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	95% CI	P	Adjusted OR	95% CI	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,

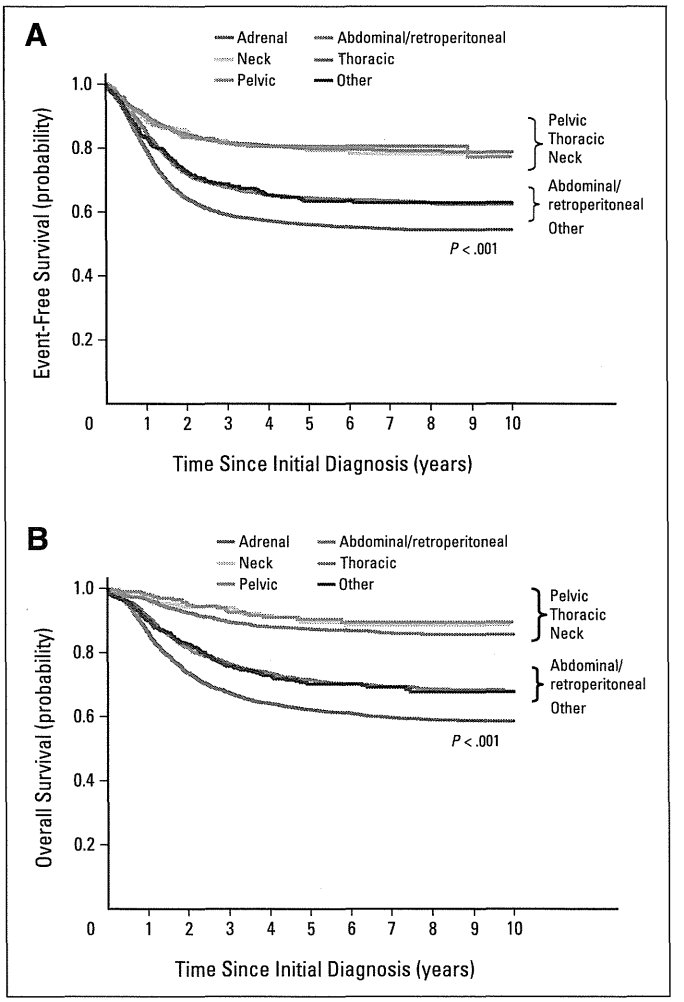

Fig 1. Kaplan-Meier estimated (A) event-free survival and (B) overall survival from time of diagnosis according to primary tumor site.

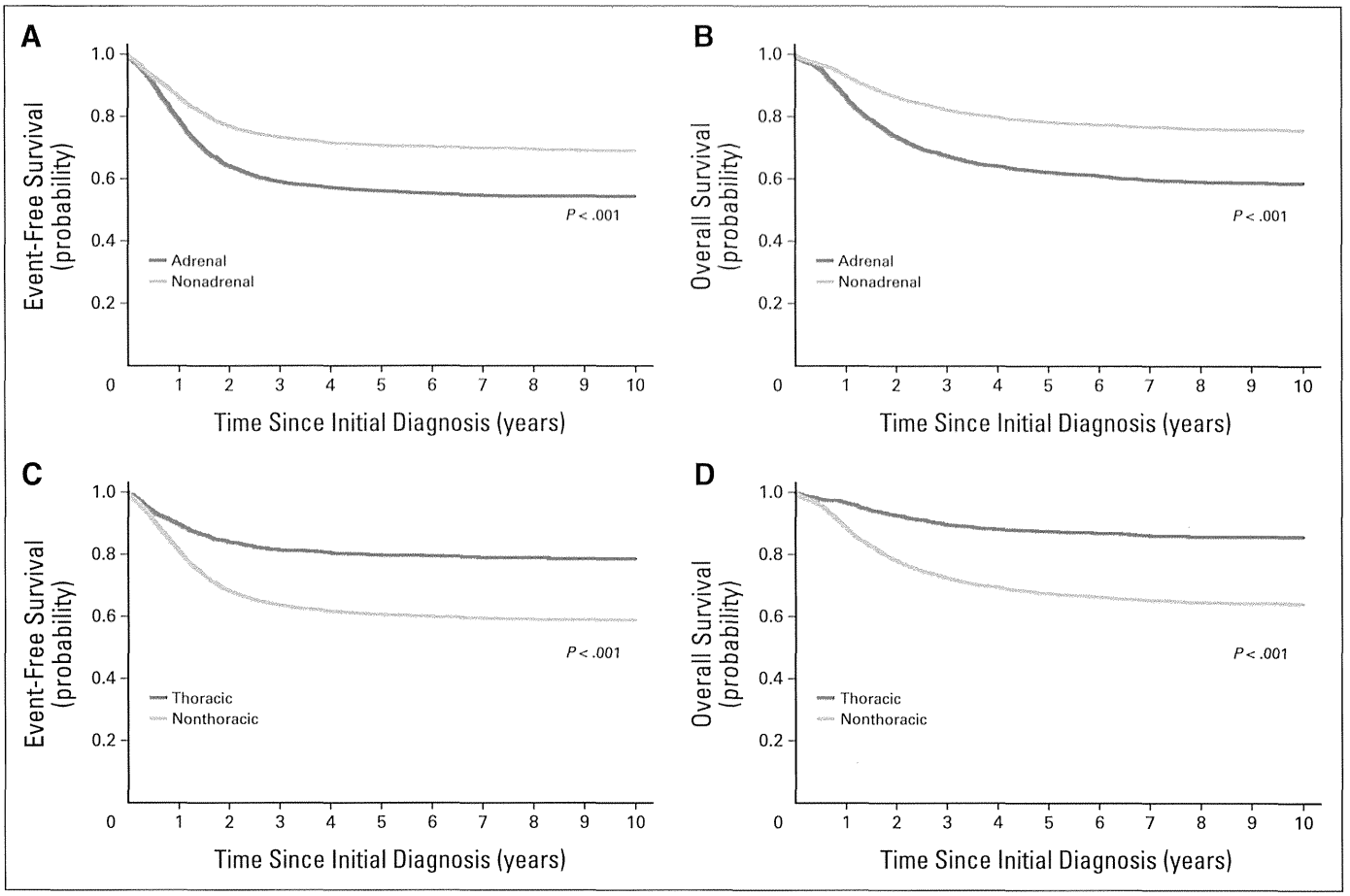

Fig 2. Kaplan-Meier estimated (A) event-free survival and (B) overall survival from time of diagnosis for patients with adrenal versus nonadrenal primary tumor sites. Kaplan-Meier estimated (C) event-free survival and (D) overall survival from time of diagnosis for patients with thoracic versus nonthoracic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

Table 4. Cox Proportional Hazards Regression Analysis of the Association Between Primary Tumor Site and Event-Free and Overall Survival Without (unadjusted HR) and With Adjustment (adjusted HR) for Age at Diagnosis, *MYCN* Status, and INSS Stage

	Unadjusted			Adjusted		
Primary Tumor Site	HR	95% CI	*P*	HR	95% CI	*P*
			Event-Free Survival			
Adrenal	1	Ref	Ref	1	Ref	Ref
Abdominal/retroperitoneal	0.75	0.69 to 0.83	< .001	0.94	0.85 to 1.04	.225
Neck	0.40	0.30 to 0.54	< .001	0.98	0.70 to 1.36	.886
Thoracic	0.39	0.34 to 0.45	< .001	0.76	0.64 to 0.89	.001
Pelvic	0.39	0.29 to 0.51	< .001	0.89	0.64 to 1.24	.503
Other	0.75	0.65 to 0.87	< .001	0.85	0.72 to 1.02	.079
Nonadrenal	1	Ref	Ref	1	Ref	Ref
Adrenal	1.67	1.55 to 1.80	< .001	1.13	1.03 to 1.23	.008
Nonthoracic	1	Ref	Ref	1	Ref	Ref
Thoracic	0.46	0.40 to 0.52	< .001	0.79	0.67 to 0.92	.003
			Overall Survival			
Adrenal	1	Ref	Ref	1	Ref	Ref
Abdominal/retroperitoneal	0.70	0.63 to 0.77	< .001	0.94	0.84 to 1.06	.313
Neck	0.21	0.14 to 0.33	< .001	0.54	0.31 to 0.94	.029
Thoracic	0.28	0.24 to 0.33	< .001	0.65	0.52 to 0.80	< .001
Pelvic	0.20	0.13 to 0.30	< .001	0.65	0.39 to 1.07	.090
Other	0.70	0.59 to 0.83	< .001	0.86	0.70 to 1.06	.157
Nonadrenal	1	Ref	Ref	1	Ref	Ref
Adrenal	1.97	1.81 to 2.14	< .001	1.17	1.05 to 1.29	.003
Nonthoracic	1	Ref	Ref	1	Ref	Ref
Thoracic	0.34	0.29 to 0.40	< .001	0.68	0.56 to 0.84	< .001

Abbreviations: HR, hazard ratio; INSS, International Neuroblastoma Staging System; Ref, reference.

our knowledge, our finding that more thoracic tumors were diagnosed before 1996 is novel, and may reflect the impact of earlier NB screening efforts that identified a higher proportion of patients with favorable disease.[17,18] Previous studies have shown that the pelvic primary site in NB is a favorable location, with lower rates of *MYCN* amplification and advanced stage than nonpelvic primary tumor sites.[6,7] Given the rarity of occurrence, previous studies of primary NBs of the neck and cervical region are limited to small case series that suggest that favorable clinical and biologic features are also associated with these tumors, including lower-stage disease and less *MYCN* amplification.[19,20] To date, there are no indications that molecular events involved in tumorigenesis are distinct in NB according to primary site, although no genomic studies comparing DNA mutations by primary site have been performed.

It is known that *MYCN*-amplified NBs are characterized by highly aggressive behavior with unfavorable outcome. Perhaps the most striking biologic difference observed in the current study was in the proportion of *MYCN* amplification across primary tumor sites. Although other groups have reported such differences, we show for the first time, to our knowledge, that the substantially different rates of *MYCN* amplification according to primary tumor site are independent of other factors associated with *MYCN* amplification, including age, stage, and grade of differentiation. It is not clear whether developing neuroblasts in the adrenal medulla might be more susceptible to amplification at the *MYCN* locus or if our findings simply reflect a greater number of cells at risk for undergoing *MYCN* amplification at that site because of its size compared with other sympathetic tissues.

We also confirmed previous smaller analyses that demonstrated differences in outcomes according to primary site. For example, the superior unadjusted EFS and OS rates of thoracic primary tumors found in this study (80.0% and 87.6%, respectively) are comparable with those seen in single-center or cooperative group studies with overall survival rates ranging from 71.2% to 100%.[8,15,16,21] The biologically favorable profile of thoracic tumors may explain the better prognosis in these tumors. In one study, *MYCN*-amplified tumors with a thoracic primary were shown to have a better outcome compared with all nonthoracic NB tumors in a previous univariable survival analysis; however, a multivariable Cox analysis was not conducted in that study.[16] A key advantage of our study is our ability to control for potential confounding variables that might be associated both with primary tumor site and prognosis. As the largest multivariable analysis addressing the prognostic impact of primary tumor site, our study demonstrated that the inferior outcomes for patients with adrenal tumors and the superior outcomes for patients with thoracic and neck tumors are independent of differences in age, stage, and *MYCN* status associated with these sites. Interestingly, patients with neck tumors were at decreased risk of death, but not at decreased risk of an analytic event. One reason for this finding may be that, whereas cervical and cervicothoracic NBs have favorable prognostic features, their anatomic localization makes it difficult to completely resect them in many patients, and these tumors tend to recur locally.[19,22-24]

We have confirmed that primary tumor site plays an important role in the heterogeneity of NB. To address our aims, we used the INRG database to evaluate the largest available cohort of patients with NB. However, there are several limitations to analyzing data from a tumor registry. We were limited to the available variables in the registry. As such, we were unable to report on important variables, such as extent of surgery and chemotherapy/radiation treatment used. Although we were able to describe the frequency of stage 3 disease by primary site, we were not able to assess whether a patient was deemed

to have stage 3 disease because of tumor crossing the midline, contralateral node involvement, or both. In addition, we were unable to confirm the primary tumor site designation of the "other" category, which may represent rare primary tumor sites, multifocal primary tumors, large tumors that may cross two or more anatomic compartments, or tumors of unknown origin. Along these lines, some large so-called abdominal/retroperitoneal tumors may have had ambiguous origins and may have actually arisen from adjacent adrenal or pelvic sites. It should be noted that the INRG database contains data from multiple cooperative groups, and it is possible that some patients who were included in our analysis were reported in previous studies on this topic. In addition, contributing cooperative groups may have used slightly different definitions of sites of disease. Moreover, our classification of segmental chromosomal aberrations included only the LOH/aberration at 1p, gain of 17q, and 11q aberration. Our evaluation of these variables separately and also as a pooled variable may differ from previous smaller studies, but these genetic aberrations have been shown to have prognostic significance in previous INRG studies.[12,13] Finally, some clinical and biologic variables were missing for some patients, as noted in Tables 1 and 2, and we could only report on what was available in the INRG database.

On the basis of our findings, we conclude that there are statistically significant differences in clinical features, biologic characteristics, and outcomes between primary tumor sites in NB. Our results suggest that there is something distinctive about the tumor in these specific sites of origin that leads to or reflects different biology and clinical behavior. Further study of the developmental biology of the neural crest and sympathetic nervous system may elucidate the etiology for these observed differences. Likewise, additional efforts should be directed at elucidating the disordered mechanisms of embryonal tumorigenesis in NB.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

AUTHOR CONTRIBUTIONS

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Data analysis and interpretation: Kieuhoa T. Vo, Katherine K. Matthay, John Neuhaus, Wendy B. London, Peter F. Ambros, Akira Nakagawara, Doug Miniati, Kate Wheeler, Andy D.J. Pearson, Susan L. Cohn, Steven G. DuBois
Manuscript writing: All authors
Final approval of manuscript: All authors

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GLOSSARY TERMS

Cox proportional hazards regression model: a statistical model for regression analysis of censored survival data, examining the relationship of censored survival distribution to one or more covariates. This model produces a baseline survival curve, covariate coefficient estimates with their standard errors, risk ratios, 95% CIs, and significance levels.

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.

logistic regression analysis: a multivariable regression model in which the log of the odds of a time-fixed outcome event (eg, 30-day mortality) or other binary outcome is related to a linear equation.

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

MYCN: gene encoding for c-myc.

overall survival: the duration between random assignment and death.

Appendix

Table A1. Sites of Metastases of Patients With Stage 4 Neuroblastoma by Primary Tumor Site (n = 2,899)

	All (n = 2,899)		Primary Tumor Site												
			Adrenal (n = 1,773)		Abdominal/ Retroperitoneal (n = 628)		Neck (n = 38)		Thoracic (n = 258)		Pelvic (n = 34)		Other (n = 171)		
Metastatic Site	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	*P**
Bone marrow	2,146	74	1,362	77	455	72	24	63	166	64	22	71	117	68	< .001
Bone	1,783	61	1,158	65	361	57	16	42	132	51	14	45	102	60	< .001
Distant lymph nodes	1,000	34	635	36	199	32	14	37	85	33	9	29	58	34	.499
Liver	514	18	359	20	100	16	3	8	27	10	3	10	22	13	< .001
Lung	99	3	68	4	22	3	0	0	3	1	1	3	5	3	.269
CNS	76	3	55	3	10	2	2	5	4	2	0	0	5	3	.199
Skin	80	3	48	3	15	2	1	3	7	3	0	0	9	5	.390
Other	820	28	520	29	143	23	11	29	72	28	9	29	65	38	.003

*P value refers to χ^2 test.

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ORIGINAL REPORT

Metastatic Neuroblastoma Confined to Distant Lymph Nodes (stage 4N) Predicts Outcome in Patients With Stage 4 Disease: A Study From the International Neuroblastoma Risk Group Database

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ABSTRACT

Purpose
The presence of distant metastases is one of the most powerful predictors of outcome in patients with neuroblastoma. However, the pattern of metastatic spread is not incorporated into current risk stratification systems. Small case series have suggested that patients with neuroblastoma who have metastatic disease limited to distant lymph nodes (4N disease) may have improved outcomes.

Patients and Methods
We analyzed retrospective data from the International Neuroblastoma Risk Group database for patients diagnosed from 1990 to 2002. 4N patients were compared with the remaining stage 4 patients (non-4N), excluding those with missing metastatic site data.

Results
In all, 2,250 International Neuroblastoma Staging System stage 4 patients with complete data were identified, of whom 146 (6.5%) had 4N disease. For 4N patients, event-free survival (EFS; 5-year, 77% ± 4%) and overall survival (OS; 5-year, 85% ± 3%) were significantly better than EFS (5-year, 35% ± 1%) and OS (5-year, 42% ± 1%) for non-4N stage 4 patients ($P < .001$). 4N patients were more likely to be younger ($P < .001$) and have tumors with favorable characteristics, including absence of *MYCN* amplification (89% *v* 69%; $P < .001$). In a multivariable analysis, 4N disease remained a significant predictor of outcome (hazard ratio for non-4N *v* 4N: 3.40 for EFS and 3.69 for OS). Within subgroups defined by age at diagnosis and tumor *MYCN* status, 4N disease was significantly associated with improved outcomes.

Conclusion
4N represents a subgroup with better outcome than that of other patients with metastatic disease. These findings suggest that the biology and treatment response of 4N tumors differ from other stage 4 tumors, and less intensive therapy should be considered for this cohort. Future exploration of biologic factors determining the pattern of metastatic spread is warranted.

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INTRODUCTION

Risk stratification is a key principle of current neuroblastoma treatment protocols, and therapy is determined by prognostic factors, including patient age, tumor stage, histology, ploidy, and *MYCN* amplification (MNA) status.[1] The value of incorporating additional genetic markers (ie, segmental chromosome aberrations [SCAs] such as loss of 11q) is currently being explored. Patients older than age 18 months with metastatic (stage 4) disease, most commonly involving bone and bone marrow, typically have a poor prognosis despite intensive multimodal therapy.[1] Although the prognostic significance of metastatic spread to specific sites has not been extensively studied, case reports and small case series have raised the possibility that patients with metastatic disease confined to distant lymph nodes (4N disease; previously IV-N) may have a better outcome.[2-6]

In a single-institution series of six patients with Evans stage IV neuroblastoma and extensive lymph node metastases but no extranodal disease, three patients were long-term survivors in comparison to none of the 40 patients with standard stage IV disease (extranodal involvement).[2] Subsequently,

Yamada et al[3] reported 52 patients with stage III to IV disease, of whom eight had N3 disease (distant nodal involvement). Three of these had metastatic disease that was limited to nodal sites. Among patients with stage IV disease, there was a trend toward better overall survival (OS) in those with N3 disease compared with other groups. There was also a trend toward an association between N3 stage IV disease and the absence of MNA (zero of four MNAs) compared with other stage IV patients (11 of 22 MNAs). Abramson et al[4] reported a series of eight patients with abdominal primary tumors and specific distant node involvement of the left supraclavicular lymph nodes (Virchow's node). Four were long-term survivors (3 to 11 years). In a separate case report, a 10-year-old patient with stage IV-N, non-MNA neuroblastoma was also a long-term survivor.[5] In an analysis of the prognostic impact of different metastatic sites in 434 patients older than 1 year of age with stage 4 neuroblastoma, 11 patients (2.5%) had 4N disease, with a nonsignificant trend toward improved event-free survival (EFS) for these individuals.[6] Loss of heterozygosity (LOH) at 19q13 has been suggested as a marker for locoregional disease with reported increased frequency in patients with stage 3 or stage 4N disease.[7] In that series, 19q LOH was detected in four (67%) of six stage 4N patients but in only four (7%) of 55 non-4N stage 4 patients. Finally, in an analysis of 218 patients with stage 4 disease treated with high-dose chemotherapy and stem-cell rescue, Hartmann et al[8] reported the absence of bone marrow metastases at diagnosis as a favorable prognostic marker, although it is important to note that these patients cannot be formally identified as stage 4N (ie, they may have had extralymphatic metastases to other sites).

The International Neuroblastoma Risk Group (INRG) database brings together patient data from groups in North America, Europe, Australia, and Japan and is the largest single source of data on neuroblastoma, containing information on more than 8,800 children.[1] This resource therefore provides a unique opportunity to establish whether stage 4N neuroblastoma represents a defined subgroup of patients with metastatic disease and to examine differences in prognostic factors and outcome for this rare cohort.

PATIENTS AND METHODS

Patient Cohort

The INRG database includes data from the Children's Oncology Group (COG; North America/Australia), Society of Paediatric Oncology European Neuroblastoma Group (SIOPEN; predominantly Europe), German, and Japanese cooperative study groups. Patients age younger than 21 years with pathologically confirmed neuroblastoma diagnosed between January 1, 1990, and December 31, 2002, are currently included. Of the total 8,800 patients, 3,244 (37%) had stage 4 disease. Of these, 994 were excluded because of incomplete or inconsistent metastatic site data, leaving 2,250 patients in the final analytic cohort (26% of all patients in the database). Patient age, site of primary tumor, and follow-up data were available for all patients. Other variables, including serum ferritin, lactate dehydrogenase (LDH), MNA, and cytogenetic characteristics, were analyzed for those patients for whom data were available. Histology was classified as favorable or unfavorable according to International Neuroblastoma Pathology Classification (INPC) or the Shimada system.[1,9] The cohort of 4N patients was defined as those with positive distant lymph nodes, but no bone marrow, bone, liver, lung, CNS, skin, or other metastatic disease. Patients with missing or unknown pattern of metastatic disease were excluded. The INRG uses International Neuroblastoma Staging System (INSS)[10] or Evans stage[11] if INSS unknown, as the staging criteria.[1,12] Consequently, patients with regional lymph node involvement were not considered to have metastatic disease and thus do not meet criteria for inclusion in this analysis.

Statistical Methods

Time to event for EFS was defined as time from diagnosis to first relapse, progression, second malignancy, or death or until time of last contact if no event occurred. Time to event for OS was similarly defined as time from diagnosis to death or time of last contact if patient was alive. Estimates for 5-year EFS and OS were generated by using the Kaplan-Meier method, and curves were compared by using a log-rank test.[13] For univariable analyses to identify factors prognostic of EFS or OS, a 5% significance level was used without adjustment for multiple testing, except for primary tumor site for which Sidak adjustment for multiple comparisons was used. Patient characteristics and prognostic factors were compared by using t test or Mann-Whitney U test for continuous variables and Fisher's exact test or χ^2 test for binary or other categorical variables as appropriate. Variables such as age, LDH, and ferritin were dichotomized as per previous INRG database analyses.[1,14] Cox proportional hazards regression models were used to identify the most significant factors prognostic of outcome in multivariable analyses.

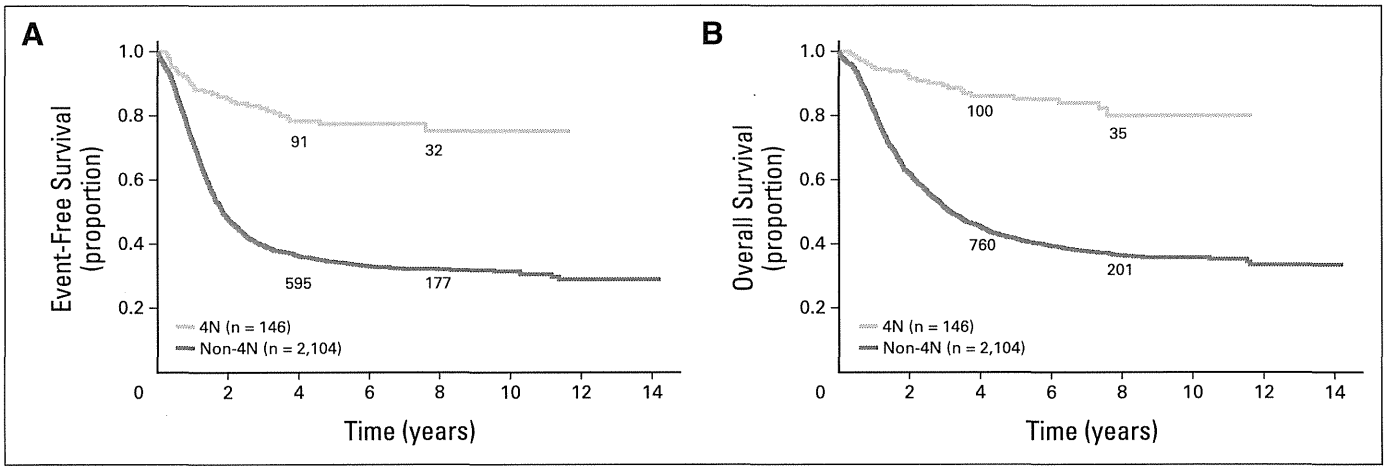

Fig 1. Patients with stage 4 neuroblastoma. (A) Event-free survival and (B) overall survival curves for patients with 4N disease (metastatic spread limited to distant lymph nodes) versus the balance of stage 4 patients (non-4N). $P < .001$ for both event-free and overall survival. The numbers of patients at risk for an event are shown along the curves at years 4 and 8.

RESULTS

Stage 4N Cohort

Data from 3,244 patients with stage 4 disease from the INRG database were analyzed. Those with missing or inconsistent data relating to metastatic site (n = 994) were excluded, leaving a final cohort of 2,250 patients. Comparison of EFS and OS showed that these excluded patients had a significantly worse outcome compared with the final analytic cohort ($P = .0024$ for EFS; $P < .001$ for OS; Figure A1, online only). Of the final group, 146 (6.5%) had a 4N pattern of disease (metastatic spread limited to distant lymph nodes), and the remaining 2,104 non-4N stage 4 patients served as the comparison cohort. For the 4N patients, estimated 5-year EFS and OS were 77% ± 4% and 85% ± 3%, both significantly better than those for non-4N stage 4 patients (EFS, 35% ± 1%; OS, 42% ± 1%; $P < .001$ for both EFS and OS; Fig 1). Comparison of clinical features demonstrated important differences between the two groups (Table 1; Appendix Table A1, online only). Stage 4N patients were younger (median age, 423 *v* 929 days; $P < .001$) and had tumors with more favorable histology, including INPC/Shimada histologic classification, grade of tumor differentiation, and mitosis karyorrhexis index (MKI). MNA was less frequent in stage 4N patients (11% *v* 31%; $P < .001$). Other cytogenetic features, including ploidy, 1p or 11q loss, or 17q gain, were not significantly different between the 4N and non-4N groups, although data were unavailable for many patients (see Appendix, online only). Patients with 4N disease were less likely to have an adrenal primary (40% *v* 60% $P < .001$) and more likely to have a thoracic tumor (26% *v* 10%; $P < .001$), consistent with increased frequency of thoracic primary tumors in patients age younger than 547 days.[15] Within the total stage 4 population, primary tumor was thoracic in 15% of patients age younger than 547 days versus 9.9% in those age ≥ 547 days ($P < .001$). Consistent with the more favorable outcome observed, 4N patients also had lower mean serum ferritin (147 *v* 324 ng/mL; $P < .001$) and LDH (1,207 *v* 1,763 U/L; $P = .0192$). Year of diagnosis was earlier for patients with 4N disease, with 77% diagnosed before 1996 (*v* 63% for non-4N patients; $P < .001$). In terms of therapy, 4N patients were less likely to receive intensive initial therapy than non-4N patients; therefore treatment differences are unlikely to account for the improved outcome of the 4N group (Appendix Table A2, online only).

The importance of the 4N pattern of disease as a prognostic factor was explored in a multivariable analysis by using Cox proportional hazards. A model incorporating known prognostic variables (INSS stage, age, *MYCN* status, year of diagnosis, serum ferritin, and LDH) for which adequate data were available (n = 952) confirmed that stage 4N is independently statistically significantly prognostic of improved EFS and OS after adjusting for these variables (Table 2). Similar results were obtained after the incorporation of histology, ploidy, grade, MKI, and 11q, 1p, and 17q status into the model, each with a category for unknown (Appendix Table A3, online only). The Cox model was also used to calculate the hazard ratios for stage 4N versus non-4N (range, 0.24 to 0.36) when tested individually with each prognostic factor in separate models (Appendix Table A4, online only).

Table 1. Comparison of Characteristics for 4N and Non-4N Stage 4 Patients

Characteristic	4N (n = 146) No.	%	Non-4N (n = 2,104) No.	%	*P*
Age					
Median, days	423		929		< .001
< 18 months	85	58	640	30	
≥ 18 months	61	42	1,464	70	< .001
Year of diagnosis					
1990-1995	113	77	1,314	62	
1996-2002	33	23	790	38	< .001
Ferritin/LDH (± SD)					
Mean ferritin, ng/mL	147 ± 261		324 ± 461		< .001
Mean LDH, U/L	1,207 ± 1,859		1,763 ± 2,236		.0192
Histologic category					
Favorable	45	63	219	26	
Unfavorable	27	37	609	74	< .001
Histologic grade					
Differentiating	9	21	44	8	
Undifferentiated/poorly differentiated	33	79	537	92	.0058
MKI					
Low	28	76	240	45	
Intermediate	6	16	158	29	
High	3	8	138	26	.0011
MYCN status					
Nonamplified	120	89	1,145	69	
Amplified	15	11	511	31	< .001
Cytogenetics					
Ploidy					
Hypodiploid/diploid	22	27	231	38	
Hyperdiploid	60	73	385	62	.0666
1p loss					
Yes	7	35	183	36	
No	13	65	318	64	1.0
17q gain					
Yes	3	50	100	64	
No	3	50	57	36	.6703
11q loss					
Yes	3	30	114	42	
No	7	70	154	58	.5270
Site of primary*					†
Adrenal	59	40	1273	60	< .001
Abdomen	38	26	498	24	N/S
Neck	6	4	25	1	N/S
Thorax	38	26	220	10	< .001
Pelvis	3	2	28	1	N/S
Other	4	2	78	4	N/S
Initial treatment					
None/surgery/conventional	71	77	502	30	
Intensive ± SCT	21	23	1168	70	< .001

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.

Prognostic Factors Within 4N Cohort

Many of the factors previously reported to affect outcome within the whole neuroblastoma population were also prognostic when examined within the 4N cohort (Table 3). Most significant in a univariable analysis for factors determining OS were patient age (using a cutoff at 547 days[14]; $P < .001$), tumor MNA status ($P < .001$), and INPC/Shimada histology classification ($P < .001$). Serum ferritin, LDH, tumor MKI, and initial treatment were also significant at the 5%

Table 2. Multivariable Cox Proportional Hazards Models (one model for EFS and one for OS) in 952 Patients Who Had Complete Data

	EFS			OS		
Risk Factor*	HR	95% CI	*P*	HR	95% CI	*P*
Stage						
4N disease	1	—		1	—	
Non-4N disease	3.40	2.00 to 5.81	< .001	3.69	2.02 to 6.71	< .001
Year of diagnosis						
1996-2002	1	—		1	—	
1990-1995	1.29	1.09 to 1.51	< .001	1.34	1.13 to 1.59	< .001
Age at diagnosis, days						
< 547	1	—		1	—	
≥ 547	2.16	1.74 to 2.68	< .001	2.25	1.79 to 2.84	< .001
MYCN amplification						
Nonamplified	1	—		1	—	
Amplified	1.76	1.47 to 2.10	< .001	1.93	1.60 to 2.32	< .001
Serum ferritin, ng/mL						
< 92	1	—		1	—	
≥ 92	1.54	1.26 to 1.89	< .001	1.48	1.19 to 1.84	< .001
Serum LDH, U/L						
< 580	1	—		1	—	
≥ 580	1.32	1.08 to 1.60	.0062	1.58	1.27 to 1.95	< .001

Abbreviations: EFS, event-free survival; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival.
*Other risk factors (histology, grade, mitosis karyorrhexis index, and ploidy) were not included in the model because missing data dramatically reduced the sample size and the model became uninformative.

level. Year of diagnosis was not correlated with outcome within the 4N cohort.

Subgroup Analysis

Because age and presence of MNA are independently prognostic of outcome within the INSS stage 4 population and are used for risk stratification within current international studies, we further evaluated the prognostic significance of 4N disease pattern in four subgroups defined by patient age (cutoff, 547 days) and tumor *MYCN* status. 4N patients had significantly improved EFS and OS compared with non-4N patients in each subgroup (Fig 2), except for patients age younger than 547 days with MNA tumors, a subgroup in which there were only four stage 4N patients. For patients age younger than 547 days with non-MNA tumors, 5-year EFS and OS were 92% ± 3% and 99% ± 1% for 4N disease compared with 83% ± 2% and 88% ± 2% for non-4N disease ($P = .03$ and $P = .004$, respectively; Fig 2A). The differences were more pronounced for patients age ≥ 547 days with non-MNA tumors; estimated 5-year EFS and OS were 63% ± 8% and 74% ± 7% for those with 4N disease, both significantly better than for non-4N patients (EFS, 27% ± 2%; OS, 38% ± 2%; $P < .001$ for both EFS and OS; Fig 2B). Within this subgroup of patients age ≥ 547 days with non-MNA tumors, comparison of characteristics between 4N and non-4N patients revealed no differences in patient age or site of primary tumor. However, patients with 4N disease were more likely to have tumors with favorable histologic characteristics, including Shimada/INPC classification, grade, and MKI (Table 4). Insufficient data were available to allow comparison of tumor ploidy or incidence of SCAs between 4N and non-4N patients. Finally, in the subgroup of patients age ≥ 547 days with MNA tumors, 5-year EFS and OS were again better for 4N patients (both 64% ± 15%) than for non-4N patients (EFS, 17% ± 2% [$P = .0133$]; OS, 22% ± 2% [$P = .0278$]).

DISCUSSION

Numerous prognostic factors for neuroblastoma have been identified, including patient characteristics (particularly age at diagnosis), disease extent (INSS stage), and tumor biology. The most significant predictive genetic factors are MNA[1,16] and SCAs, including 1p and 11q deletions.[17,18] For patients with stage 4 disease, the pattern of metastatic spread may also influence outcome, and several case reports and small case series have suggested that patients with only distant nodal metastatic involvement (4N disease) may have better outcomes.[2-5] Although an analysis of the prognostic significance of specific metastatic sites demonstrated that the presence of bone marrow metastases was predictive of poor outcome,[19] this report did not examine outcomes for patients with disease limited to a particular metastatic site, such as lymph nodes.

The INRG database represents the largest data set for patients with neuroblastoma, and the analysis presented here provides the most comprehensive analysis of 4N patients to date. These data demonstrate that patients with 4N disease have a markedly better outcome compared with other stage 4 patients. Although published cases suggested that 4N disease may be more common in older patients (median age of published cases, 4 years), this is not supported by our larger data set, in which more than half of 4N patients were infants age younger than 18 months. 4N disease is inversely correlated with MNA and, consequently, the prognostic significance of 4N disease can be at least partly explained by the association with younger age and absence of MNA—both factors strongly associated with improved outcome in stage 4 disease.[1] Nevertheless, both the subgroup and multivariable analyses confirm that 4N disease remains independently associated with improved outcome even after adjusting for age and *MYCN* status. The hazard ratio for non-4N disease (compared with 4N) of

Table 3. Univariable Analyses of Prognostic Factors for 4N Patients

Characteristic	Total		5-Year EFS			5-Year OS		
	No.	%	%	SE	*P*	%	SE	*P*
Overall patients	146		77	4		85	3	
Age, days								
< 547	85	58	91	3	< .001	98	2	< .001
≥ 547	61	42	59	6		69	6	
Year of diagnosis								
1990-1995	113	77	78	4	.7646	86	3	.5466
1996-2002	33	23	77	8		82	8	
MYCN status								
Nonamplified	120	89	81	4	.0172	90	13	< .001
Amplified	15	11	64	13		63	3	
Ferritin, ng/mL								
< 92	38	49	89	6	.0012	93	5	.0021
≥ 92	39	51	62	8		70	8	
LDH, U/L								
< 580	43	46	79	7	.1842	92	4	.0273
≥ 580	50	54	73	7		74	7	
Ploidy								
Hyperdiploid	60	73	82	5	.2485	89	4	.0776
Diploid/hypodiploid	22	27	73	10		73	11	
Histology								
Favorable	45	62	89	5	.0127	98	2	< .001
Unfavorable	27	38	61	10		68	10	
Histologic grade								
Differentiating	9	21	78	14	.9454	100		.0864
Undifferentiated/poorly differentiated	33	79	74	8		76	8	
MKI								
Low/intermediate MKI	34	92	79	7	.0407	87	6	.0062
High MKI	3	8	33	27		33	27	
Initial treatment								
None/surgery only	35	38	91	5		97	3	
Conventional chemotherapy	36	39	80	7		91	5	
Intensive ± SCT	21	23	59	11	.0065	69	10	.0024

Abbreviations: EFS, event-free survival; LDH, lactate dehydrogenase; MKI, mitosis karyorrhexis index; OS, overall survival; SCT, stem cell transplantation; SE, standard error.

approximately 3.5 for both EFS and OS is larger than for any of the other variables tested, which demonstrates that this metastatic pattern is powerfully prognostic of outcome within the stage 4 population. The overall frequency of 4N disease is low (6.5% of stage 4 patients); however, the risk reduction associated with 4N disease suggests that this metastatic pattern may need to be considered differently within the current risk stratification system. Recent efforts have attempted to identify subgroups of high-risk patients with the poorest outcomes, so-called "ultra-high-risk patients." Our findings suggest that, in contrast, there may also be subsets of patients such as those with 4N disease in which further treatment intensification may not be warranted or treatment reduction may be considered. Current standard therapy for high-risk patients includes chemotherapy, surgery, myeloablative therapy with stem-cell rescue, radiotherapy, immunotherapy, and differentiation therapy and is associated with significant short- and long-term toxicities. The definition of high-risk disease has already undergone several revisions, with it long being recognized that infants (age younger than 12 months) with neuroblastoma have a considerably better outcome, even if presenting with metastatic disease.[11] Consequently, these patients (provided their disease does not have MNA) are not considered high risk. More recently, the definition of high-risk disease has been further refined with those age 12 to 18 months with non-MNA metastatic disease (approximately 6% of all stage 4 patients) also excluded from the high-risk group.[1,14] Patients older than age 18 months with 4N disease may represent another subgroup that could be reclassified.

The improved outcome for 4N patients likely represents underlying biologic differences in the tumor, with pattern of metastatic spread being a surrogate marker for these differences. Comparison of histologic features between 4N and non-4N populations (within both the entire cohort and in subgroups of patients age ≥ 547 days and without MNA) confirmed that 4N disease is associated with differentiating grade, low MKI, and favorable histology, all characteristics of a more favorable tumor biology.[20] Ultimately, these variables likely reflect underlying genetic and chromosomal abnormalities, and 4N tumors may have a specific pattern of these abnormalities that distinguish them from other stage 4 neuroblastoma. There is limited cytogenetic information within the current INRG data set, and numbers were insufficient to demonstrate associations among 1p and 11q loss, 17q gain, or other SCAs and the 4N pattern of disease (see Appendix). Many preclinical studies and gene expression analyses in cancers, including breast cancer and melanoma, have demonstrated that specific messenger RNA expression signatures predict patterns or sites of

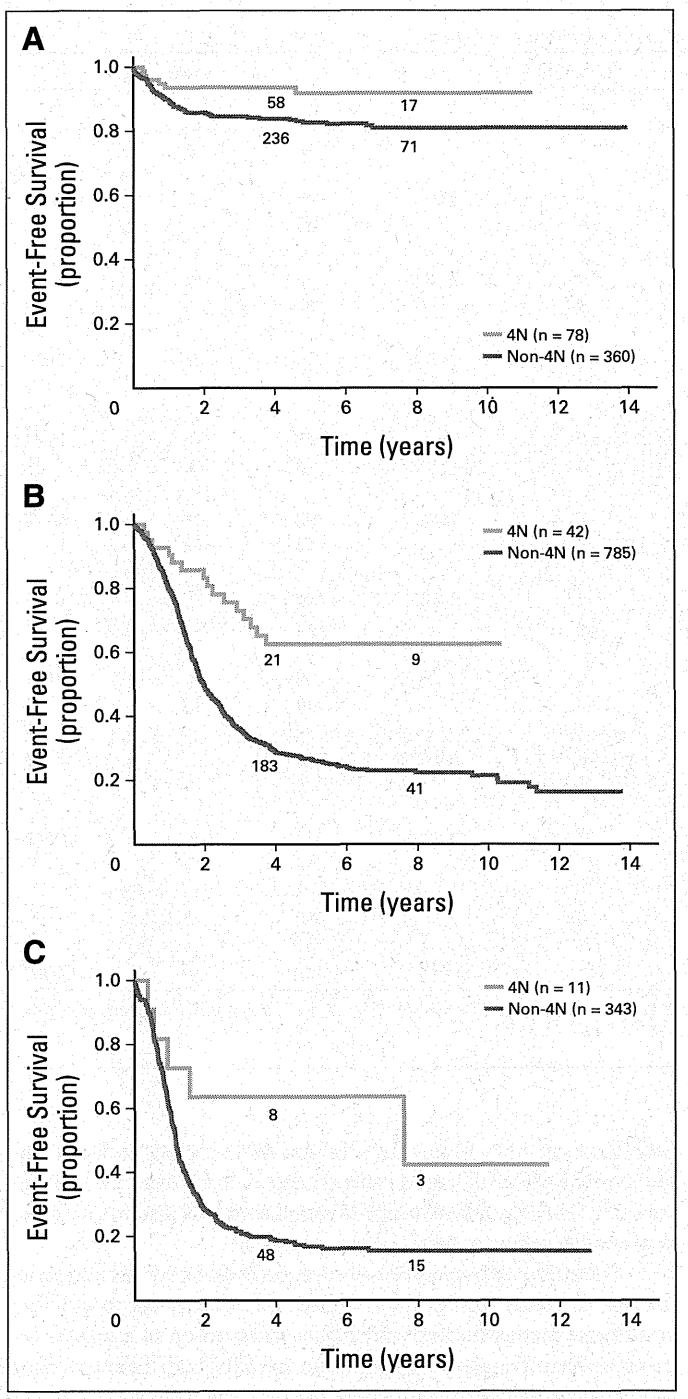

Fig 2. Event-free survival curves for patients with 4N versus non-4N disease for subgroups based on patient age at diagnosis and tumor *MYCN* status. (A) Patients age younger than 547 days with *MYCN* nonamplified tumors (hazard ratio [HR] for 4N disease, 0.51; 95% CI, 0.28 to 0.95; $P = .03$). (B) Patients 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.

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.

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.

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)		Non-4N (n = 2,104)		Excluded (n = 994)		*P* Excluded *v* 4N	*P* Excluded *v* Non-4N
	No.	%	No.	%	No.	%		
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.

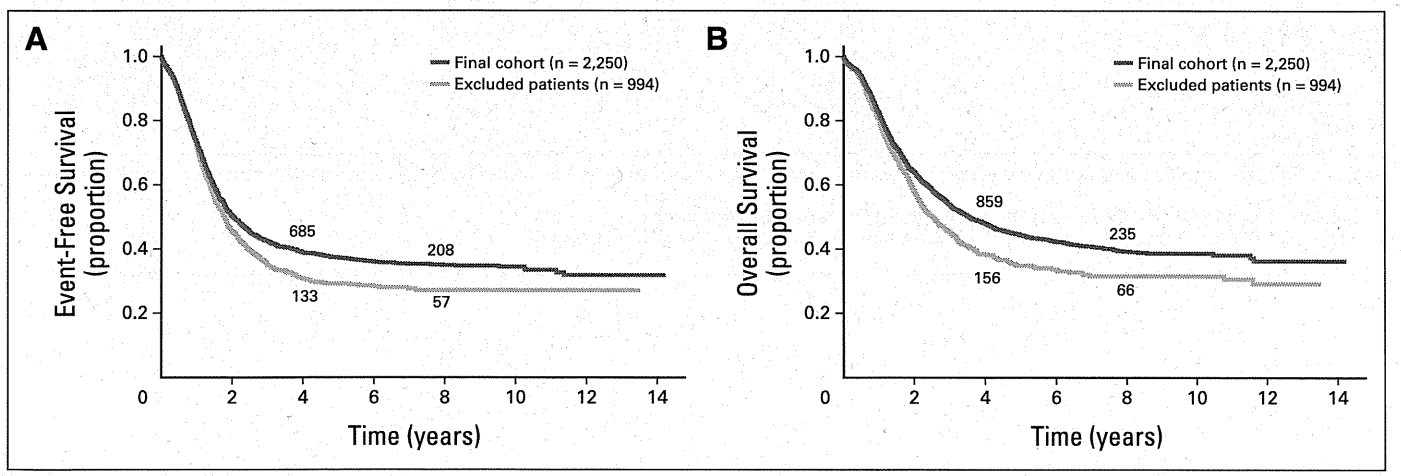

Fig A1. (A) Event-free survival and (B) overall survival curves for final analytic cohort of stage 4 patients (n = 2,250) versus stage 4 patients excluded from analysis because of missing/inconsistent metastatic site data (n = 994). $P = .0024$ for event-free survival; $P < .001$ for overall survival.