abbreviations: CI, confidence interval; SCA, segmental chromosome aberration; Undiff. = undifferentiated.

^aThe reference level for calculation of the hazard ratios is MYCN not amplified. The HR absolute difference (HRdiff) is calculated by subtracting the HR for the prognostic factor subgroup that has the better outcome from the subgroup with the worse outcome.

TABLE 4

Univariate and bivariate Cox models of overall survival for patients in the Test cohort

Univariate model	n	P-value	HR (95% CI on HR)
MYCN: MYCN-A vs MYCN-NA	6223	<0.0001	6.3 (5.7, 7.0)
Bivariate models: MYCN + additional covariate			
	n	P-value	HR (95% CI on HR)
Age (18 months vs <18 months)	6223	<0.0001	3.7 (3.2, 4.2)
INSS stage (stage 4 vs others)	6078	<0.0001	6.7 (5.8, 7.7)
Primary site adrenal (yes vs no)	5598	<0.0001	1.5 (1.3, 1.7)
Primary site thoracic (no vs yes)	5600	<0.0001	2.0 (1.6, 2.5)
Bone metastasis (yes vs no)	2940	<0.0001	3.4 (2.9, 3.9)
Bone marrow metastasis (yes vs no)	2939	<0.0001	4.4 (3.8, 5.2)
LDH (1400 U/L vs < 1400 U/L)	2990	<0.0001	3.0 (2.4, 3.7)
Ferritin (30 ng/mL vs < 30 ng/mL)	2966	<0.0001	2.4 (1.9, 2.9)
Ploidy (diploid vs hyperdiploid)	4162	<0.0001	1.8 (1.5, 2.0)
1p aberration (yes vs no)	1911	<0.0001	1.8 (1.3, 2.4)
11q aberration (yes vs no)	1385	<0.0001	3.0 (2.2, 3.9)
SCA (yes vs no)	1477	<0.0001	3.5 (2.6, 4.7)
Histology (unfavorable vs favorable)	4211	<0.0001	9.6 (7.7, 12.1)
MKI (high vs intermediate/low)	3133	0.0015	1.4 (1.1, 1.8)
Grade of differentiation (undifferentiating vs differentiating)	3298	<0.0001	2.9 (2.0, 4.4)

SCA, segmental chromosome aberration.

TABLE 5
Bivariate Cox models of overall survival including interaction terms for patients in the Test cohort

Additional covariate (reference group shown in parentheses and represents the more favorable subgroup)	P-value	HR (95% confidence interval)			
		HR for death for patients with MYCN-A compared to MYCN-NA, in subgroups defined by covariate		HR for death for patients with unfavorable covariate result compared to favorable covariate result in subgroups defined by MYCN status	
		Covariate reference group (favorable)	Covariate nonreference group (unfavorable)	MYCN-A	MYCN-NA
Age (< 18 months)	<0.0001	<0.0001	<0.0001	1.1 (0.9, 1.3)	8.7 (7.2, 10.5)
INSS stage (not stage 4)	<0.0001	<0.0001	<0.0001	2.0 (1.6, 2.5)	10.8 (9.1, 12.9)
Primary site adrenal (not adrenal)	<0.0001	<0.0001	0.004	1.2 (1.0, 1.5)	1.7 (1.5, 2.0)
Primary site thoracic (yes thoracic)	<0.0001	<0.0001	0.74	1.9 (1.1, 3.1)	2.1 (1.6, 2.6)
Bone metastasis (no mets)	<0.0001	<0.0001	<0.0001	1.5 (1.2, 1.9)	5.4 (4.5, 6.5)
Bone marrow metastasis (no mets)	<0.0001	<0.0001	<0.0001	1.8 (1.4, 2.3)	6.9 (5.7, 8.4)
LDH (<1400 U/L)	<0.0001	<0.0001	<0.0001	1.6 (1.2, 2.1)	4.5 (3.6, 5.7)
Ferritin (<30 ng/mL)	<0.0001	<0.0001	<0.0001	1.2 (0.9, 1.7)	3.1 (2.4, 4.0)
Ploidy (hyperdiploid)	<0.0001	<0.0001	<0.0001	1.2 (0.9, 1.5)	2.3 (1.9, 2.8)
1p aberration (no aberration)	<0.0001	<0.0001	0.0021	1.1 (0.8, 1.6)	2.5 (1.8, 3.4)
11q aberration (no aberration)	<0.0001	<0.0001	0.0001	0.9 (0.4, 1.9)	4.4 (3.2, 6.2)
SCA (no SCA)	<0.0001	<0.0001	<0.0001	1.1 (0.6, 1.9)	4.6 (3.3, 6.4)
Histology (favorable)	<0.0001	<0.0001	<0.0001	1.9 (1.3, 3.0)	12.8 (9.9, 16.5)
MKI (intermediate/low)	<0.0001	<0.0001	0.0029	1.1 (0.9, 1.5)	2.1 (1.5, 3.0)

Additional covariate (reference group shown in parentheses and represents the more favorable subgroup)	<i>P</i> -value	HR (95% confidence interval)					HR for death for patients with unfavorable covariate result compared to favorable covariate result in subgroups defined by <i>MYCN</i> status
		HR for death for patients with <i>MYCN</i> -A compared to <i>MYCN</i> -NA, in subgroups defined by covariate					
				Interaction term (<i>MYCN</i> * covariate)	Covariate reference group (favorable)	Covariate nonreference group (unfavorable)	
Grade of neuroblastic differentiation (differentiating)	<0.0001	<0.0001	0.049	14.9 (6.0, 36.8)	5.9 (5.0, 6.9)	1.4 (0.6, 3.1)	3.4 (2.2, 5.3)

Abbreviations: SCA, segmental chromosome aberration.