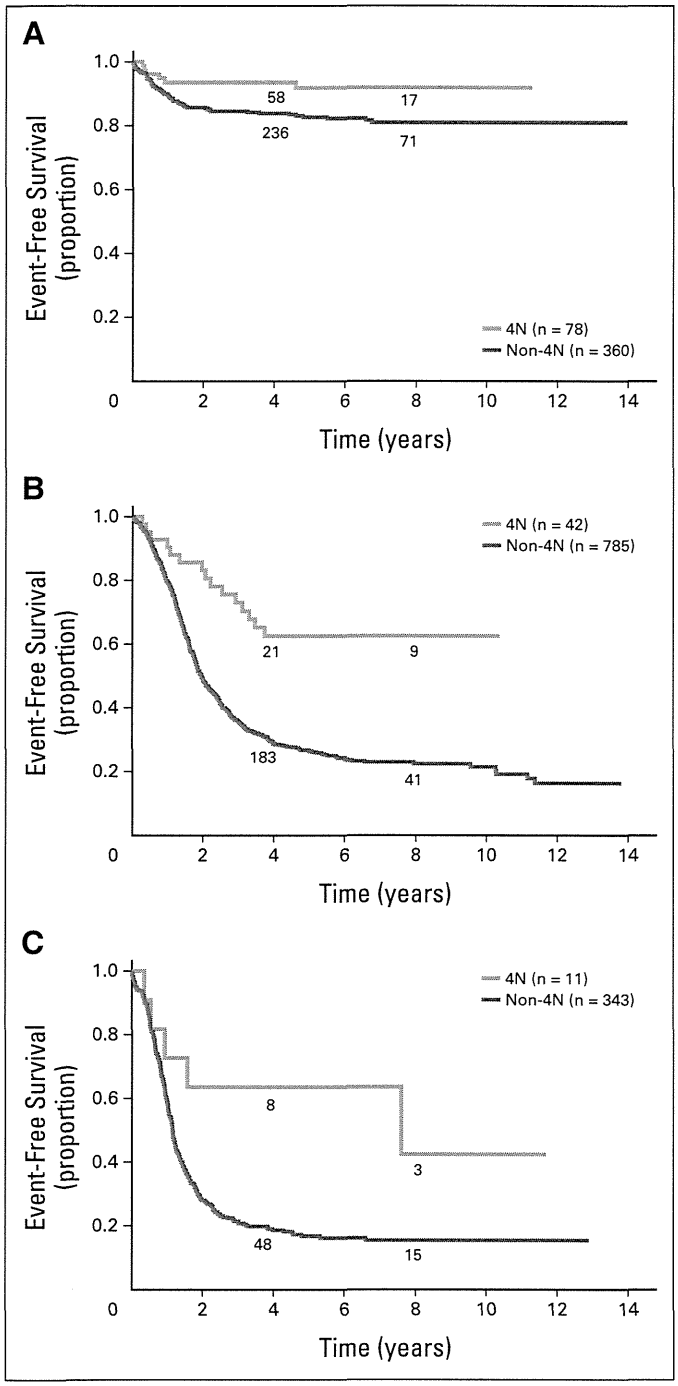

Fig 2. Event-free survival curves for patients with 4N versus non-4N disease for subgroups based on patient age at diagnosis and tumor *MYCN* status. (A) Patients age younger than 547 days with *MYCN* nonamplified tumors (hazard ratio [HR] for 4N disease, 0.51; 95% CI, 0.28 to 0.95; $P = .03$). (B) Patients age ≥ 547 days with *MYCN* nonamplified tumors (HR, 0.49; 95% CI, 0.36 to 0.67; $P < .001$). (C) Patients age ≥ 547 days with *MYCN* amplified tumors (HR, 0.50; 95% CI, 0.29 to 0.87; $P = .013$). The numbers of patients at risk for an event are shown along the curves at years 4 and 8.

metastases (eg, CNS *v* bone), lending insight into the molecular mechanisms governing metastases.[21] Future studies to explore genomic and gene expression differences between 4N and non-4N tumors are planned and may provide important insights into the pathways regulating metastatic spread and organ-specific tropisms in neuroblastoma.

Table 4. Comparison of Characteristics for 4N and Non-4N Stage 4 Patients Age ≥ 547 Days at Diagnosis and With *MYCN* Nonamplified Tumors

Characteristic	Stage 4N (n = 42) No.	%	Non-4N (n = 785) No.	%	*P*
Age, years					
Median	3.6		3.8		.5344
< 5	28	67	558	71	
≥ 5	14	33	227	29	.6012
Year of diagnosis					
1990-1995	32	76	460	59	
1996-2002	10	24	325	41	.0241
Ferritin/LDH (± SD)					
Mean ferritin, ng/mL	122 ± 153		349 ± 421		.0194
Mean LDH, U/L	1032 ± 2361		1077 ± 1290		.8740
Ploidy					
Hypodiploid/diploid	7	37	103	42	
Hyperdiploid	12	73	144	58	.8107
Histologic category					
Favorable	10	45	33	10	
Unfavorable	12	55	285	90	< .001
Histologic grade					
Differentiating	5	45	15	8	
Undifferentiated/poorly differentiated	6	55	179	92	.0017
MKI					
Low	10	91	95	52	
Intermediate	1	9	60	33	
High	0		28	15	.0397
Initial treatment					
None/surgery/ conventional	18	72	120	19	
Intensive ± SCT	7	28	502	81	< .001

Abbreviations: LDH, lactate dehydrogenase; MKI, mitosis karyorrhexis index; SCT, stem cell transplantation; SD, standard deviation.

In addition to underlying biologic differences, consideration must also be given to potential confounders in explaining the improved outcome of 4N disease. For this analysis, patients with any missing metastatic site data were excluded. Comparison of EFS and OS showed that these excluded patients had a significantly worse outcome than the whole final analytic cohort (Appendix Figure A1). Thus, the observed differences between outcome for 4N and non-4N patients may be an underestimate because our analytic cohort represents a group with a better outcome than unselected stage 4 patients. Although the ideal analysis would have been conducted with all stage 4 patients, this was not feasible because 4N patients cannot be identified unless metastatic site data are known. In addition, patients within the INRG data set did not necessarily undergo a uniform set of investigations. In particular, although metaiodobenzylguanidine scintigraphy (MIBG scintigraphy) is now routinely used to characterize metastatic spread of neuroblastoma, the database includes patients diagnosed in the early 1990s, at which time the use of MIBG imaging was not universal. It is possible that without MIBG imaging, metastatic sites may not have been detected, leading to the understaging of patients as having 4N disease. Indeed, the frequency of 4N disease is greater among patients diagnosed before 1996 (7.9% *v* 4.0% for those diagnosed from 1996 to 2002; $P < .001$), suggesting that increased imaging sensitivity has led to identification of more metastatic sites of disease. However, any

understaging of stage 4 patients as 4N would serve to reduce the observed effect size, non-4N patients having a worse prognosis than 4N patients. Furthermore, any bias introduced by 4N disease being more frequent in the early diagnostic period (1990 to 1995) would be countered by improved prognosis overall for later diagnostic years.[1] Consequently, both factors would be anticipated to reduce, rather than increase, the effect size for 4N favorable outcome.

In conclusion, for patients with metastatic spread limited to distant lymph nodes, our data support use of this pattern as a prognostic factor. For those with 4N disease, outcome in terms of both EFS and OS is significantly better than for other stage 4 patients. Consideration should therefore be given to whether these 4N patients might be eligible for different classification in the current risk stratification system. In particular, they may not require further therapeutic escalation that is likely necessary to improve outcomes for the remaining high-risk stage 4 groups (those age $\geq$ 547 days or infants with metastatic MNA disease) and thus may reduce adverse late effects in these patients. Of further interest is the likelihood that the tumors of patients with 4N disease are biologically distinct. The data presented here indicate that MNA is particularly uncommon within the 4N group. Insufficient data limit the analysis of the potential role of established SCAs.[22] However, future studies comparing chromosomal aberrations, messenger RNA expression profiles, and host genetic factors may reveal valuable insights into the processes governing neuroblastoma metastatic spread.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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GLOSSARY TERMS

event-free survival: calculated from the date of diagnosis to the date of the first event, which is resistance, relapse, death, or second malignant neoplasm.

loss of heterozygosity a situation in which one chromosome has a normal allele of a gene and one chromosome has a mutant or deleted allele.

MIBG scintigraphy: a nuclear medicine scan using iodine-123 metaiodobenzylguanidine (MIBG) scintigraphy to identify neuroblastoma or pheochromocytoma lesions.

overall survival: the duration between random assignment and death.

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Appendix

Future Development of the International Neuroblastoma Risk Group Database

The International Neuroblastoma Risk Group (INRG) database includes information relating to 36 prognostic variables for more than 11,500 children with neuroblastoma enrolled onto studies conducted in North America, Europe, Japan, and Australia between 1974 and 2002. Most published analyses, including the INRG classification system itself,[1] are based on the subset of 8,800 patients diagnosed between 1990 and 2002. The aim is to update the follow-up data on the existing patients in the INRG database and to import the next set of data for patients diagnosed after 2002. For this cohort, more genomic and detailed treatment information will be included in the INRG database. In addition, the data are now available through a Web-based interface with an advanced query engine and technology that facilitates linkage with other databases, both on- and off-site. This will greatly improve the consistency in collection of data regarding sites of disease and other elements. We have successfully established a link to the Children's Oncology Group Biobank and are in the process of connecting to databases that contain host and tumor genomic information. This Interactive INRG database (iINRGdb) will provide a resource for complex biologic studies based on data generated from genome-wide assays and next-generation sequencing.

Table A1. Comparison of Characteristics for 4N and Non-4N Stage 4 Patients and Those Stage 4 Patients Excluded From the Final Analysis Because of Missing/Inconsistent Metastatic Site Data

Characteristic	4N (n = 146) No.	%	Non-4N (n = 2,104) No.	%	Excluded (n = 994) No.	%	*P* Excluded *v* 4N	*P* Excluded *v* Non-4N
Age								
Median, days	423		929		932			
< 18 months	85	58	640	30	259	26		
> 18 months	61	42	1,464	70	735	74	< .001	.0141
Year of diagnosis								
1990-1995	113	77	1,314	62	313	31		
1996-2002	33	23	790	38	681	69	< .001	< .001
Ferritin/LDH (± SD)								
Mean ferritin, ng/mL	147 ± 261		324 ± 461		360 ± 708		.0093	N/S
Mean LDH, U/L	1,207 ± 1,859		1,763 ± 2,236		2,893 ± 4,284		< .001	< .001
Histologic category								
Favorable	45	63	219	26	98	24		
Unfavorable	27	37	609	74	303	76	< .001	N/S
Histologic grade								
Differentiating	9	21	44	8	24	5		
Undifferentiated/poorly differentiated	33	79	537	92	503	95	< .001	.0444
MKI								
Low	28	76	240	45	217	50		
Intermediate	6	16	158	29	101	23		
High	3	8	138	26	117	27	.0073	N/S
MYCN status								
Nonamplified	120	89	1,145	69	453	67		
Amplified	15	11	511	31	223	33	< .001	N/S
Cytogenetics								
Ploidy								
Hypodiploid/diploid	22	27	231	38	231	50		
Hyperdiploid	60	73	385	62	233	50	< .001	< .001
1p loss								
Yes	7	35	183	36	83	43		
No	13	65	318	64	112	57	N/S	N/S
17q gain								
Yes	3	50	100	64	1			
No	3	50	57	36	0		N/S	N/S
11q loss								
Yes	3	30	114	42	36	33		
No	7	70	154	58	72	67	N/S	N/S
Site of primary*							†	
Adrenal	59	40	1,273	60	554	60	< .001	N/S
Abdomen	38	26	498	24	189	20	N/S	N/S
Neck	6	4	25	1	11	1	N/S	N/S
Thorax	38	26	220	10	57	6	< .001	.0018
Pelvis	3	2	28	1	6	1	N/S	N/S
Other	4	2	78	4	109	12	.0018	< .001
Initial treatment								
None/surgery/conventional	71	77	502	30	73	11		
Intensive ± SCT	21	23	1,168	70	567	89	< .001	< .001

NOTE. The excluded patients are similar to the non-4N group on the basis of similar clinical and biological characteristics (age, ferritin, histology, mitosis karyorrhexis index [MKI], and *MYCN* status) and as reflected by their worse overall outcomes (Fig A1). They also have characteristics that correlate with aggressive disease (lactate dehydrogenase [LDH], grade, ploidy) and that are detected more commonly in the non-4N cohort. Thus, this analysis suggests that the excluded patients are unlikely to include substantial numbers of 4N patients. Furthermore, the fact that these excluded patients have a worse outcome than the final cohort would serve to reduce the observed effect of more favorable outcome for the 4N patients compared with the non-4N group. Thus, the exclusion of these 994 patients with incomplete data does not lead to a more pronounced effect.

Abbreviations: LDH, lactate dehydrogenase; MKI, mitosis karyorrhexis index; N/S, not significant; SCT, stem cell transplantation; SD, standard deviation.

*A small number of patients had primary tumors in multiple sites; therefore, totals vary from actual number of individual patients.

†*P* values corrected by using Sidak adjustment for multiple comparisons.

Table A2. Comparison of Treatment Approaches for 4N and Non-4N Patients

Treatment Category*	4N (n = 146) No.	4N (n = 146) %	Non-4N (n = 2,104) No.	Non-4N (n = 2,104) %	*P*†
Observation only	34	37	14	1	< .001
Surgery only	1	1	24	1	N/S
Conventional chemotherapy ± surgery	36	39	464	28	N/S
Intensive multimodal therapy, specific type unknown	7	8	265	16	N/S
Intensive multimodal therapy, no SCT	5	5	354	21	< .001
Intensive multimodal therapy plus SCT	9	10	549	33	< .001

NOTE. INRG data relating to treatment regimens must be interpreted with caution since patients included within the database were managed by several different cooperative groups during different periods, and a variety of clinical trials and protocols were used. Nevertheless, these data demonstrate that patients with 4N disease were significantly more likely than non-4N patients to be managed with observation alone, and non-4N patients were significantly more likely to receive intensive chemotherapy ± SCT. Consequently, the observed better outcome for 4N patients is not the result of more intensive treatment for this group.

Abbreviations: INRG, International Neuroblastoma Risk Group; N/S, not significant; SCT, stem-cell transplantation.

*Treatment categories are according to INRG classification.

†*P* values corrected using Sidak adjustment for multiple comparisons.

Table A3. Multivariable Cox Proportional Hazards Model of EFS in the Overall Cohort of 2,250 Patients

Risk Factor*	HR	95% CI	*P*
Disease stage			
4N	1	—	
Non-4N	2.86	2.01 to 4.07	< .001
Year of diagnosis			
1996-2002	1	—	
1990-1995	1.28	1.13 to 1.45	< .001
Age at diagnosis, days			
< 547	1	—	
≥ 547	1.89	1.64 to 2.19	< .001
MYCN amplification			
Nonamplified	0.68	0.59 to 0.78	< .001
Amplified	1.30	1.11 to 1.51	.001
Unknown	1	—	
Serum ferritin, ng/mL			
< 92	0.77	0.63 to 0.95	.0124
≥ 92	1.30	1.12 to 1.51	< .001
Unknown	1	—	
Serum LDH, U/L			
< 580	0.74	0.61 to 0.89	.002
≥ 580	0.95	0.80 to 1.12	.5483
Unknown	1	—	
Histology			
Favorable	0.39	0.29 to 0.52	< .001
Unfavorable	1.10	0.97 to 1.25	.1321
Unknown	1	—	

NOTE. To permit inclusion of all patients within the multivariable model, a dummy variable was created for unknown category of each factor for which there were missing data.

Abbreviations: EFS, event-free survival; HR, hazard ratio; LDH, lactate dehydrogenase.

*Initial model also included ploidy, grade, mitosis karyorrhexis index, 1p loss, 17q gain, and 11q loss.

Table A4. Univariable Cox Models of EFS Testing the Presence of Stage 4N Disease With Adjustment for Each Other Variable

Variable	No.	For Comparator Variable			For Stage 4N (*v* non-4N)		
		HR	95% CI	*P*	HR	95% CI	*P*
Total	2,250				0.24	0.17 to 0.34	< .001
Age, days	2,250						
≥ 547		2.59	2.26 to 2.98	< .001	0.29	0.20 to 0.42	< .001
< 547		1	—				
Year of diagnosis	2,250						
1996-2002		0.80	0.71 to 0.90	< .001	0.23	0.16 to 0.32	< .001
1990-1995		1	—				
MYCN status	1,791						
Amplified		2.31	2.04 to 2.62	< .001	0.26	0.17 to 0.37	< .001
Nonamplified		1	—				
Ferritin, ng/mL	1,255						
≥ 92		2.32	1.94 to 2.77	< .001	0.31	0.19 to 0.50	< .001
< 92		1	—				
LDH, U/L	1,447						
≥ 580		1.76	1.52 to 2.04	< .001	0.28	0.18 to 0.43	< .001
< 580		1	—				
Histologic category	900						
Unfavorable		5.25	3.95 to 6.96	< .001	0.36	0.22 to 0.61	< .001
Favorable		1	—				
Histologic grade	623						
Differentiating		0.58	0.37 to 0.93	.0225	0.32	0.17 to 0.60	< .001
Undifferentiated or poorly differentiated		1	—				
MKI	573						
High		1.77	1.40 to 2.25	< .001	0.33	0.17 to 0.65	.0011
Low or intermediate		1	—				
Ploidy	698						
Hypodiploid/diploid		1.55	1.26 to 1.90	< .001	0.24	0.15 to 0.40	< .001
Hyperdiploid		1	—				
1p LOH	521						
Present		1.70	1.36 to 2.14	< .001	N/S		.7061
Absent		1	—				
17q aberration (present *v* absent)	163	N/S		.1602	N/S		.3303
11q aberration (present *v* absent)	278	N/S		.9971	N/S		.3158

NOTE. Each table row is a separate model.
Abbreviations: EFS, event-free survival; HR, hazard ratio; LDH, lactate dehydrogenase; LOH, loss of heterozygosity; MKI, mitosis karyorrhexis index; N/S, not shown because not statistically significant.

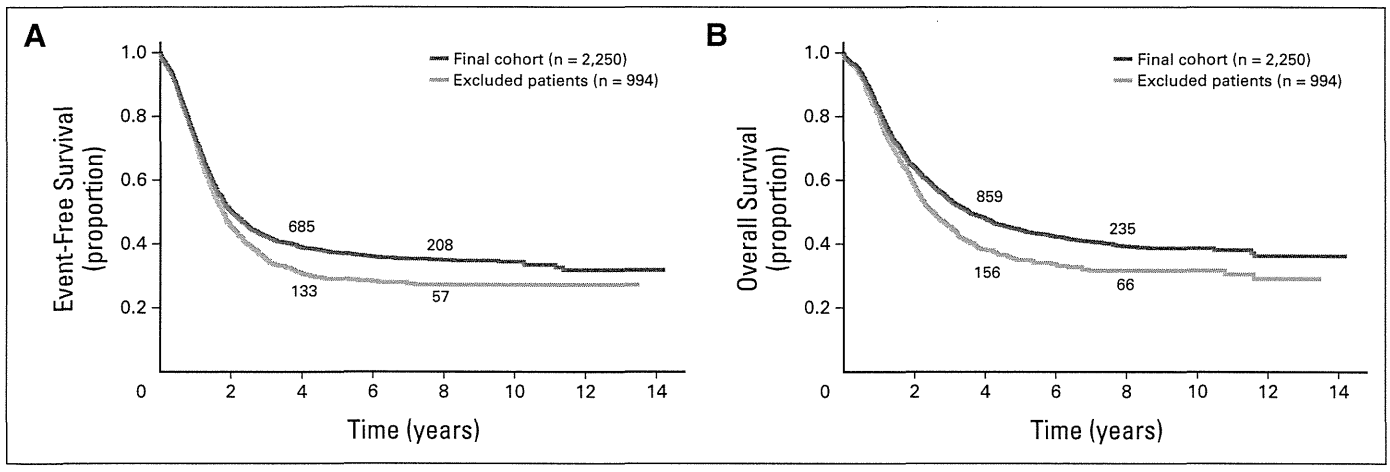

Fig A1. (A) Event-free survival and (B) overall survival curves for final analytic cohort of stage 4 patients (n = 2,250) versus stage 4 patients excluded from analysis because of missing/inconsistent metastatic site data (n = 994). $P = .0024$ for event-free survival; $P < .001$ for overall survival.

Pediatr Blood Cancer 2014;61:1932–1939

Significance of Clinical and Biologic Features in Stage 3 Neuroblastoma: A Report from the International Neuroblastoma Risk Group Project

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Background. International Neuroblastoma Staging System (INSS) Stage 3 neuroblastoma is a heterogeneous disease. Data from the International Neuroblastoma Risk Group (INRG) database were analyzed to define patient and tumor characteristics predictive of outcome. ***Procedure.*** Of 8,800 patients in the INRG database, 1,483 with INSS Stage 3 neuroblastoma and complete follow-up data were analyzed. Secondary analysis was performed in 1,013 patients (68%) with *MYCN*-non-amplified (NA) tumors. Significant prognostic factors were identified via log-rank test comparisons of survival curves. Multivariable Cox proportional hazards regression model was used to identify factors independently predictive of event-free survival (EFS). ***Results.*** Age at diagnosis ($P < 0.0001$), tumor *MYCN* status ($P < 0.0001$), and poorly differentiating/undifferentiated histology ($P = 0.03$) were independent predictors of EFS. Compared to other Stage 3 subgroups, outcome was inferior for patients ≥547 days with *MYCN*-NA neuroblastoma ($P < 0.0001$), and within this cohort, serum ferritin ≥96 ng/ml was associated with inferior EFS ($P = 0.02$). For patients <547 days of age with *MYCN*-NA tumors, serum ferritin levels were prognostic of overall survival (OS) ($P = 0.04$) and chromosome 11q aberration was prognostic of EFS ($P = 0.03$). ***Conclusions.*** Among patients with INSS Stage 3 neuroblastoma patients, age at diagnosis, *MYCN* status and histology predict outcome. Patients <547 days of age with *MYCN*-NA tumors that lack chromosome 11q aberrations or those with serum ferritin <96 ng/ml have excellent prognosis and should be considered for therapy reduction. Prospective clinical trials are needed to identify optimal therapy for those patients ≥547 days of age with undifferentiated histology or elevated serum ferritin. Pediatr Blood Cancer 2014;61: 1932–1939.

Key words: biologic factor; neuroblastoma; treatment outcome

INTRODUCTION

Patients with neuroblastoma can be classified as low-, intermediate-, and high-risk for relapse based upon age at diagnosis, International Neuroblastoma Staging System (INSS) stage, histopathologic characteristics, tumor cell ploidy (DNA index), and *MYCN* amplification [1–4]. Patients with INSS Stage 3 neuroblastoma represent a heterogeneous population with respect to disease presentation and prognosis and controversy exists regarding the most effective treatment algorithms. Prior Children's Cancer Group (CCG) trials demonstrated that age (<1 year of age at diagnosis) and favorable histology were statistically significantly predictive of excellent event-free survival (EFS) and overall survival (OS) for patients with Stage 3 neuroblastoma while *MYCN* amplification, unfavorable histopathology and elevated serum ferritin portended a worse prognosis [5]. Modifying therapy based upon prognostic factors has led to an improvement in outcome for patients with Stage 3 neuroblastoma [6–10]. Although small series have indicated that some patients with Stage 3 neuroblastoma may be effectively managed with surgery alone or close clinical observation [8,11], this treatment approach has not been adopted internationally. Children with INSS Stage 3 neuroblastoma characterized by *MYCN* amplification or older age at diagnosis have a less favorable outcome [5,12]. There is consensus across current clinical trials that patients with *MYCN*-amplified INSS Stage 3 tumors should be treated with high-risk therapy. Treatment of tumors without *MYCN* amplification has varied, ranging from surgery alone to use of intensive, multi-modality therapy with autologous hematopoietic stem cell transplant [8,12,13].

Biomarkers that provide more precise prognostication are needed to ensure that treatment is appropriately tailored and outcome for children with Stage 3 neuroblastoma is improved. The International Neuroblastoma Risk Group (INRG) database, which contains information on over 8,800 children with neuroblastoma, provides a unique resource for analyzing smaller cohorts of patients. In this study, we mined the INRG data for patients with

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Stage 3 disease to identify clinical and biologic markers predictive of relapse and OS.

METHODS

INRG Database

The INRG database includes a total of 8,800 patients younger than 21 years of age with pathologically confirmed neuroblastoma, diagnosed between January 1, 1990 and December 31, 2002. Patients were enrolled on neuroblastoma studies in Germany, Japan, Italy, Spain, the UK or were enrolled on a Children's Oncology Group (COG) study or the International Society of Pediatric Oncology Europe Neuroblastoma Group (SIOPEN) LNESG1 study [14]. Members of INRG are listed in Table SI (online only). In addition to date of diagnosis and follow-up data, information on 35 established risk factors are included in the database.

Of the 8,800 patients, 1,483 (17%) had INSS Stage 3 neuroblastoma with complete follow-up data and comprised the cohort for analysis. The primary objective was to determine clinical and biologic features prognostic of EFS and OS in the overall Stage 3 cohort. The prognostic value of age at diagnosis (<547 vs. ≥547 days) [15], histopathologic features (Shimada/International Neuroblastoma Pathology Classification [INPC] histology) [16], INPC diagnostic category [17], INPC grade of tumor differentiation, INPC mitosis-karyorrhexis index (MKI), *MYCN* status (amplified, non-amplified) [18,19], tumor cell ploidy defined as DNA index >1.0 or ≤1.0, chromosome 1p and 11q (aberration [loss of heterozygosity [LOH], deletion, unbalanced LOH], no aberration [no loss, balanced]), chromosome 17q gain, serum ferritin and serum lactate dehydrogenase (LDH) levels were analyzed. Of these factors, *MYCN* status and age at diagnosis are the most highly prognostic of outcome [14,20]. Secondary analyses were performed in 1,013 (68%) patients with Stage 3 disease with *MYCN*-non-amplified (NA) tumors, with additional cohort studies of children <547 days of age (654 patients) versus ≥547 days of age (359 patients).

Statistical Methods

Events for the EFS analysis were defined as relapse, progressive disease, secondary malignancy, or death from any cause. Time to event was calculated as time from diagnosis to first event or to time of last patient contact if no event occurred. Time to event for OS analysis was time from enrollment until death or time of last contact if the patient was alive. The methods of Kaplan–Meier were used to generate survival curves, and curves were compared using a log-rank test (*P*-values less than 0.05 were considered statistically significant). EFS and OS are expressed as the 5-year point estimate ± the standard error, with standard errors calculated per the methods of Peto and Peto [21]. For LDH and serum ferritin, the median values from the overall INRG cohort were 580 U/L and 96 ng/ml, respectively. These thresholds were used to divide patients into two categories: elevated (at or above the median value) and not elevated (below the median value).

A Cox proportional hazards regression model was used to test for factors independently predictive of EFS and calculate the hazard ratio (HR). Variables were selected for testing in the Cox model if they were highly statistically significant in univariate analysis. The INPC and Shimada histology systems use age at diagnosis and histological features of the tumor to categorize tumors as favorable versus unfavorable. This results in a duplication of the prognostic contribution ("confounding") of age when histology is used in a model or risk-group schema that includes age. To eliminate confounding, INPC tumor grade, MKI, histological category were tested in the model with age instead of INPC histologic classification (favorable, unfavorable). Once the independent prognostic factors were identified in the Cox model (Model A), an additional model (Model B), including a term for the level of initial treatment, was tested. This is referred to as "adjusting" the level of effect that that prognostic factors have on outcome, because in Model B, some of the effect on outcome is explained by the term for treatment instead of by the terms for the prognostic factors. Initial treatment was categorized as: none, surgery alone, moderate-dose chemotherapy plus surgery, multi-modality therapy (type unknown), multi-modality therapy without autologous hematopoietic stem cell transplant (AHSCT), or multi-modality therapy with AHSCT. Information on chemotherapy agents utilized, radiation treatment and specifics of surgery (i.e., complete or partial resection; nodal resection) were not captured in this data set.

RESULTS

In the overall INSS Stage 3 cohort, the 5-year EFS and OS were 74 ± 1% and 81 ± 1%, respectively (n = 1,483) (Table I), with median follow-up of 5.5 years in patients without an event. In univariate analysis, statistically significant differences in EFS and OS were identified for age at diagnosis, initial treatment, *MYCN* status, tumor cell ploidy, 11q aberrations (EFS only, not OS), 1p aberrations, INPC histologic classification, INPC diagnostic category, INPC grade of differentiation, INPC MKI, serum ferritin, and LDH (Table I).

Over all INSS Stage 3 patients, at least one initial therapy group was statistically different from the others in terms of EFS and OS ($P < 0.0001$; $P < 0.0001$, respectively). However, within the subset of patients with *MYCN*-NA tumors <547 days of age at diagnosis, at least one initial therapy group differed significantly in terms of EFS ($P < 0.0001$), but not OS ($P = 0.7$). Within patients with *MYCN*-NA tumors ≥547 days of age at diagnosis, there was no evidence of difference in EFS or OS by initial therapy ($P = 0.9$; $P = 0.9$, respectively) (Table II).

In multivariable analysis of the overall Stage 3 cohort, age at diagnosis, *MYCN* status, and INPC grade of tumor differentiation were independently prognostic of EFS (Table III, Model A). After adjustment for assignment at diagnosis to intensive chemotherapy and AHSCT, these three variables remained independently statistically significant (Table III, Model B).

Five-year EFS and OS were 81 ± 2% and 89 ± 1%, respectively, for the 1,013 (68%) patients with *MYCN*-NA Stage 3 tumors (Fig. 1A; Table IV). Within this cohort, patients <547 days old had better EFS and OS (90 ± 2% and 95 ± 1%) than those ≥547 days of age (64 ± 3% and 76 ± 3%; $P < 0.0001$, Fig. 1B and C). For patients <547 days old with *MYCN*-NA tumors: (i) the presence of 11q aberrations was prognostic of inferior EFS (64 ± 22% vs. 88 ± 5% at 5 years; $P = 0.02$) and OS (73 ± 22% vs. 96 ± 3% at 5 years; $P = 0.003$) (Table IV; Fig. 2A and B); and (ii) elevated serum ferritin (≥96 ng/ml) was associated with significantly worse 5-year OS (88 ± 4% vs. 96 ± 2%; $P = 0.04$), but the EFS was not statistically significantly different (Table IV). In multivariable analysis of EFS, only 11q aberration was prognostic of poor

TABLE I. Outcome of INSS Stage 3 Patients From the INRG Database, by Risk Factors (n = 1,483)

Factors	EFS hazard ratio (95% CI)	n (%)	5-year EFS ± SE (%)	EFS *P*-value	5-year OS ± E (%)	OS *P*-value
Overall INSS Stage 3		1,483 (100%)	74 ± 1		81 ± 1	
Age						
<547 days	3.5	850 (57)	86 ± 2	<0.0001	91 ± 1	
≥547 days	(2.8, 4.4)	633 (43)	58 ± 3		66 ± 2	<0.0001
MYCN status						
Not amplified	4.0	1,013 (82)	81 ± 2		89 ± 1	
Amplified	(3.1, 5.0)	217 (18)	45 ± 4	<0.0001	48 ± 4	<0.00001
Unknown		253				
Ploidy						
>1 (hyperdiploid)	2.1	152 (25)	82 ± 3		86 ± 3	
≤1 (diploid, hypodiploid)	(1.4, 3.0)	452 (75)	65 ± 6	<0.0001	66 ± 6	<0.0001
Unknown		879				
11q						
No aberration or balanced	2.1	152 (81)	77 ± 5		85 ± 4	
Deletion, imbalance, or unbalanced	(1.2, 3.8)	35 (19)	51 ± 11	0.012	75 ± 11	0.1
Unknown		1,296				
1p						
No loss or no aberration	2.7	313 (78)	77 ± 3		88 ± 3	
LOH, deletion, or imbalance	(1.8, 4.0)	90 (22)	52 ± 7	<0.0001	61 ± 7	<0.0001
Unknown		1,080				
17q gain						
Gain	1.2	16 (25)	75 ± 13		94 ± 7	
No gain	(0.4, 3.6)	47 (75)	74 ± 7	0.8	85 ± 6	0.4
Unknown		1,420				
Histologic classification						
Favorable	6.0	435 (61)	91 ± 2		96 ± 1	
Unfavorable	(4.1, 8.8)	281 (39)	55 ± 4	<0.0001	62 ± 4	<0.0001
Unknown		767				
Diagnostic category (INPC, Shimada if INPC missing)						
1 = NB, stroma-poor	UD	635 (92)	76 ± 2		82 ± 2	
2 = GanglioNB, intermixed, stroma-rich		18 (3)	100		100	
3 = GanglioNB, well diff., stroma-rich		6 (1)	100		100	
4 = GanglioNB, nodular (composite)		31 (4)	73 ± 11	0.1	89 ± 8	0.2
Unknown		793				
(1, 4 vs. 2, 3)						
2 and 3	UD	24 (3)	100		100	
1 and 4		666 (97)	76 ± 2	0.01	83 ± 2	0.04
Grade of NB differentiation (INPC, Shimada if INPC missing)						
Differentiating	2.4	97 (16)	87 ± 5		93 ± 3	
Undifferentiated	(1.3, 4.5)	494 (84)	74 ± 3	0.004	81 ± 3	0.004
Unknown		892				
MKI (INPC, Shimada if INPC missing)						
Low, intermediate	2.2	470 (86)	80 ± 3		86 ± 2	
High	(1.4, 3.4)	77 (14)	63 ± 8	0.0002	62 ± 8	<0.0001
Unknown		936				
Ferritin (ng/ml)						
<96	1.9	390	77 ± 3		88 ± 2	
≥96	(1.5, 2.6)	276	61 ± 4	<0.0001	64 ± 4	<0.0001
Unknown		817				
LDH (U/L)						
<580	1.6	499	78 ± 2		88 ± 2	
≥580	(1.3, 2.1)	460	67 ± 3	<0.0001	72 ± 3	<0.0001
Unknown		524				

UD, undefined.

outcome (HR [95% CI] = 3.8 [1.2, 12.5]; $P = 0.03$; Table III, Model C).

For patients ≥547 days of age with *MYCN*-NA tumors, unfavorable histologic classification, poorly/undifferentiated grade, and elevated ferritin were associated with significantly inferior EFS and OS in univariate analyses (Table IV, Fig. 2C and D). Only ferritin (EFS: HR [95% CI] = 1.8 [1.1, 3.0], $P = 0.02$; OS: HR [95% CI] = 3.2 [1.7, 5.8], $P = 0.0002$) was independently statistically

TABLE II. Outcome of INSS Stage 3 Patients by Treatment: Overall, and by Age Within Patients Who Have *MYCN*-Non-Amplified Tumors

	Overall (regardless of MYCN status), n = 1,483			MYCN non-amplified (age <547 days), n = 654			MYCN non-amplified (age ≥547 days), n = 359		
Treatment group	n	5-year EFS ± SE (%)	5-year OS ± SE (%)	n	5-year EFS ± SE (%)	5-year OS ± SE (%)	n	5-year EFS ± SE (%)	5-year OS ± SE (%)
None	9	78 ± 14	100	7	71 ± 17	100	2	100	100
Surgery alone	97	69 ± 5	82 ± 4	60	75 ± 6	93 ± 3	25	72 ± 9	74 ± 9
2–8 cycles of chemo, plus surgery	794	81 ± 1	87 ± 1	407	92 ± 1	96 ± 1	174	64 ± 4	78 ± 3
Multi-modality therapy: type unknown	28	35 ± 10	43 ± 10	3	67 ± 27	100	6	67 ± 19	67 ± 19
Multi-modality therapy: no AHSCT	169	62 ± 4	68 ± 4	37	92 ± 4	92 ± 4	42	56 ± 8	71 ± 7
Multi-modality therapy: plus AHSCT	75	63 ± 7	75 ± 6	7	100	100	23	39 ± 19	72 ± 17
Unknown	311	71 ± 3	71 ± 3	133	91 ± 4	91 ± 5	87	70 ± 6	76 ± 6
Log-rank test that at least one treatment group has a survival curve that is different from the others[a]		$P < 0.0001$	$P < 0.0001$		$P < 0.0001$	$P = 0.67$		$P = 0.9$	$P = 0.9$

[a]Excluding the "Unknown" treatment group.

significant in multivariable analyses (Table III, Models D and E, respectively).

Among the 272 patients with *MYCN*-NA tumors who were ≥547 days of age and for whom initial treatment assignment was known, EFS and OS, respectively, were not statistically significantly different by transplant status: (AHSCT 39 ± 22% vs. no AHSCT 63 ± 4%; $P = 0.99$) and (AHSCT 72 ± 19% vs. no AHSCT 76 ± 3%; $P = 0.65$). Further, EFS was not statistically significantly different when comparing surgery or chemotherapy alone versus multi-modal therapy with or without AHSCT for patients ≥567 days of age with ferritin ≥96 ng/ml or for those with tumors with poorly/undifferentiated grade (surgery/2–8 cycles

TABLE III. Multivariable Survival Analyses in INSS Stage 3 Patients From the INRG Database

	N[b]	Hazard ratio (95% CI)	*P*-value
Model A[a]—EFS in all INSS Stage 3			
Age ≥547 days	519	2.9 (1.9, 4.4)	<0.0001
MYCN-amplified		2.6 (1.7, 3.9)	<0.0001
Poorly/undifferentiated grade		2.3 (1.1, 4.7)	0.03
Model B—EFS in all INSS Stage 3; Model A plus a term to "adjust" for assignment at diagnosis to receive AHSCT			
Age ≥547 days	388	2.8 (1.7, 4.5)	<0.0001
MYCN-amplified		2.1 (1.3, 3.5)	0.004
Poorly/undifferentiated grade		2.2 (1.0, 4.9)	0.05
Assignment at diagnosis to AHSCT		1.1 (0.6, 1.8)	0.8
Model C—EFS in INSS Stage 3, *MYCN* non-amplified, <547 days old			
11q aberration	89	3.8 (1.2, 12.5)	0.03
Model D—EFS in INSS Stage 3, *MYCN* non-amplified, ≥547 days old			
Elevated (≥96 ng/ml) ferritin	153	1.8 (1.1, 3.0)	0.02
Model E—OS in INSS Stage 3, *MYCN* non-amplified, ≥547 days old			
Elevated (≥96 ng/ml) ferritin	153	3.2 (1.7, 5.8)	0.0002

CI, confidence interval. The following factors were tested in a given model and found not significant: Models A and B: ploidy, 11q, 1p, 17q, histologic classification, diagnostic category, MKI, ferritin, and LDH; Model C: ploidy, 1p, 17q, histologic classification, diagnostic category, MKI, ferritin, and LDH; Models D and E: ploidy, 11q, 1p, 17q, histologic classification, diagnostic category, MKI, and LDH. [a]For each model, the category(ies) of increased risk for the significant factor(s) is(are) presented. For example, in Model D, patients with ferritin ≥96 ng/ml have a 1.8 times greater risk of an event than do patients with ferritin <96 ng/ml. [b]This is the number of patients included in the model, which equals the number of patients who have non-missing values for all the terms included in the model. For example, in Model D, 153 patients have a known value of ferritin.

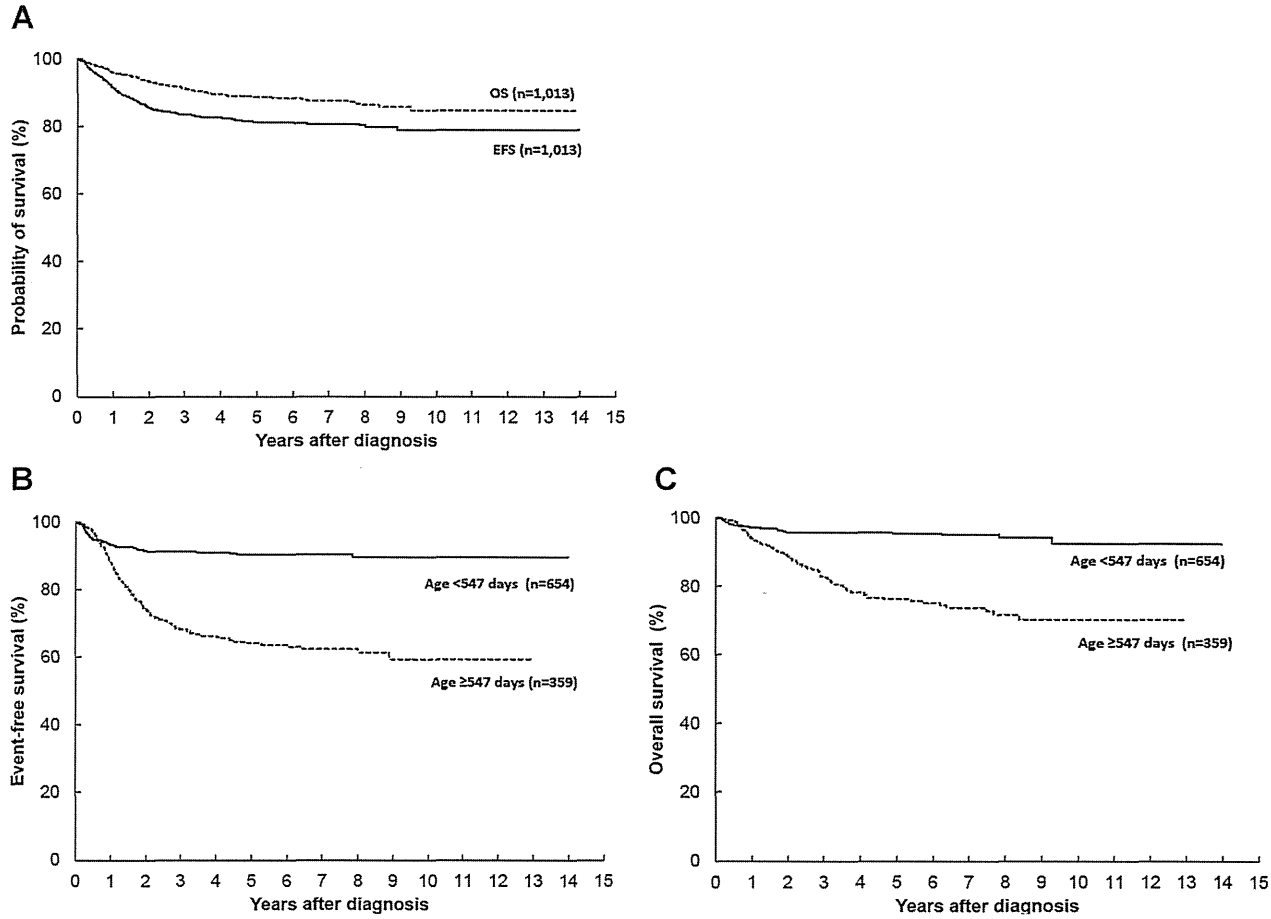

Fig. 1. Kaplan–Meier survival curves in patients with INSS Stage 3, *MYCN*-non-amplified neuroblastoma. **A**: Event-free survival (EFS) and overall (OS) in all patients (n = 1,013 patients). (**B**) Event-free survival (EFS) and (**C**) overall survival (OS) stratified by age at diagnosis; <547 days (n = 654 patients) and ≥547 days (n = 359 patients).

chemotherapy [n = 74] vs. multi-modal therapy [n = 57]; $P = 0.34$, Fig. 2E).

DISCUSSION

Utilizing the INRG database, we have analyzed the largest group of INSS Stage 3 neuroblastoma patients that has been reported. There are limitations to this retrospective review as disease stage was based on the INSS staging system and in upcoming studies patients will be classified according to the INRG system. In addition, detailed treatment data were not available. However, we have shown that clinical and biologic markers can accurately predict prognosis for patients with INSS Stage 3 neuroblastoma. Compared to the balance of patients, worse outcome is observed in patients ≥547 days at diagnosis and those with tumors with *MYCN* amplification or poorly differentiating/undifferentiated histologic grade. Furthermore, these factors remain prognostic regardless of the initial treatment intensity assigned at diagnosis (intensive chemotherapy and AHSCT). Age at diagnosis remains prognostic within the subset of INSS Stage 3 patients with *MYCN*-NA tumors, consistent with previously published clinical trials [22] and analyses of the entire INRG database [23]. Importantly, the prognostic variables differ for patients <547 versus ≥547 days of age at diagnosis with Stage 3 *MYCN*-NA neuroblastoma.

Within the *MYCN*-NA, Stage 3 cohort, although outcome was excellent for most patients <547 days, inferior survival was seen in the subset of patients <547 days with 11q aberrations. Genomic data were not available for many patients; however, the effect of 11q aberration on survival reached statistical significance. Prior retrospective analyses have demonstrated 11q loss as an independent predictor of EFS and OS in patients with neuroblastoma [24–27]. Analyses of patients enrolled onto a COG biology repository trial demonstrated an inferior 3-year EFS and OS for those with unbalanced 11q LOH ($50 \pm 5\%$ and $66 \pm 5\%$, respectively), as compared to those without LOH (EFS $74 \pm 2\%$ and OS $83 \pm 2\%$, respectively) [26]. Spitz et al. [27] demonstrated that loss of chromosome 11q predicted for decreased EFS and OS ($P < 0.001$) in the entire NBL population and specifically in those patients with *MYCN*-NA Stage 1, 2, 3, and 4S disease ($P < 0.001$). More recently, analyses of the overall genomic pattern (segmental or whole chromosome aberrations) has been evaluated and shown to be prognostic [28,29]. The presence of at least one segmental chromosome aberration in patients ≥547 days of age was shown to correlate with a poor EFS and OS [30,31] and may serve as a surrogate marker for more general segmental chromosomal

TABLE IV. Characteristics of Patients With INSS Stage 3, *MYCN*-Non-Amplified Tumors Neuroblastoma

	Age <547 days					Age ≥547 days				
Characteristics	N (%)	5-year EFS ± SE (%)	*P*-value[a]	5-year OS ± SE (%)	*P*-value[a]	N (%)	5-year EFS ± SE (%)	*P*-value[a]	5-year OS ± SE (%)	*P*-value[a]
INSS Stage 3 *MYCN* non-amplified	654 (100)	90 ± 2		95 ± 1		359 (100)	64 ± 3		76 ± 3	
Ploidy										
>1 (hyperdiploid)	281 (88)	93 ± 2		96 ± 2		100 (65)	71 ± 8		77 ± 7	
≤1 (diploid, hypodiploid)	40 (12)	82 ± 11	0.2	86 ± 10	0.3	53 (35)	68 ± 10	0.5	66 ± 9	0.2
Unknown	333					206				
11q										
No aberration or balanced	78 (88)	88 ± 5	0.02	96 ± 3	0.003	41 (67)	73 ± 10	0.2	90 ± 7	0.6
LOH, deletion, or unbalanced	11 (12)	64 ± 22		73 ± 22		20 (32)	49 ± 14		76 ± 14	
Unknown	565					298				
1p										
No aberration or balanced	183 (93)	87 ± 3		94 ± 2		95 (83)	65 ± 6		80 ± 6	
LOH, deletion, or unbalanced	13 (7)	85 ± 13	0.7	92 ± 10	0.8	19 (17)	51 ± 15	0.4	72 ± 14	0.7
Unknown	458					245				
Histologic classification[b]										
Favorable	320 (94)	92 ± 2	0.2	98 ± 1	0.1	63 (31)	94 ± 4	<0.0001	98 ± 2	<0.0001
Unfavorable	22 (6)	86 ± 10		91 ± 8		139 (69)	57 ± 6		66 ± 6	
Unknown	312					157				
Grade of differentiation[b]										
Differentiating	42 (15)	91 ± 6		97 ± 2		35 (23)	89 ± 7		96 ± 4	
Poorly or undifferentiated	243 (85)	91 ± 3	0.6	97 ± 3	0.6	119 (77)	58 ± 7	0.002	70 ± 6	0.004
Unknown	369					205				
MKI										
Low, intermediate	268 (95)	91 ± 3	0.3	96 ± 2	0.5	121 (85)	67 ± 6	0.7	78 ± 6	0.2
High	13 (5)	85 ± 17		92 ± 13		21 (15)	64 ± 14		62 ± 13	
Unknown	373					217				
Ferritin (ng/ml)										
<96	200 (68)	89 ± 3		96 ± 2		104 (68)	65 ± 5		84 ± 4	
≥96	96 (32)	84 ± 5	0.2	88 ± 4	0.04	49 (32)	46 ± 8	0.01	53 ± 9	<0.0001
Unknown	358					206				
LDH (U/L)										
<580	266 (60)	87 ± 3		95 ± 2		125 (59)	59 ± 5		78 ± 5	
≥580	181 (40)	89 ± 3	0.5	93 ± 2	0.4	87 (41)	57 ± 6	0.6	66 ± 6	0.2
Unknown	207					147				

[a]*P*-values per log-rank test. [b]INPC, Shimada if INPC missing.

aberrations. Modest numbers of patients with complete genomic data, in particular those with data for prognostic markers of 1p LOH [32,33] and 17q gain [34,35], limited our ability to detect their prognostic value. Current prospective clinical trials being performed by North American and European international cooperative groups will more clearly determine the prognostic relevance of specific chromosomal gains and losses as well as patterns of chromosomal aberration using whole genomic techniques.

Our analysis of the INRG database confirms prior reports of excellent EFS and OS in young patients with Stage 3 disease without *MYCN* amplification and differentiated/differentiating tumor histologic features. Individual clinical trial results have previously demonstrated a similarly excellent 4-year EFS and OS (90% and 93%, respectively) for patients with Stage 3 neuroblastoma <12 months of age at diagnosis without *MYCN* amplification and unfavorable histology [5]. Park et al. [12] demonstrated a trend for improved 5-year OS for patients who were <18 months of age as compared to patients ≥18 months of age regardless of histology, 100% and 74 ± 9%, respectively ($P = 0.1545$). Our analysis of the largest cohort of Stage 3 patients ever reported, confirms that patients <547 days of age at diagnosis have an excellent outcome regardless of the tumor histologic features, supporting further reduction of therapy for this cohort.

For patients >547 days of age, tumor grade and serum ferritin were prognostic of outcome. Patients with poorly differentiated/undifferentiated tumor histologic grade have significantly worse EFS and OS than those with histologic evidence of tumor differentiation. Goto et al. [36] similarly reported a statistically significant difference in OS for patients ≥12 months of age with Stage 3, *MYCN*-NA neuroblastoma and favorable histology as compared to unfavorable, 100% and 81.8%, respectively ($P = 0.01$). Serum ferritin ≥96 ng/ml was also found to be predictive of worse EFS and OS in this older cohort. Univariate analysis of patients with neuroblastoma enrolled on the Austrian A-NB87 study demonstrated elevated ferritin (>300 ng/ml) to be a statistically significant unfavorable risk factor ($P = 0.007$) [37]. The predictive power was found to be greater in patients with localized disease as compared to Stage 4 patients. Elevated serum ferritin (>142 ng/ml) was associated with worse progression-free survival (PFS) in a multivariable analysis of prognostic factors in patients with Evans Stage III and IV disease [38] and in patients with INSS Stage 3 neuroblastoma with *MYCN*-NA neuroblastoma [5]. It has been proposed that serum ferritin may act as a surrogate for tumor burden, but also may reflect tumor biologic characteristics or environmental factors that could alter prognosis [38]. Ferritin may prove to be an important factor for patient risk stratification.

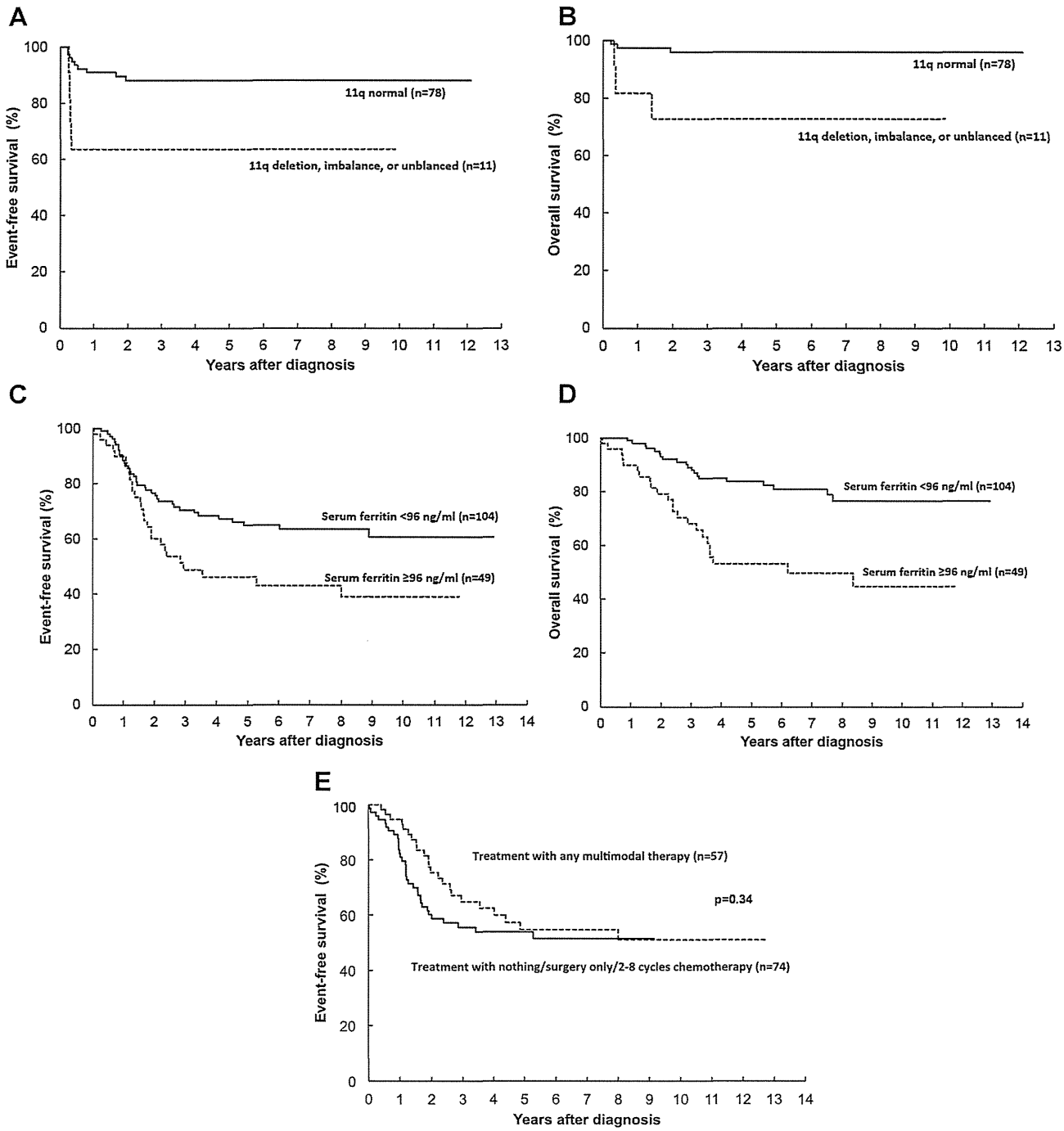

Fig. 2. Kaplan–Meier survival curves in patients with INSS Stage 3, *MYCN*-non-amplified neuroblastoma. (**A**) Event-free survival (EFS) and (**B**) overall (OS) in patients <547 days of age stratified by 11q no aberration (n = 78) versus 11q aberration (n = 11). (**C**) Event-free survival (EFS) and (**D**) overall (OS) in patients ≥547 days of age stratified by serum ferritin; <96 ng/ml (n = 104) and ≥96 ng/ml (n = 49). (**E**) Event-free survival (EFS) in patients ≥547 days of age with unfavorable histology or elevated ferritin (≥96 ng/ml) stratified by therapy received.

Our study, together with other reports, demonstrates that Stage 3 patients ≥547 days with elevated serum ferritin and/or poorly differentiated/undifferentiated tumors are at increased risk for relapse, indicating that this cohort may benefit from more intensive therapy. Although survival was similar in our analysis of the INRG data for those who received surgery with or without two to eight cycles of moderate dose chemotherapy versus multi-modality therapy, the retrospective nature of this analysis, the incomplete details regarding therapy received, the confounding of prognostic factors with initial treatment, and the small numbers of patients limit our ability to interpret the data about treatment effect. An international consensus as to the appropriate therapy for Stage 3 patients ≥547 days with unfavorable histology has not been reached and there is wide variation in the intensity of treatment. Collecting

detailed information on therapy received in these patients would permit analysis of the effect of treatment on outcome and inform best practice.

Our data validate current approaches that incorporate both clinical and biologic features into prognostication and treatment assignment for patients with Stage 3 neuroblastoma. Outcome remains poor for a subset of older patients with Stage 3 *MYCN*-NA tumors and more effective treatment is needed for these children. Gene expression profiling may provide more precise prognostication in patients with localized *MYCN*-NA neuroblastoma [39,40]. If these molecular signatures are validated in prospective studies, they may further refine risk classification and identify the subset of patients who will benefit from alternative therapies. Patients with more favorable clinical and biologic features, specifically those <18 months of age without unfavorable chromosomal features, have an excellent EFS and OS. A change in the current standard practice should be considered through further decreases in exposure to chemotherapy sparing these children the potential acute and long-term toxicities associated with treatment.

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Clinical, Biologic, and Prognostic Differences on the Basis of Primary Tumor Site in Neuroblastoma: A Report From the International Neuroblastoma Risk Group Project

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ABSTRACT

Purpose
Neuroblastoma (NB) is a heterogeneous tumor arising from sympathetic tissues. The impact of primary tumor site in influencing the heterogeneity of NB remains unclear.

Patients and Methods
Children younger than age 21 years diagnosed with NB or ganglioneuroblastoma between 1990 and 2002 and with known primary site were identified from the International Neuroblastoma Risk Group database. Data were compared between sites with respect to clinical and biologic features, as well as event-free survival (EFS) and overall survival (OS).

Results
Among 8,369 children, 47% had adrenal tumors. All evaluated clinical and biologic variables differed statistically between primary sites. The features that were > 10% discrepant between sites were stage 4 disease, *MYCN* amplification, elevated ferritin, elevated lactate dehydrogenase, and segmental chromosomal aberrations, all of which were more frequent in adrenal versus nonadrenal tumors ($P < .001$). Adrenal tumors were more likely than nonadrenal tumors (adjusted odds ratio, 2.09; 95% CI, 1.67 to 2.63; $P < .001$) and thoracic tumors were less likely than nonthoracic tumors (adjusted odds ratio, 0.20; 95% CI, 0.11 to 0.39; $P < .001$) to have *MYCN* amplification after controlling for age, stage, and histologic grade. EFS and OS differed significantly according to the primary site ($P < .001$ for both comparisons). After controlling for age, *MYCN* status, and stage, patients with adrenal tumors had higher risk for events (hazard ratio, 1.13 compared with nonadrenal tumors; 95% CI, 1.03 to 1.23; $P = .008$), and patients with thoracic tumors had lower risk for events (HR, 0.79 compared with nonthoracic; 95% CI, 0.67 to 0.92; $P = .003$).

Conclusion
Clinical and biologic features show important differences by NB primary site, with adrenal and thoracic sites associated with inferior and superior survival, respectively. Future studies will need to investigate the biologic origin of these differences.

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INTRODUCTION

One of the hallmarks of neuroblastoma (NB) is its clinical and biologic heterogeneity. The likelihood of cure is dependent on widely varying factors, including age, disease stage, tumor site, and biologic features.[1-3] The impact of the primary site of disease in influencing the heterogeneity of NB remains unclear.

Previous work has suggested that extra-abdominal NB tumors (cervical, thoracic, pelvic) may be associated with more favorable clinical and biologic characteristics and therefore a better outcome compared with NBs that originate from the abdomen.[4] In a retrospective analysis of 143 patients with NB, the frequency of stage 4 disease, tumor *MYCN* gene amplification, elevated lactate dehydrogenase (LDH), and elevated ferritin were all significantly lower in the extra-abdominal group than in the abdominal group. Not surprisingly, the probability of 5-year event-free survival (EFS) was higher in the extra-abdominal group (94%) than in the abdominal group (69%); however, a multivariable analysis was not performed in this study.[4] Studies focused on pelvic NB have shown conflicting results. One study observed that pelvic primary tumor sites were mainly associated with advanced disease.[5] Other studies reported that pelvic tumors represent a more favorable prognostic subgroup, particularly among patients with higher-stage disease.[6,7]

Previous work has suggested that thoracic NBs are a distinct subset of tumors that present at an earlier age and localized stages and have a more favorable outcome.[8,9]

These previous studies indicate that primary tumor site may account for some of the heterogeneity in clinical features, tumor biology, and clinical outcomes in NB. Given the small size and limited scope of these previous studies, a clear understanding of the impact of primary tumor site has not been possible. We therefore performed a comprehensive analysis of primary tumor site in NB. We used the largest available cohort of patients with this disease, those registered in the International Neuroblastoma Risk Group (INRG) database, to assess whether clinical features, tumor biologic features, and survival differ between primary tumor sites.

PATIENTS AND METHODS

Patients

A total of 8,800 patients younger than age 21 years with pathologically confirmed NB or ganglioneuroblastoma who were diagnosed/enrolled between 1990 and 2002 comprise the INRG database.[10] An enrollment cutoff of 2002 was chosen to allow for sufficient follow-up time. Patients provided consent and were enrolled onto one or more NB clinical or biologic trials in Germany, Japan, Italy, Spain, or the United Kingdom or onto a North American Children's Oncology Group study or the International Society of Pediatric Oncology Europe Neuroblastoma Group (SIOPEN) Localized Neuroblastoma European Study (LNESG1). Each country, cooperative group, and treating institution obtained institutional review board approval and informed patient consent for their respective studies. In addition to the date of diagnosis and follow-up data, information on 35 potential risk factors is included in the INRG database.[10]

Of the 8,800 patients, only those patients with an assigned primary tumor site were included in the analytic cohort for this report (N = 8,369). The six primary tumor site categories included adrenal, abdominal/retroperitoneal, neck, thoracic, pelvic, and other. The "other" primary tumor site category (n = 664) comprised patients who were originally assigned an "other" designation in the INRG database (n = 507) as well as those patients who were assigned more than one of the six primary tumor site categories listed (n = 157).

Statistical Analysis

Primary tumor site was the predictor variable of interest in this analysis. The adrenal gland was the most common site. Of the sites (neck, thoracic, pelvic) that may be associated with more favorable clinical and biologic characteristics and outcome, thoracic tumors comprise the largest group. Therefore, primary tumor site was analyzed using the six categories described and also as separate grouped binary variables: adrenal versus nonadrenal and thoracic versus nonthoracic.

Clinical and biologic dependent variables described in the INRG at initial diagnosis and analyzed in this study are listed in Table 1. For LDH and ferritin, median values from the entire INRG cohort (580 U/L and 96 ng/mL, respectively) were used to dichotomize patients as having elevated or not elevated levels following the convention used for previous INRG analyses.[10] The INRG

Table 1. Clinical and Biologic Characteristics of the INRG Analytic Cohort by Primary Tumor Site (N = 8,369)

			Primary Tumor Site												
	All (N = 8,369)†		Adrenal (n = 3,966)		Abdominal/ Retroperitoneal (n = 1,991)		Neck (n = 229)		Thoracic (n = 1,266)		Pelvic (n = 253)		Other‡ (n = 664)		
Characteristic*	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	P§
Mean age at diagnosis, months	26.4		26.6		27.9		19.5		24.5		24.4		27.7		< .001
Age ≥ 18 months at diagnosis	3,812 of 8,369	46	1,882	47	963	48	66	29	492	39	103	41	306	46	< .001
Tumor diagnosis of neuroblastoma, nodular‖	3,833 of 8,369	46	1,918	48	838	42	114	50	491	39	116	46	356	54	< .001
Enrollment/diagnosis before 1996	4,173 of 8,369	50	2,008	51	993	50	113	49	719	57	109	43	231	35	< .001
INSS stage 4	3,298 of 8,186	40	1,963	50	718	37	44	20	268	22	34	14	271	42	< .001
Serum ferritin ≥ 92 ng/mL	2,192 of 4,270	51	1,239	59	533	49	35	32	188	34	37	35	160	51	< .001
LDH ≥ 587 U/L	2,540 of 5,144	49	1,332	55	681	49	54	39	271	36	54	35	148	55	< .001
MYCN amplified¶	1,114 of 6,811	16	718	23	290	17	4	2	32	3	6	3	64	12	< .001
Ploidy ≤ 1 (diploid, hypodiploid)	1,044 of 3,541	29	485	33	279	30	21	22	121	25	17	17	121	28	.001
LOH at 1p	4,78 of 2,107	23	314	30	94	18	5	11	28	10	5	11	32	20	< .001
Gain of 17q	168 of 346	49	115	61	32	43	2	33	16	27	1	14	2	18	< .001
11q aberration	218 of 1,026	21	125	26	57	24	1	5	21	14	1	10	12	11	< .001
Pooled segmental chromosomal aberration															
LOH at 1p, gain of 17q and/or 11q aberration	681 of 2,141	32	416	39	156	29	6	13	53	19	7	15	43	27	< .001
Unfavorable INPC pathology classification	1,422 of 3,989	36	720	41	354	39	22	20	141	22	31	21	154	39	< .001
High MKI	378 of 3,047	12	219	15	96	14	7	8	21	5	3	3	32	11	< .001
Undifferentiated/poorly differentiated	2,726 of 3,239	84	1,346	85	619	85	78	90	332	78	75	68	276	88	< .001

Abbreviations: ANOVA, analysis of variance; INPC, International Neuroblastoma Pathology Classification; INRG, International Neuroblastoma Risk Group; INSS, International Neuroblastoma Staging System; LDH, lactate dehydrogenase; LOH, loss of heterozygosity; MKI, Mitosis Karyorrhexis Index.

*For each variable, only the percent with the adverse risk factor is shown.

†Adverse risk factor sample size over the total sample size with data available for the variable of interest.

‡The "other" primary tumor site category (n = 664) comprised patients who were originally assigned an "other" designation in the INRG database (n = 507) as well as those patients who were assigned more than one primary tumor site category among the adrenal, abdominal/retroperitoneal, neck, thoracic, pelvic, and other categories (n = 157).

§*P* value refers to a one-way ANOVA test (for continuous age variable) or χ^2 test for all other variables (age, tumor diagnosis, year of enrollment, INSS stage, serum ferritin, LDH, *MYCN* status, ploidy, LOH at 1p, gain of 17q, 11q aberration, pooled segmental chromosomal classification, INPC pathology classification, and MKI and grade of differentiation categories).

‖INPC diagnostic category[11]: neuroblastoma or ganglioneuroblastoma, nodular versus ganglioneuroblastoma, intermixed; ganglioneuroma, maturing subtype; or ganglioneuroblastoma, well differentiated.

¶The number of *MYCN*-amplified adrenal tumors in this study differs slightly from previous INRG studies because those patients who were assigned more than one primary tumor site were included in the "other" category for this study.[12]

Table 2. Clinical and Biologic Characteristics of the INRG Analytic Cohort by Adrenal Versus Nonadrenal and Thoracic Versus Nonthoracic Primary Tumor Sites (N = 8,369)

Characteristic*	Primary Tumor Site: Adrenal (n = 3,966) No.	%	Primary Tumor Site: Nonadrenal (n = 4,403) No.	%	P†	Primary Tumor Site: Thoracic (n = 1,266) No.	%	Primary Tumor Site: Nonthoracic (n = 7,103) No.	%	P†
Mean age at diagnosis, months	26.6		26.3		.59	24.5		26.8		.018
Age ≥ 18 months at diagnosis	1,882	47	1,930	44	.001	492	39	3,320	47	< .001
Neuroblastoma or ganglioneuroblastoma, nodular‡	1,918	48	1,915	43	< .001	491	39	3,342	47	< .001
Enrollment/diagnosis before 1996	2,008	51	2,165	49	.182	719	57	3,454	49	< .001
INSS stage 4	1,963	50	1,335	31	< .001	268	22	3,030	44	< .001
Serum ferritin ≥ 92 ng/mL	1,239	59	953	44	< .001	188	34	2,004	54	< .001
LDH ≥ 587 U/L	1,332	55	1,208	44	< .001	271	36	2,269	52	< .001
MYCN amplified	718	23	396	11	< .001	32	3	1,082	19	< .001
Ploidy ≤ 1 (diploid, hypodiploid)	485	33	559	27	.001	121	25	923	30	.032
LOH at 1p	314	30	164	16	< .001	28	10	450	25	< .001
Gain of 17q	115	61	53	34	< .001	16	27	152	53	< .001
11q aberration	125	26	93	17	.001	21	14	197	23	.015
Pooled segmental chromosomal aberration										
LOH at 1p, gain of 17q, and/or 11q aberration	416	39	265	25	< .001	53	19	628	34	< .001
Unfavorable INPC pathology classification	720	41	702	32	< .001	141	22	1,281	38	< .001
High MKI	219	15	159	10	< .001	21	5	357	14	< .001
Undifferentiated/poorly differentiated	1,346	85	1,380	83	.059	332	78	2,394	85	< .001

Abbreviations: INPC, International Neuroblastoma Pathology Classification; INRG, International Neuroblastoma Risk Group; INSS, International Neuroblastoma Staging System; LDH, lactate dehydrogenase; LOH, loss of heterozygosity; MKI, Mitosis Karyorrhexis Index.

*For each variable, only the percent with the adverse risk factor is shown.

†*P* value refers to a Student's *t* test (for continuous age variable) or χ^2 test for all other variables (age, tumor diagnosis, year of enrollment, INSS stage, serum ferritin, LDH, *MYCN* status, ploidy, LOH at 1p, gain of 17q, 11q aberration, pooled segmental chromosomal classification, INPC pathology classification, and MKI and grade of differentiation categories).

‡INPC diagnostic category[11]: neuroblastoma or ganglioneuroblastoma, nodular versus ganglioneuroblastoma, intermixed; ganglioneuroma, maturing subtype; or ganglioneuroblastoma, well differentiated.

database includes data on loss of heterozygosity (LOH)/aberration at 1p, gain of 17q, and 11q aberration. We evaluated each of these variables separately and also created a pooled variable reflecting the presence of segmental chromosomal aberration if at least one of these aberrations was present.[13] Clinical and biologic features were compared between groups defined by primary tumor site using χ^2 tests (for categorical variables) or *t* test or analysis of variance between groups (for continuous variables). We fit logistic regression models to describe the odds of having *MYCN* amplification according to primary tumor site after controlling for key potential confounders.

Clinical outcome variables that were available for analysis in the INRG database were EFS and overall survival (OS). EFS was defined as the time from study enrollment at diagnosis to first occurrence of relapse, progression, secondary malignancy, or death. Patients without an event were censored at the time of last patient contact. OS was defined as time from study enrollment until death, with living patients censored at the time of last contact. EFS and OS were estimated using Kaplan-Meier methods with survival distributions compared according to primary tumor site using a two-sided log-rank test.[14] Cox proportional hazards regression models were used to calculate the hazard ratio (HR) for increased risk of event or death while controlling for key potential confounders. Time-dependent covariates were used to test the proportional hazards assumption. Any variables that did not satisfy the proportional hazards assumption were removed as covariates from the model and instead used

Table 3. Logistic Regression Analysis of the Association Between Primary Tumor Site and *MYCN* Amplification Without (unadjusted OR) and With Adjustment (adjusted OR) for Age at Diagnosis, INSS Stage, and Grade of Differentiation

Primary Tumor Site	Unadjusted OR	Unadjusted 95% CI	Unadjusted P	Adjusted OR	Adjusted 95% CI	Adjusted P
Adrenal	1	Ref	Ref	1	Ref	Ref
Abdominal/retroperitoneal	0.70	0.60 to 0.81	< .001	0.76	0.58 to 0.98	.038
Neck	0.072	0.027 to 0.20	< .001	0.13	0.030 to 0.53	.005
Thoracic	0.11	0.074 to 0.15	< .001	0.16	0.083 to 0.31	< .001
Pelvic	0.10	0.044 to 0.23	< .001	0.14	0.033 to 0.57	.006
Other	0.44	0.33 to 0.58	< .001	0.39	0.26 to 0.60	< .001
Nonadrenal	1	Ref	Ref	1	Ref	Ref
Adrenal	2.47	2.16 to 2.82	< .001	2.09	1.67 to 2.63	< .001
Nonthoracic	1	Ref	Ref	1	Ref	Ref
Thoracic	0.14	0.096 to 0.20	< .001	0.20	0.11 to 0.39	< .001

Abbreviations: INSS, International Neuroblastoma Staging System; OR, odds ratio; Ref, reference.

as stratification variables, including age, stage, and *MYCN* status. All statistical analyses were performed using STATA, version 13 (STATA, College Station, TX).

RESULTS

Clinical and Biologic Features Differ by Primary Tumor Site

The clinical and biologic characteristics at diagnosis of the 8,369 patients in the INRG analytic cohort with an assigned primary tumor site are listed in Table 1, and include 47% with adrenal, 24% with abdominal/retroperitoneal, 15% with thoracic, 3% with pelvic, 3% with neck, and 8% with other primary tumor sites. Each of the evaluated clinical and biologic features showed statistically significant differences when compared across all six primary site categories ($P <$ .001 for all comparisons; Table 1). The most prominent differences (> 10% difference) seemed to be a lower proportion of patients with stage 4 disease, elevated ferritin, elevated LDH, *MYCN* amplification, LOH/aberration at 1p, gain of 17q, 11q aberration, pooled segmental chromosomal aberrations at these loci, and unfavorable International Neuroblastoma Pathology Classification category in thoracic, neck, and pelvic primary tumor sites compared with adrenal primary tumors (Table 1).[11] We also assessed the frequency of International Neuroblastoma Staging System stage 3 tumors across primary sites (n = 1,440 stage 3 tumors in total). Pelvic tumors had the highest frequency of stage 3 disease (41% of pelvic tumors were stage 3), followed by abdominal/retroperitoneal (28%), other (19%), thoracic (18%), adrenal (11%), and neck (10%) sites.

To evaluate some of these differences more closely, we compared features according to the group site variables: adrenal versus nonadrenal and thoracic versus nonthoracic (Table 2). Patients with adrenal tumors had statistically significantly higher proportions of most unfavorable risk factors compared with patients with nonadrenal tumors. In contrast, patients with thoracic tumors had statistically significantly lower proportions of most unfavorable risk factors compared with patients with nonthoracic tumors. Interestingly, there was a higher proportion of thoracic tumors observed in the earlier era (before 1996) compared with more recently diagnosed patients.

Given the striking differences in the incidence of *MYCN* amplification between primary tumor sites, we used logistic regression analysis to assess whether these differences were independent of differences in other features associated with *MYCN* amplification, including age, stage, and grade of differentiation (Table 3). Adrenal primary tumors had double the odds of having *MYCN* amplification compared with nonadrenal primary tumors after controlling for these potential confounders (adjusted odds ratio, 2.09; 95% CI, 1.67 to 2.63; $P <$ .001). Conversely, thoracic primary tumors had one fifth the odds of having *MYCN* amplification compared with nonthoracic primary tumors (adjusted odds ratio, 0.20; 95% CI, 0.11 to 0.39; $P <$ .001).

We also evaluated whether metastatic pattern differs according to primary tumor site. Of the 3,298 patients with stage 4 disease, only 2,899 patients had documented site(s) of metastases in the INRG database and were included in this analysis (Appendix Table 1, online only). Only incidence rates of bone marrow, bone, liver, and "other" metastatic sites showed statistically significant differences across all six primary tumor categories. Specifically, the highest proportion of metastases to the bone marrow (77%), bone (65%), and liver (20%) originated from adrenal primary tumor sites. Bone marrow metastases were also common in patients with abdominal/retroperitoneal (72%) and pelvic (71%) stage 4 tumors. Patients with neck, pelvic, and thoracic stage 4 tumors had lower rates of bone metastasis. Patients with adrenal or abdominal/retroperitoneal metastatic tumors were more likely to have liver metastasis compared with patients with other primary sites.

EFS and OS Differ According to Primary Tumor Site

We next evaluated potential differences in EFS and OS according to primary tumor site. Log-rank tests detected statistically significant differences in times to both outcomes according to primary tumor site. The unadjusted 5-year EFS and OS rates according to the six primary tumor sites were as follows (± SE): adrenal, 56% ± 0.8% and 62% ± 0.8%; abdominal/retroperitoneal, 64% ± 1.1% and 72% ± 1.1%; neck, 79% ± 2.8% and 90% ± 2.2%; thoracic, 80% ± 1.2% and 88% ± 1.0%; pelvic, 81% ± 2.6% and 91% ± 2.0%; and other, 63% ± 2.2% and 70% ± 2.2%, respectively (Figs 1A and 1B; $P <$ .001). Evaluating adrenal versus nonadrenal tumors, we again observed statistically significant differences; the unadjusted 5-year EFS and OS rates were significantly lower for adrenal (estimates previously given) versus nonadrenal primary tumors (EFS and OS for nonadrenal,

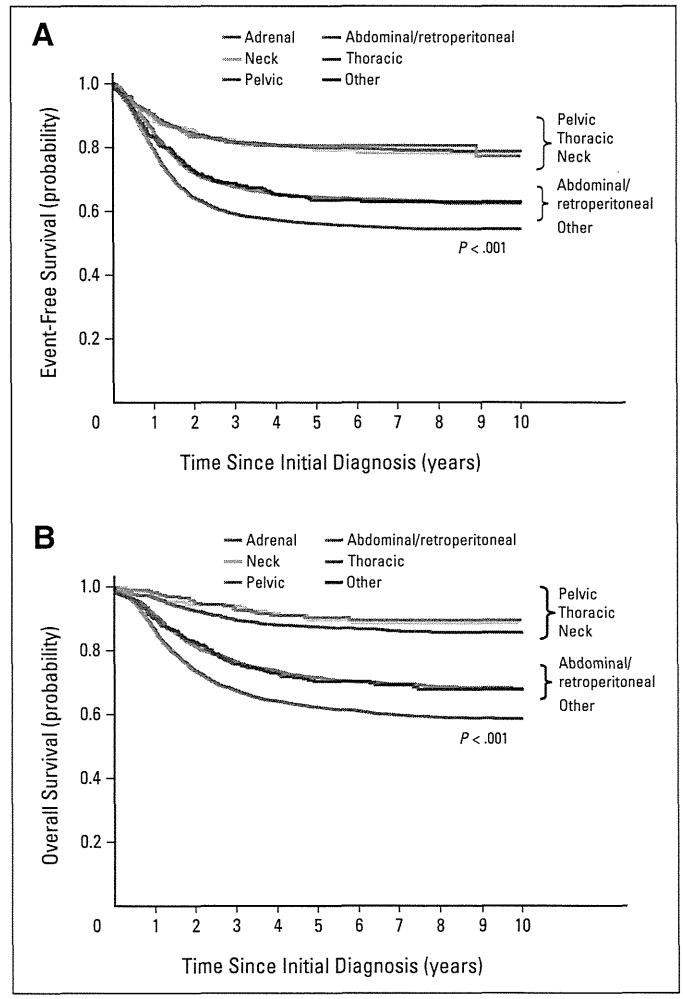

Fig 1. Kaplan-Meier estimated (A) event-free survival and (B) overall survival from time of diagnosis according to primary tumor site.

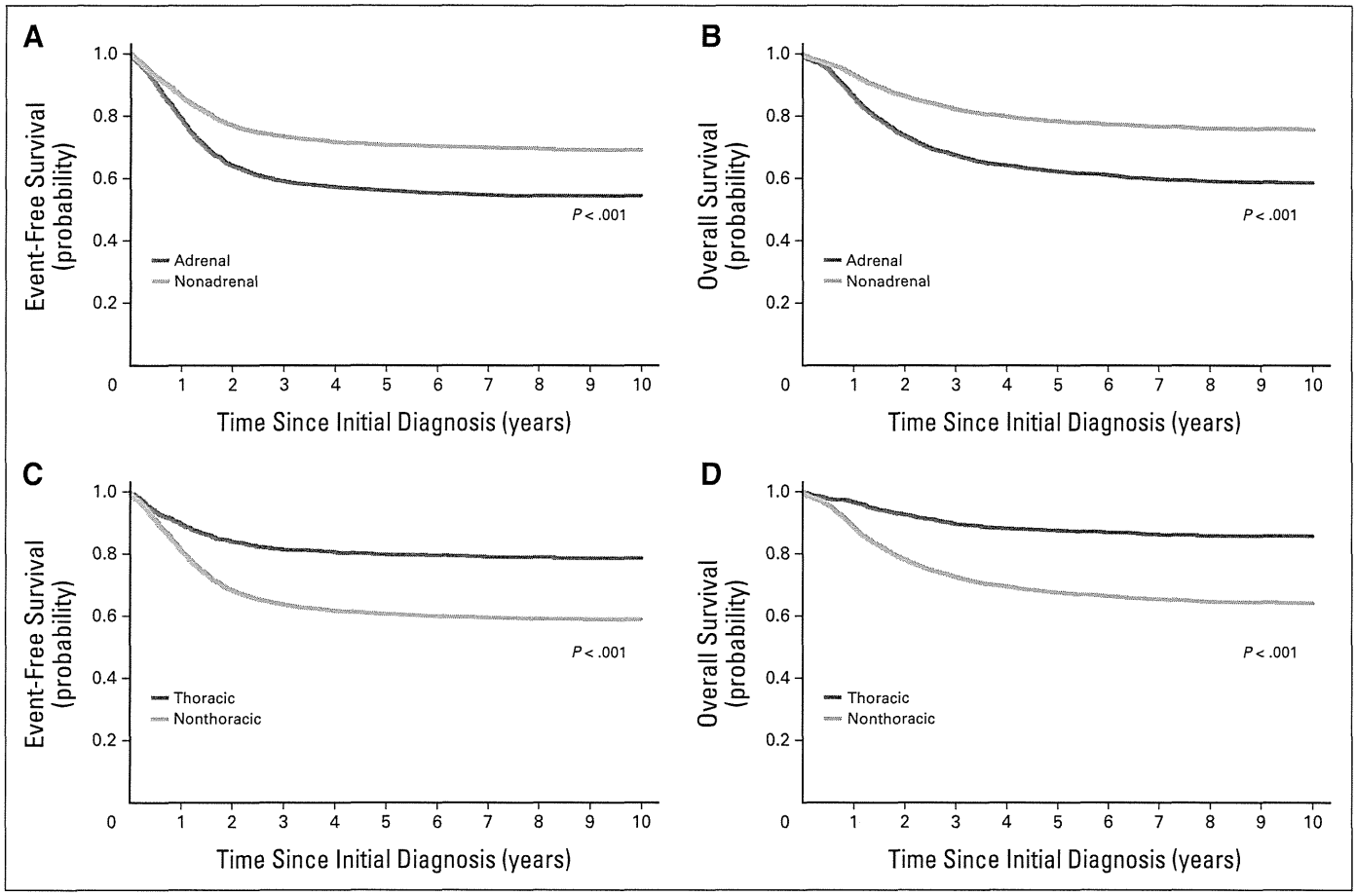

Fig 2. Kaplan-Meier estimated (A) event-free survival and (B) overall survival from time of diagnosis for patients with adrenal versus nonadrenal primary tumor sites. Kaplan-Meier estimated (C) event-free survival and (D) overall survival from time of diagnosis for patients with thoracic versus nonthoracic primary tumor sites.

71% ± 0.7% and 78% ± 0.7%; $P < .001$; Figs 2A and 2B). The opposite was true for thoracic (estimates previously given) versus nonthoracic primary tumors (EFS and OS for nonthoracic, 61% ± 0.6% and 68% ± 0.6%; $P < .001$; Figs 2C and 2D).

Our finding that the three main prognostic factors in NB (age, stage, and *MYCN* status) also differed significantly according to primary tumor site raised the possibility that these differences confounded our univariable observation of differential EFS and OS according to primary tumor site. We therefore constructed Cox proportional hazards models to control for these differences in age, *MYCN* status, and stage (Table 4). In a model evaluating EFS in all six primary tumor sites, only patients with thoracic tumors remained at a decreased risk for an event compared with the reference group of patients with adrenal tumors (adjusted HR, 0.76; 95% CI, 0.64 to 0.89; $P = .001$). Using a similar model for OS, patients with thoracic (adjusted HR, 0.65; 95% CI, 0.52 to 0.80; $P < .001$) or neck (adjusted HR, 0.54; 95% CI, 0.34 to 0.94; $P = .029$) primary tumor sites were at decreased risk for death compared with patients with adrenal tumors. In similar models evaluating adrenal versus nonadrenal tumors, patients with adrenal tumors remained at increased risk for event (adjusted HR, 1.13; 95% CI, 1.03 to 1.23; $P = .008$) and death (adjusted HR, 1.17; 95% CI, 1.05 to 1.29; $P = .003$) compared with patients with nonadrenal tumors. Conversely, patients with thoracic tumors remained at decreased risk for event (HR, 0.79; 95% CI, 0.67 to 0.92; $P = .003$) and death (adjusted HR, 0.68; 95% CI, 0.56 to 0.84; $P < .001$) compared with patients with nonthoracic tumors.

DISCUSSION

In this large comprehensive analysis of primary tumor site in NB, we observed that the primary tumor site may influence some of the heterogeneity in the clinical features, tumor biology, and clinical outcomes in NB. We found statistically significant differences in clinical and biologic characteristics between primary tumor sites. We also observed that patients with primary adrenal tumors had inferior EFS and OS independent of age at diagnosis, *MYCN* status, and International Neuroblastoma Staging System stage. This is in contrast to patients with primary thoracic tumors, who had superior EFS and OS when controlling for these same variables.

Our findings that clinical and biologic features differ according to primary tumor site confirm and extend previous observations. For example, our findings that adrenal tumors are associated with unfavorable prognostic features were also shown in previous smaller studies.[4,5] Likewise, other groups have shown that thoracic primary tumors are associated with younger age, *MYCN* nonamplified tumors, hyperdiploid tumors, and normal LDH and ferritin values.[15,16] To