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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.

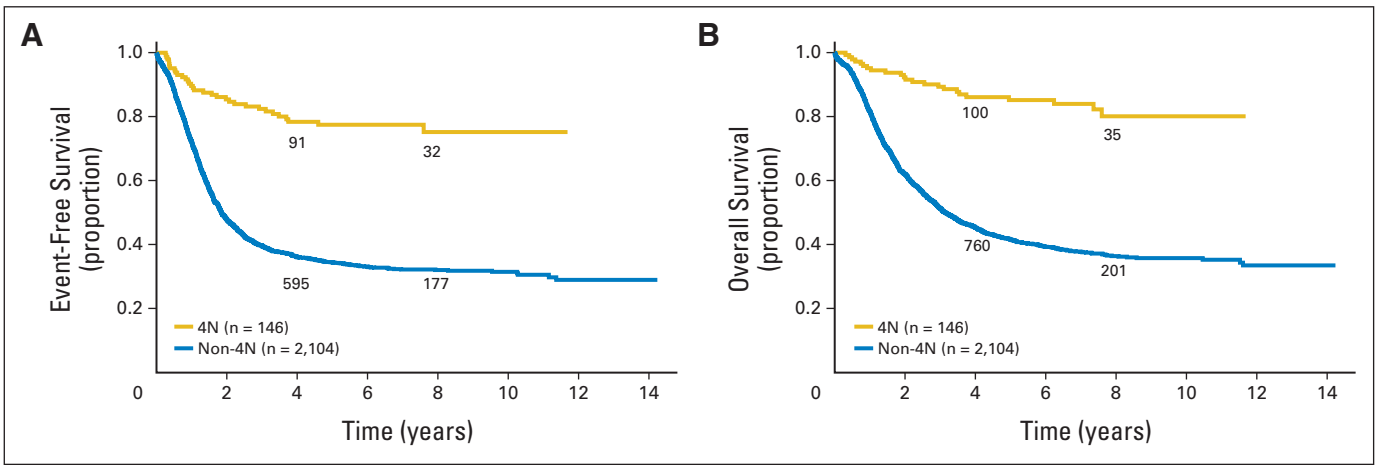

Fig 1. Patients with stage 4 neuroblastoma. (A) Event-free survival and (B) overall survival curves for patients with 4N disease (metastatic spread limited to distant lymph nodes) versus the balance of stage 4 patients (non-4N). $P < .001$ for both event-free and overall survival. The numbers of patients 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.	4N %	Non-4N (n = 2,104) No.	Non-4N %	*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

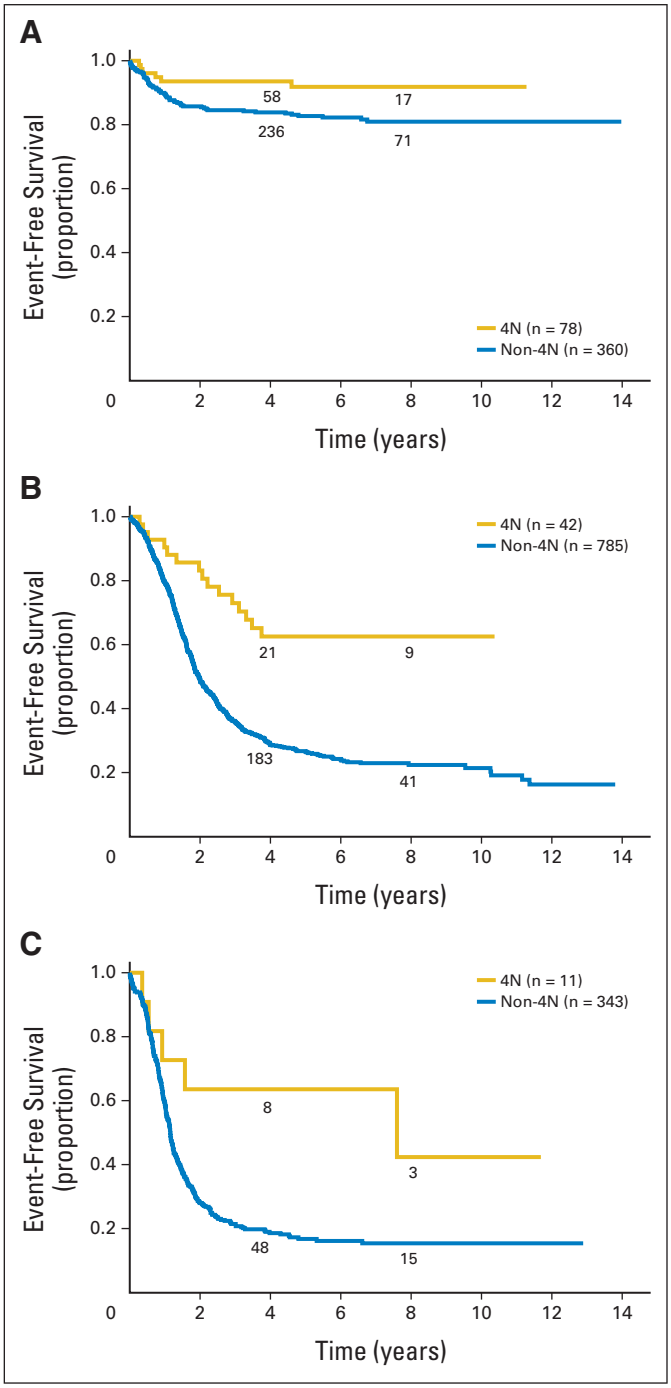

Fig 2. Event-free survival curves for patients with 4N versus non-4N disease for subgroups based on patient age at diagnosis and tumor *MYCN* status. (A) Patients age younger than 547 days with *MYCN* nonamplified tumors (hazard ratio [HR] for 4N disease, 0.51; 95% CI, 0.28 to 0.95; $P = .03$). (B) Patients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

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)		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.

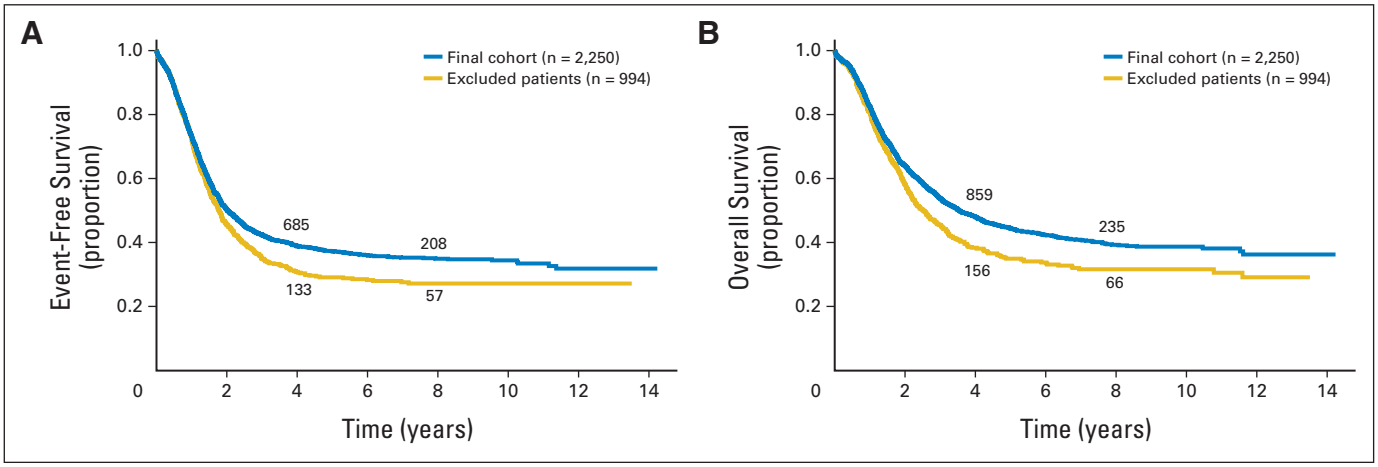

Fig A1. (A) Event-free survival and (B) overall survival curves for final analytic cohort of stage 4 patients (n = 2,250) versus stage 4 patients excluded from analysis because of missing/inconsistent metastatic site data (n = 994). $P = .0024$ for event-free survival; $P < .001$ for overall survival.