original reports

Age Dependency of the Prognostic Impact of Tumor Genomics in Localized Resectable *MYCN*-Nonamplified Neuroblastomas. Report From the SIOPEN Biology Group on the LNESG Trials and a COG Validation Group

Inge M. Ambros, MD[1]; Gian-Paolo Tonini, PhD[2]; Ulrike Pötschger, MSc[1]; Nicole Gross, PhD[3]; Véronique Mosseri, MD[4]; Klaus Beiske, MD, PhD[5]; Ana P. Berbegall, PhD[6,7]; Jean Bénard, PhD[8]; Nick Bown, PhD[9]; Huib Caron, MD, PhD[10]; Valérie Combaret, PhD[11]; Jerome Couturier, MD[12]; Raffaella Defferrari, PhD[13]; Olivier Delattre, MD, PhD[14]; Marta Jeison, PhD[15]; Per Kogner, MD, PhD[16]; John Lunec, PhD[17]; Barbara Marques, MSc[18]; Tommy Martinsson, PhD[19]; Katia Mazzocco, PhD[13]; Rosa Noguera, MD, PhD[6,7]; Gudrun Schleiermacher, MD, PhD[14,20]; Alexander Valent, PhD[8]; Nadine Van Roy, PhD[21]; Eva Villamon, PhD[6,7]; Dasa Janousek, MSc[1]; Ingrid Pribill, PhD[1]; Evgenia Glogova, MSc[1]; Edward F. Attiyeh, MD[22]; Michael D. Hogarty, MD[22]; Tom F. Monclair, MD, PhD[23]; Keith Holmes, ChM, DCH[24]; Dominique Valteau-Couanet, MD, PhD[25]; Victoria Castel, MD, PhD[26]; Deborah A. Tweddle, MD, PhD[27]; Julie R. Park, MD[28]; Sue Cohn, MD[29]; Ruth Ladenstein, MD[1,30]; Maja Beck-Popovic, MD[31]; Bruno De Bernardi, MD[32]; Jean Michon, MD[20]; Andrew D. J. Pearson, MD[33]; and Peter F. Ambros, PhD[1,30]

abstract

PURPOSE For localized, resectable neuroblastoma without *MYCN* amplification, surgery only is recommended even if incomplete. However, it is not known whether the genomic background of these tumors may influence outcome.

PATIENTS AND METHODS Diagnostic samples were obtained from 317 tumors, International Neuroblastoma Staging System stages 1/2A/2B, from 3 cohorts: Localized Neuroblastoma European Study Group I/II and Children's Oncology Group. Genomic data were analyzed using multi- and pangenomic techniques and fluorescence in-situ hybridization in 2 age groups (cutoff age, 18 months) and were quality controlled by the International Society of Pediatric Oncology European Neuroblastoma (SIOPEN) Biology Group.

RESULTS Patients with stage 1 tumors had an excellent outcome (5-year event-free survival [EFS] ± standard deviation [SD], 95% ± 2%; 5-year overall survival [OS], 99% ± 1%). In contrast, patients with stage 2 tumors had a reduced EFS in both age groups (5-year EFS ± SD, 84% ± 3% in patients < 18 months of age and 75% ± 7% in patients ≥ 18 months of age). However, OS was significantly decreased only in the latter group (5-year OS ± SD in < 18months and ≥ 18months, 96% ± 2% and 81% ± 7%, respectively; $P = .001$). In < 18months, relapses occurred independent of segmental chromosome aberrations (SCAs); only 1p loss decreased EFS (5-year EFS ± SD in patients 1p loss and no 1p loss, 62% ± 13% and 87% ± 3%, respectively; $P = .019$) but not OS (5-year OS ± SD, 92% ± 8% and 97% ± 2%, respectively). In patients ≥ 18 months, only SCAs led to relapse and death, with 11q loss as the strongest marker (11q loss and no 11q loss: 5-year EFS ± SD, 48% ± 16% and 85% ± 7%, $P = .033$; 5-year OS ± SD, 46% ± 22% and 92% ± 6%, $P = .038$).

CONCLUSION Genomic aberrations of resectable non–*MYCN*-amplified stage 2 neuroblastomas have a distinct age-dependent prognostic impact. Chromosome 1p loss is a risk factor for relapse but not for diminished OS in patients < 18 months, SCAs (especially 11q loss) are risk factors for reduced EFS and OS in those > 18months. In older patients with SCA, a randomized trial of postoperative chemotherapy compared with observation alone may be indicated.

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ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Neuroblastoma, the commonest extracranial solid tumor in infancy and childhood, accounts for 8%-10% of pediatric neoplasms and is responsible for 10% of childhood cancer deaths. As a result of the broad and divergent clinical spectrum of these tumors, prognostic markers are used to stratify therapy, which ranges from a wait-and-see strategy,[1-3] to surgery as the only treatment,[4-6] to high-dose chemotherapy with hematopoietic stem cell rescue.[7] Age at diagnosis and

CONTEXT

Key Objective

To determine whether detailed genomic information in localized, resectable, non–*MYCN*-amplified neuroblastoma, treated by surgery alone, provides a more precise therapeutic classification.

Knowledge Generated

Genomic analyses of localized, resectable neuroblastomas from two consecutive European studies and a North American cohort revealed a different prognostic impact of tumor genomics depending on patient age (< or ≥ 18 months). The presence of segmental chromosome aberrations, especially 11q loss, significantly reduced survival in patients ≥18 months of age with stage 2 neuroblastoma, but not in the cohort < 18 months.

Relevance

This study provides the rationale for more precise treatment decisions, by the inclusion of tumor genomic aberrations (segmental chromosome aberrations), for localized non–*MYCN*-amplified neuroblastoma than previously possible.

MYCN amplification (MNA) have both been related to the biologic tumor behavior. Eighteen months is the most effective age cutoff for risk-group stratification.[8-10] MNA confers an inferior prognosis to all patients with neuroblastoma (except metastatic disease at age > 18 months treated with current high-dose chemotherapy), was the first genomic marker used for therapy stratification,[11,12] and is used by cooperative groups for therapeutic decisions.[13] MNA can be classified as homogeneous (homMNA) when the vast majority of tumor cells show > 4-fold increase in *MYCN* signals (related to a reference probe on 2q) and virtually no tumor cells without *MYCN* gain or amplification. In heterogeneous tumors, MNA and nonMNA tumor-cell clones are found side by side.[14]

In 20% to 30% of patients, the disease is localized and resectable.[8,13] Whereas patients with International Neuroblastoma Staging System (INSS) stage 1 tumors without MNA (INSS stage 1 can be translated for most clinical purposes into International Neuroblastoma Risk Group [INRG] L1)[8] have excellent relapse-free and overall survival (OS) rates,[9,10] this is not the case for patients with INSS stage 2A/B tumors (because of the inclusion of INSS 3, INRG L2 cannot be considered equivalent to INSS 2) for whom significantly higher recurrence rates are reported.[15,16] In localized disease, up to two-thirds of non-MNA neuroblastomas bear segmental chromosome aberrations (SCAs). The most commonly found SCAs affecting whole or part of chromosomal arms (ie, losses of or at chromosomal arms 1p/3p/4p/11q; gains of or at 1q/2p/17q) were designated typical SCAs (typSCAs) by the International Society of Paediatric Oncology, European Neuroblastoma (SIOPEN) Biology Group.[17] A prognostic impact for these aberrations has been shown repeatedly.[18-24] Chromosome 1p loss was the first reported recurrent SCA, especially in MNA high-stage neuroblastoma, but kept its prognostic power for non-MNA neuroblastomas.[19,25,26] The most frequently detected aberration irrespective of *MYCN* status is unbalanced gain of 17q; however, its prognostic impact is still controversial.[27,28] Chromosome 11q loss in non-MNA tumors[19,29] and 1q gain are regarded prognostically significant.[22] The INRG classification schema is based on 4 clinical and morphologic features and on the *MYCN*-, 11q- status and tumor cell ploidy.[13] These genomic features and 1p information were used in the Children's Oncology Group (COG) as stratifying elements.[30] In the ongoing Low and Intermediate Neuroblastoma Trial (LINES; ClinicalTrials.gov identifier: NCT01728155) the SIOPEN group applies *MYCN* copy-number data and typSCA status, in addition to clinical parameters, to stratify therapy.

In the first European neuroblastoma treatment protocol, Localized Neuroblastoma European Study Group (LNESG) 1 (1995-1999), excision (irrespective of a tumor residue) was the only treatment of patients with INSS stage 2A/2B disease[9,31] with non-MNA tumors. The main objective was to test whether surgery alone was an effective and safe treatment for this patient cohort[9,31,32]; secondary aims were evaluation of the prognostic impact of histopathology[33] and tumor genomics. A successor study, LNESG2 (2005-2012), had the objective to increase knowledge of prognostic factors, improve event-free survival (EFS) and OS, establish a uniform treatment of patients with disease relapse, and implement image-defined risk factors in patients with stage 1 and 2 disease.[31] Patient cohorts were both based on uniform criteria for clinical diagnosis and staging. Standardized guidelines for tumor splitting, workup, and genomic assessment were applied.[34,35] The cohorts share central review and data validation by the SIOPEN Biology Group and the availability of genomic testing in addition to fluorescence in-situ hybridization data.

In this study, we present the genomic analyses of 71 LNESG1 and 175 LNESG2 tumors from consecutively registered trial patients, together with DNA of 71 tumors from localized, resectable, non-MNA neuroblastomas from the COG Neuroblastoma Committee nucleic acid repository

selected according to inclusion and exclusion criteria similar to that used by the LNESG.

PATIENTS AND METHODS

Patients

Inclusion criteria for this study were as follows: surgery only (ie, no chemotherapy at diagnosis and no homMNA) and, for statistical analyses, only patients with complete clinical follow-up data, complete and unambiguous genetic data and without heterogeneous MNA (hetMNA). Institutions recruited patients after approval of the trial by national regulatory authorities and ethical committees. Parents or guardians and patients provided written informed consent or assent, when applicable. Note that hereafter, the term MNA is used exclusively for homMNA. Three genetic subtypes for statistical analyses were defined as follows:

1. Numeric chromosome aberrations (NCAs) only
2. typSCA: losses of or at chromosomal arms 1p/3p/4p/11q; gains of or at 1q/2p/17q (tumors may show ≥ 1 typSCAs).
3. Atypical SCA (atypSCA), any other SCA except those defined as typSCA.

Schwann cell stroma-rich neuroblastomas, mostly found in patients ≥ 18 months of age are underrepresented because the non-neoplastic Schwann cells hamper acquisition of genetic data, especially where DNA averaging techniques were used (eg, comparative genomic hybridization, single nucleotide polymorphism array). For 11 patients, no clinical data were available. Assays were

TABLE 1. Age and Stage Distribution According to Age, Median Follow-Up Times, and Genetic Subtypes for the 3 Single Cohorts

	LNESG1[a]		LNESG2[b]		COG[c]		Total	
Parameter	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Reason for patient exclusion	123		192		91		406	
No Follow-up information			11				11	
Incomplete SCA data[d]	51		0		17		68	
hetMNA	1		6		3		10	
No. of evaluable patients	71	100	175	100	71	100	317	100
Age at diagnosis, months								
< 18	58	82	130	74	40	56	228	72
≥ 18	13	18	45	26	31	44	89	28
Stage								
1	0	0	125	71	34	48	159	50
2	71	100	50	29	37	52	158	50
Stage (age at diagnosis)								
1 (< 18 months)	0	0	96	55	15	21	111	35
1 (≥ 18 months)	0	0	29	17	19	27	48	15
2 (< 18 months)	58	82	34	19	25	35	117	37
2 (≥ 18 months)	13	18	16	9	12	17	41	13
Median follow-up, months	81		35		74		57	
Genetic subtype								
NCA	43	61	105	60	51	72	199	63
typSCA[e]	25	35	64	37	17	24	106	33
atypSCA	3	4	6	3	3	4	12	4

Abbreviations: atypSCA, atypical segmental chromosome aberration for neuroblastoma; COG, Children's Oncology Group; hetMNA, heterogeneous *MYCN* amplification; LNESG, Localized Neuroblastoma European Study Group; NCA, numeric chromosome aberration; SCA, segmental chromosome aberration; typSCA, typical segmental chromosome aberration.

[a]Median age at diagnosis of LNESG1 patients was 7 months (range, 7 days to 139 months).

[b]Median age at diagnosis of LNESG2 patients was 11 months (range, 0-214 months).

[c]Median age at diagnosis of COG patients was 13 months (range, 1 day to 75 months).

[d]No *MYCN* amplification and no hetMNA, no typSCA, but not all chromosomal regions typically involved in segmental aberrations were analyzed.

[e]Including 14 tumors with intratumor heterogeneity for typSCA detected by fluorescence in situ hybridization: 11 patients < 18 months old and 3 patients ≥ 18 months old.

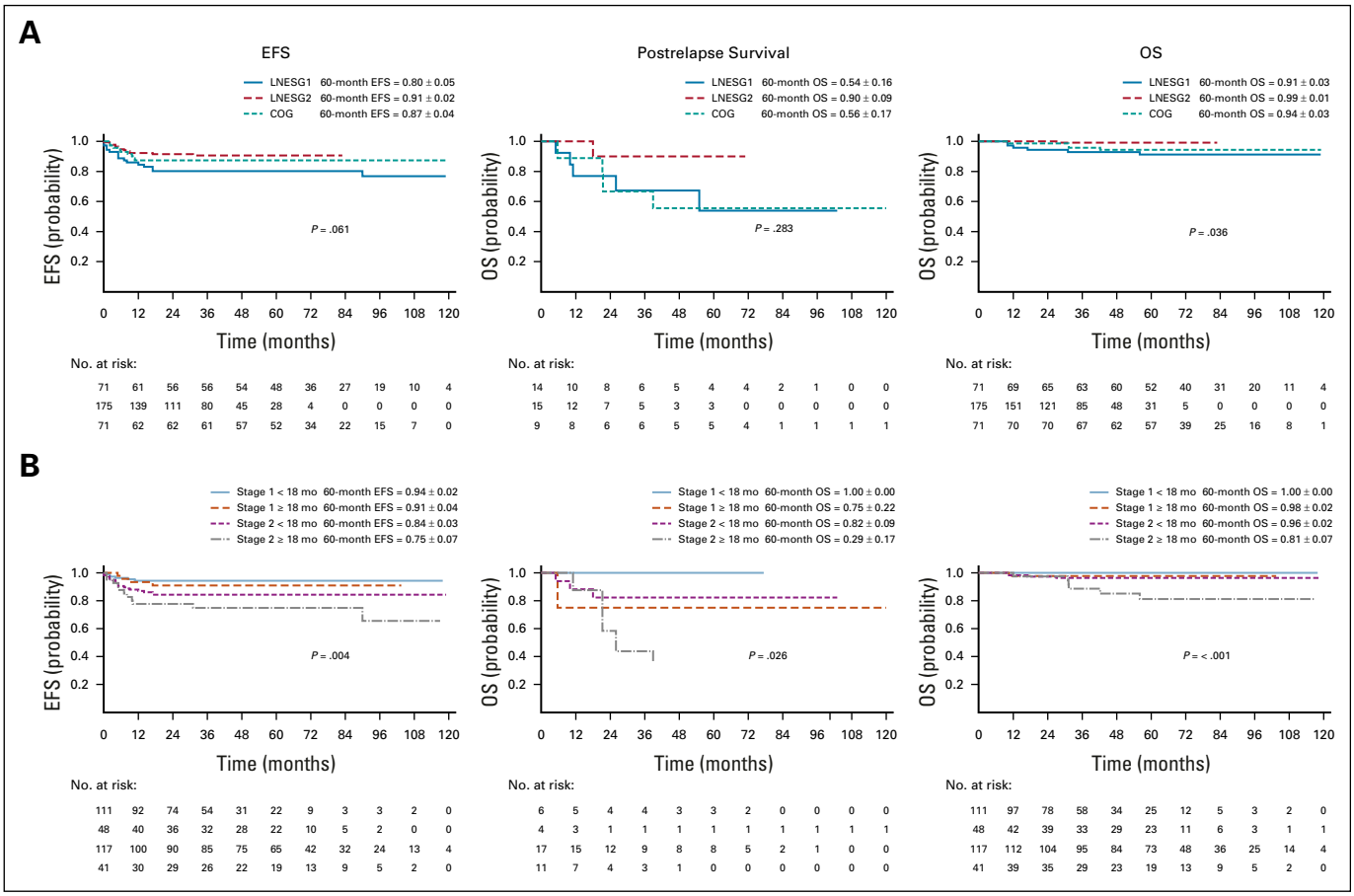

FIG 1. Event-free survival (EFS), postrelapse survival, and overall survival (OS) data according to stage and age in (A) individual cohorts and (B) according to stage and age In the LNESG1 trial, three deaths in the younger age group were not tumor related; one was due to a surgery-related complication, one due to chemotherapy-related toxicity (after relapse, and one due to therapy refusal after a local relapse in a patient with Rubinstein-Taybi syndrome).

repeated in case of unclear results and/or in tumors with higher content of normal cells (eg Schwann cells).

Patients selected for this study from COG had surgery alone, like patients in the LNESG studies and were asymptomatic. They were enrolled in the P9641 trial.[36] All the COG patients were enrolled in a biology study (P9047 before 2001; ANBL00B1 after 2001) to define risk class using clinical and molecular tumor features (ie, age, INSS stage, *MYCN* status, tumor ploidy and histopathology). The trials were approved by institutional ethics committees. For age and stage distributions, see Table 1.

Statistical Analyses

Survival curves were generated according to the Kaplan-Meier method and compared using the log-rank test, with $P < .05$ considered statistically significant. Data are reported with ± standard deviation [SD] values; when data for both age groups are reported together, the data for patients < 18 months of age are reported first.

Patients were dichotomized according to the age cutoff of 18 months at diagnosis. Survival time was calculated from the day of operation. EFS was defined as the time from diagnosis to first relapse (local or distant), progression, or death without recurrence. OS includes death from any cause. Postrelapse survival was calculated from the day of the first relapse. Patients who did not experience an event were censored at the time of last follow-up. EFS and OS probabilities were reported at 60 months with Greenwood SEs. A Cox proportional hazards model was used in multivariable analysis for EFS. Thirteen patients with atypSCA were excluded from multivariate analysis because of the rarity of this genetic subtype.

Study cohort, stage, age, genetic subtype, and the interaction between age and genetic subtype were evaluated. Because of a significant interaction between age group and genetic subtype, hazard ratios (HRs) for typSCA versus NCA were estimated in each age group separately.

RESULTS

Relapse and Postrelapse Survival Rates

The 5-year EFS, OS, and postrelapse survival data according to INSS stages and age are shown in Figure 1 for all cohorts and according to individual cohorts.

TABLE 2. Five-Year EFS and OS According to Individual typSCA, DNA Content, and Genetic Subgroup According to Tumor Stage

										Stage 2											
		Total				Stage 1				Total				Age < 18 Months				Age ≥ 18 Months			
Chromosome Aberration	Yes or No	No. of Patients	No. of Events	60-month EFS ± SD	*P*	No. of Patients	No. of Events	60-month EFS ± SD	*P*	No. of Patients	No. of Events	60-month EFS ± SD	*P*	No. of Patients	No. of Events	60-month EFS ± SD	*P*	No. of Patients	No. of Events	60-month EFS ± SD	*P*
1p	No	275	31	0.89 ± 0.02	.059	138	9	0.93 ± 0.02	.939	137	22	0.84 ± 0.03	.032	103	13	0.87 ± 0.03	.019	34	9	0.76 ± 0.07	.709
	Yes	36	8	0.77 ± 0.07	.	16	1	0.93 ± 0.06	.	20	7	0.65 ± 0.11	.	13	5	0.62 ± 0.13	.	7	2	0.71 ± 0.17	.
1 q	No	293	34	0.88 ± 0.02	.088	146	10	0.93 ± 0.02	.539	147	24	0.84 ± 0.03	.009	112	16	0.85 ± 0.03	.187	35	8	0.79 ± 0.07	.075
	Yes	10	3	0.70 ± 0.14	.	5	0	1.00 ± 0.00	.	5	3	0.40 ± 0.22	.	2	1	0.50 ± 0.35	.	3	2	0.33 ± 0.27	.
2p	No	287	32	0.89 ± 0.02	.005	146	9	0.93 ± 0.02	.405	141	23	0.84 ± 0.03	.003	108	15	0.86 ± 0.03	.060	33	8	0.78 ± 0.07	.063
	Yes	15	5	0.65 ± 0.13	.	8	1	0.88 ± 0.12	.	7	4	0.43 ± 0.19	.	4	2	0.50 ± 0.25	.	3	2	0.33 ± 0.27	.
3p	No	293	35	0.88 ± 0.02	.499	144	9	0.93 ± 0.02	.539	149	26	0.83 ± 0.03	.172	113	17	0.85 ± 0.03	.418	36	9	0.77 ± 0.07	.232
	Yes	11	2	0.81 ± 0.12	.	6	0	1.00 ± 0.00	.	5	2	0.60 ± 0.22	.	3	1	0.67 ± 0.27	.	2	1	0.50 ± 0.35	.
4p	No	298	37	0.87 ± 0.02	.546	146	9	0.93 ± 0.02	.188	152	28	0.82 ± 0.03	NA	114	18	0.84 ± 0.03	NA	38	10	0.75 ± 0.07	NA
	Yes	5	1	0.80 ± 0.18	.	5	1	0.80 ± 0.18	.												
11q	No	274	30	0.89 ± 0.02	.064	139	8	0.94 ± 0.02	.213	135	22	0.84 ± 0.03	.201	107	17	0.84 ± 0.04	.255	28	5	0.85 ± 0.07	.033
	Yes	31	7	0.76 ± 0.08	.	13	2	0.85 ± 0.10	.	18	5	0.71 ± 0.11	.	8	0	1.00 ± 0.00	.	10	5	0.48 ± 0.16	.
17q	No	238	25	0.89 ± 0.02	.103	119	6	0.95 ± 0.02	.236	119	19	0.84 ± 0.03	.210	96	15	0.84 ± 0.04	.965	23	4	0.82 ± 0.08	.154
	Yes	72	13	0.83 ± 0.05	.	37	4	0.89 ± 0.05	.	35	9	0.76 ± 0.07	.	19	3	0.83 ± 0.09	.	16	6	0.69 ± 0.12	.
Ploidy	Aneuploid	240	20	0.91 ± 0.02	.000	117	5	0.95 ± 0.02	.008	123	15	0.88 ± 0.03	.000	100	14	0.86 ± 0.04	.221	23	1	0.96 ± 0.04	.001
	Diploid	14	7	0.56 ± 0.13	.	3	1	0.67 ± 0.27	.	11	6	0.55 ± 0.15	.	2	1	0.50 ± 0.35	.	9	5	0.56 ± 0.17	.
Genetic subtype	NCA	199	18	0.91 ± 0.02	.078	97	5	0.94 ± 0.02	.619	102	13	0.87 ± 0.03	.058	85	13	0.84 ± 0.04	.834	17	0	1.00 ± 0.00	.006
	atypSCA	12	2	0.81 ± 0.12	.	8	1	0.86 ± 0.13	.	4	1	0.75 ± 0.22	.	2	0	1.00 ± 0.00	.	2	1	0.50 ± 0.35	.
	typSCA	106	19	0.82 ± 0.04	.	54	4	0.92 ± 0.04	.	52	15	0.72 ± 0.06		30	5	0.83 ± 0.07	.	22	10	0.58 ± 0.11	.

										Stage 2											
		Total				Stage 1				Total				Age < 18 Months				Age ≥ 18 Months			
Chromosome Aberration	Yes or No	No. of Patients	No. of Events	60-month OS ± SD	*P*	No. of Patients	No. of Events	60-month OS ± SD	*P*	No. of Patients	No. of Events	60-month OS ± SD	*P*	No. of Patients	No. of Events	60-month OS ± SD	*P*	No. of Patients	No. of Events	60-month OS ± SD	*P*
1p	No	275	8	0.96 ± 0.01	.091	138	1	0.99 ± 0.01	.727	137	7	0.94 ± 0.02	.089	103	3a	0.97 ± 0.02	.407	34	4	0.86 ± 0.07	.139
	Yes	36	3	0.88 ± 0.07	.	16	0	1.00 ± 0.00	.	20	3	0.82 ± 0.09	.	13	1	0.92 ± 0.08	.	7	2	0.56 ± 0.25	.
1 q	No	293	8	0.96 ± 0.01	.002	146	1	0.99 ± 0.01	.845	147	7	0.94 ± 0.02	.000	112	3a	0.97 ± 0.02	.001	35	4	0.84 ± 0.07	.093
	Yes	10	2	0.76 ± 0.15	.	5	0	1.00 ± 0.00	.	5	2	0.50 ± 0.25	.	2	1	0.50 ± 0.35	.	3	1	0.50 ± 0.35	.
2p	No	287	8	0.96 ± 0.01	.022	146	1	0.99 ± 0.01	.831	141	7	0.94 ± 0.02	.009	108	3a	0.97 ± 0.02	.027	33	4	0.84 ± 0.07	.258
	Yes	15	2	0.80 ± 0.13	.	8	0	1.00 ± 0.00	.	7	2	0.67 ± 0.19	.	4	1	0.75 ± 0.22	.	3	1	0.50 ± 0.35	.
3p	No	293	9	0.96 ± 0.01	.249	144	1	0.99 ± 0.01	.844	149	8	0.94 ± 0.02	.223	113	4a	0.96 ± 0.02	.736	36	4	0.85 ± 0.07	.111
	Yes	11	1	0.80 ± 0.18	.	6	0	1.00 ± 0.00	.	5	1	0.75 ± 0.22	.	3	0	1.00 ± 0.00	.	2	1	1.00 ± 0.00	.

(continued on following page)

TABLE 2. Five-Year EFS and OS According to Individual typSCA, DNA Content, and Genetic Subgroup According to Tumor Stage (continued)

		Total				Stage 1				Stage 2											
										Total				Age < 18 Months				Age ≥ 18 Months			
Chromosome Aberration	Yes or No	No. of Patients	No. of Events	60-month OS ± SD	*P*	No. of Patients	No. of Events	60-month OS ± SD	*P*	No. of Patients	No. of Events	60-month OS ± SD	*P*	No. of Patients	No. of Events	60-month OS ± SD	*P*	No. of Patients	No. of Events	60-month OS ± SD	*P*
4p	No	298	10	0.96 ± 0.01	.714	146	1	0.99 ± 0.01	.861	152	9	0.93 ± 0.02	NA	114	4a	0.96 ± 0.02	NA	38	5	0.82 ± 0.07	NA
	Yes	5	0	1.00 ± 0.00	.	5	0	1.00 ± 0.00	.												
11q	No	274	6	0.97 ± 0.01	.001	139	0	1.00 ± 0.00	.002	135	6	0.95 ± 0.02	.028	107	4a	0.96 ± 0.02	.597	28	2	0.92 ± 0.06	.038
	Yes	31	4	0.78 ± 0.11	.	13	1	0.92 ± 0.07	.	18	3	0.72 ± 0.14	.	8	0	1.00 ± 0.00	.	10	3	0.46 ± 0.22	.
17q	No	238	5	0.97 ± 0.01	.045	119	0	1.00 ± 0.00	.083	119	5	0.95 ± 0.02	.112	96	3a	0.97 ± 0.02	.655	23	2	0.90 ± 0.07	.318
	Yes	72	5	0.90 ± 0.05	.	37	1	0.97 ± 0.03	.	35	4	0.85 ± 0.07	.	19	1	0.94 ± 0.05	.	16	3	0.72 ± 0.14	.
Ploidy	Aneuploid	240	4	0.98 ± 0.01	.001	117	0	1.00 ± 0.00	.	123	4	0.96 ± 0.02	.017	100	3a	0.97 ± 0.02	.803	23	1	0.94 ± 0.06	.090
	Diploid	14	2	0.82 ± 0.12	.	3	0	1.00 ± 0.00	.	11	2	0.81 ± 0.12	.	2	0	1.00 ± 0.00	.	9	2	0.76 ± 0.15	.
Genetic Subtype	NCA	199	3	0.98 ± 0.01	.041	97	0	1.00 ± 0.00	.395	102	3	0.97 ± 0.02	.044	85	3a	0.96 ± 0.02	.961	17	0	1.00 ± 0.00	.034
	atypSCA	12	1	0.89 ± 0.10	.	8	0	1.00 ± 0.00	.	4	1	0.75 ± 0.22	.	2	0	1.00 ± 0.00	.	2	1	0.50 ± 0.35	.
	typSCA	106	7	0.91 ± 0.04	.	54	1	0.98 ± 0.02	.	52	6	0.86 ± 0.05	.	30	1	0.96 ± 0.04	.	22	5	0.66 ± 0.13	.

Abbreviations: atypSCA, atypical segmental chromosome aberration; EFS, event-free survival; NA, not applicable; NCA, numeric chromosome aberration; OS, overall survival; SCA, segmental chromosome aberration;
SD, standard deviation; typSCA, typical segmental chromosome aberration.

[a]The deaths of 3 patients were not tumor related.

All cohorts. Patients with stage 1 disease at any age had a higher EFS compared with patients with stage 2 disease at any age. Those with stage 2 disease in the younger age group had an excellent OS comparable to that of patients with stage 1 disease. However, the postrelapse survival in the older age group of the former was significantly inferior (29% ± 17%) as compared with the younger age group (82% ± 9%). Moreover, the 3 deaths in the younger age group were not tumor-related (Fig 1).

Stage 1. The 5-year EFS was high and comparable between the two age groups (94% ± 2% and 91% ± 4% for patients < 18 months and ≥ 18 months, respectively). In the COG cohort, patients < 18 months had lower EFS (80% ± 10%), but this patient subgroup was small (n = 15 patients; Appendix Fig A1, online only).

Stage 2. In patients < 18 months, the 5-year EFS and OS were similar in the LNESG cohorts. In patients ≥ 18 months, however, EFS was worse compared with stage 1 in all cohorts. Postrelapse survival was worse only for LNESG1 and COG patients (0% and 33% ± 27%; Appendix Fig A1).

SCA Frequency in the Different Age and Stage Subgroups

typSCA frequency. In the whole cohort, 33% of patients had typSCA (Table 1), with the highest frequency of 53.7% (n = 22 of 41) found in patients ≥ 18 months with stage 2 disease. Only 25.6% of the younger patients with stage 2 disease showed this aberration. Eighteen (37.5%) of 48 patients ≥ 18 months with stage 1 disease and 36 (32.4%) of 111patients < 18 months had typSCA (Table 2; and data not shown).

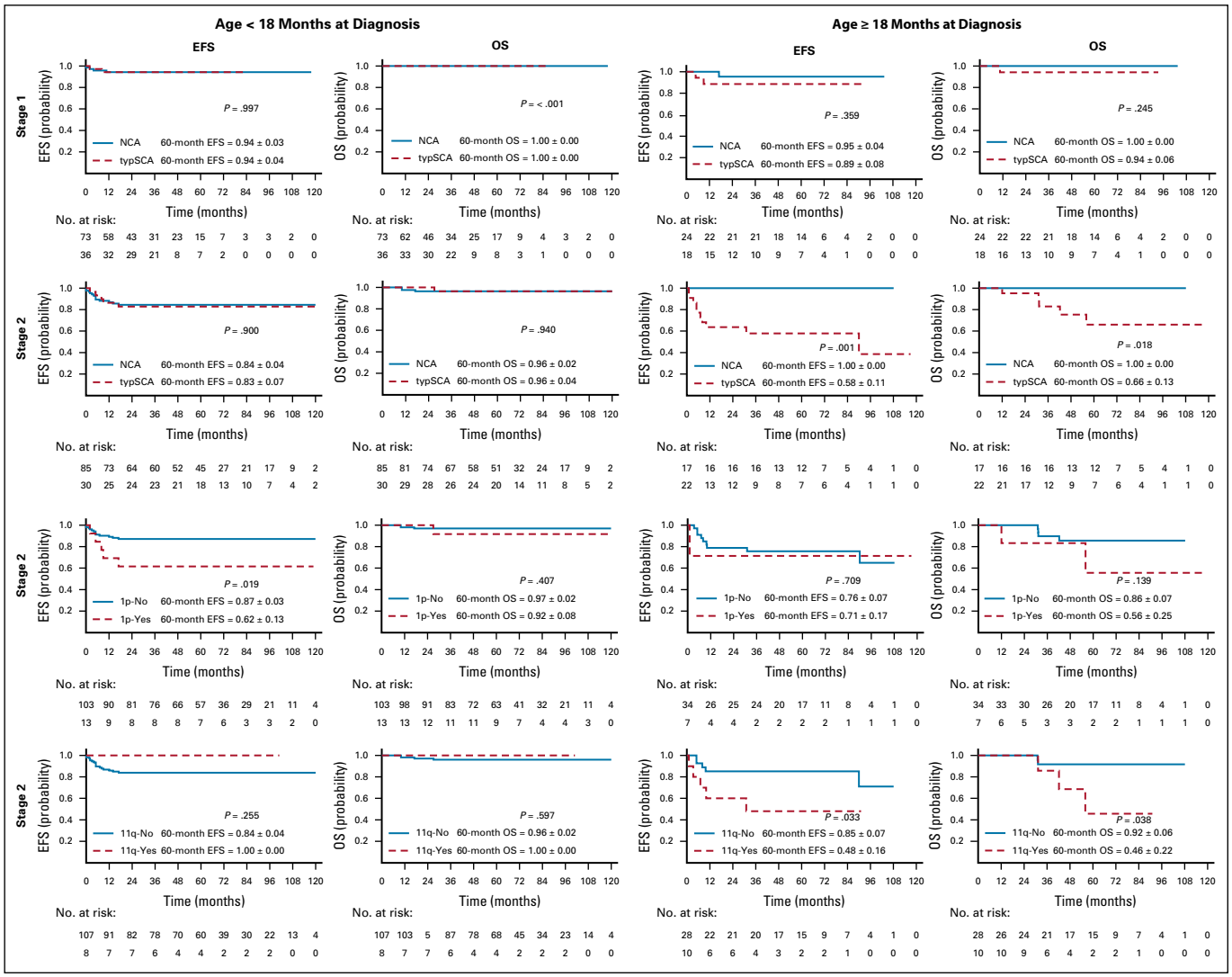

FIG 2. Event-free survival (EFS) and overall survival (OS) according to age, stage, and the genetic subtype numeric chromosome aberration (NCA), typical segmental chromosome aberration (typSCA), and the individual SCAs 1p loss and 11q loss. Patient numbers slightly differ from numbers given in the text because of lacking clinical data.

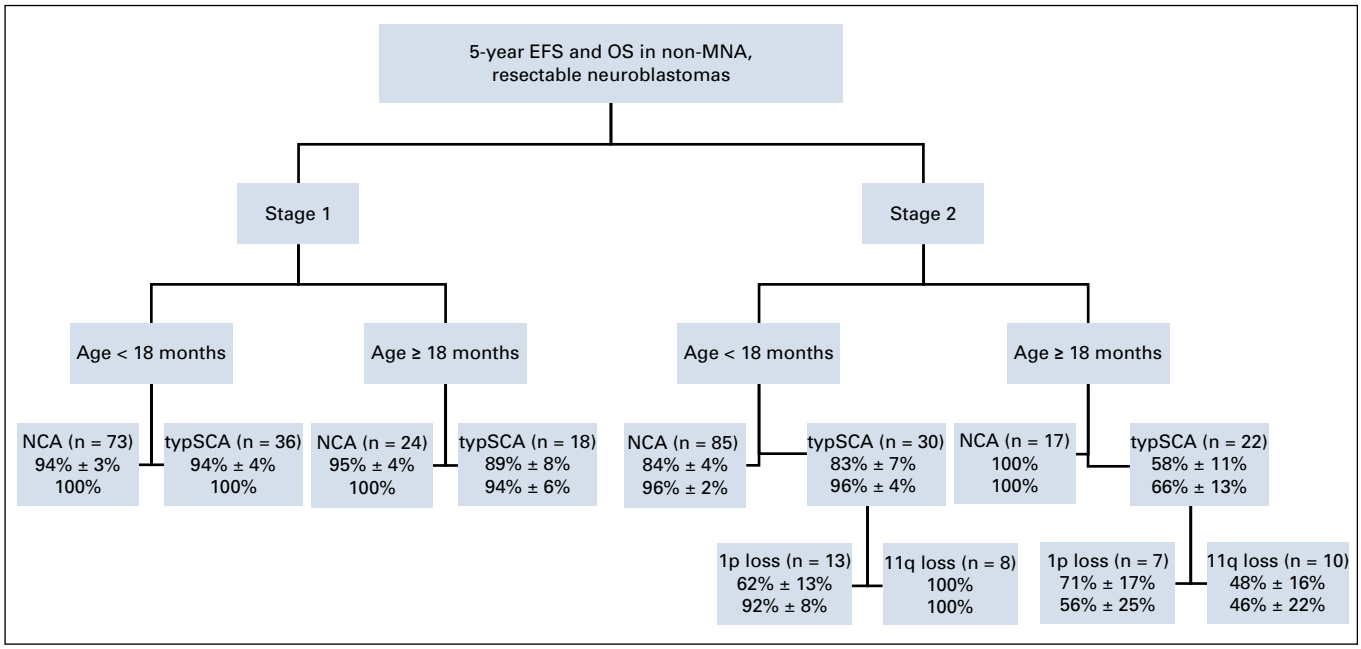

FIG 3. Subgroup analysis stratified according to stage and age as well as genetic subgroups with respect to event-free survival (EFS) and overall survival (OS). Numeric chromosome aberrations (NCA) are compared with typical segmental chromosome aberrations (typSCAs). From the seven typSCAs, only the most frequently encountered and most significant typSCA are mentioned. Patient numbers are indicated in parentheses. EFS data are listed above OS data. For stage 1 cases, none of 10 patients < 18 months of age at diagnosis with 1p loss tumors and none of eight with 11q loss experienced disease relapse. In patients ≥ 18 months of age, there was one relapse among six tumors with 1p loss, and two relapses among four tumors with 11q loss occurred (one of the relapsing tumors showed also 1p loss). For stage 2 cases, none of eight patients < 18 months of age at diagnosis with 11q loss tumors experienced a relapse. Chromosome 1p deletion was uniformly associated with relapse in both age groups. Non-MNA, nonhomogeneous *MYCN* amplification.

Individual typSCA. A higher frequency of 11q aberrations was found in the older patient group in stage 2 tumors; this was not the case for 1p loss (Table 2).

atypSCA. A total of 12 tumors (any age, both stages) showed atypSCAs as sole aberrations. In an additional 41 tumors, atypSCA were present together with typSCA.

Ploidy and typSCA. All diploid tumors had SCAs, which occurred more frequently in the older group (19% *v* 3% in patients < 18 months). Conversely, only < 10% of aneuploid tumors (n = 20 of 241) had SCAs (data not shown).

Relapse According to Age and Tumor Genomics

Altogether, 39 disease relapses were recorded: 20 local relapses, four metastatic and local, and 15 metastatic.

Patients < 18 months. In both stages, 5-year EFS was similar in NCA tumors and in typSCA tumors but lower in stage 2 tumors (stage 1: 94% ± 3% and 94% ± 4%, respectively; stage 2: 84% ± 4% and 83% ± 7%, respectively; Fig 2). Of the 23 patients (both stages) whose disease relapsed, only one patient died of disease (Fig 1). This patient had a stage 2 tumor with various typical and atypical SCAs. Disease relapse occurred in 16 patients with tumors with NCA (n = 4 stage 1; n = 12 stage 2), but there were no deaths. Seven patients had tumors with hetMNA (n = 1 relapse, no deaths; data not shown).

Patients ≥ 18 months. In stage 1 disease, 5-year-EFS did not differ significantly in NCA tumors and in typSCA tumors (95% ± 4% and 89% ± 8%, respectively). In stage 2 disease, however, EFS differed significantly between NCA and typSCA tumors (100% and 58% ± 11%, respectively; $P = .001$; Figs 2 and 3; Table 2; Data Supplement). In NCA tumors, only one of the 41 tumors (both stages) relapsed (locally), in contrast to 12 of 40 typSCA tumors (both stages; two patients' disease relapsed after 60 months, five patients with stage 2 tumors died of disease; Figs 2 and 3, Table 2). Three patients had hetMNA tumors (two patients with stage 2 tumors both had disease relapse and died of disease; data not shown).

Impact of Individual SCA Types and Tumor Cell Ploidy

Stage 1. None of the typSCAs were predictive of relapse and OS (one death in the 11q deletion group changes the *P* value), irrespective of age at diagnosis.

Stage 2. All typSCAs, except 3p, 4p, 11q, and 17q losses, were associated with decreased EFS, and all except 1p, 3p, and 17q associated with decreased OS (Table 2). No significant differences in relapse frequencies between the presence of 1 typSCA or > 1 typSCA were observed.

Patients < 18 months. Only 1p loss was associated with decreased 5-year EFS (62% ± 13% *v* 87% ± 3%; $P = .019$). Only 1 patient died of disease with a tumor bearing 1p loss and 1q, 2p, and 17q gains. Three patients with NCA

TABLE 3. Multivariate Analysis of Prognostic Factors for Event-Free Survival (n = 37 events among 305 patients with NCA or typSCA)

Parameter	Comparison	*P*	HR	95% HR Confidence Limits	
				Lower	Upper
Stage	Stage 2 *v* stage 1	.052	2.4	1.0	5.6
Study (*P* = .599)	COG *v* LNESG2	.833	1.11	0.44	2.80
	LNESG1 *v* LNESG2	.326	1.52	0.66	3.52
Interaction between genetic subtype and age, months		.026			
< 18	typSCA[a] *v* NCA	.968	1.02	0.42	2.47
≥ 18	typSCA[a] *v* NCA	.015	12.97	1.65	102.00

Abbreviations: COG, Children's Oncology Group; HR, hazard ratio; LNESG, Localized Neuroblastoma European Study Group; NCA, numeric chromosome aberration; typSCA, typical segmental chromosome aberration.

[a]Thirteen patients with atypSCA were excluded because of the rarity of this genetic subtype.

tumors died of nontumor-related causes. The 8 patients with 11q loss had 100% 5-year EFS and OS (Fig 2; Table 2), and for the 19 patients with 17q gain, EFS was 83% ± 9% and OS was 94% ± 5% (Table 2). atypSCA without typSCA occurred in 4 tumors (no relapses). Only 4 patients had diploid tumors.

Patients ≥ 18 months. In case of 11q loss, 5-year EFS was reduced to 48% ± 16% compared with 85% ± 7% without 11q loss (*P* = .033); 5-year OS was 46% ± 22% with and 92% ± 6% without 11q deletion (*P* = .038; Fig 2; Table 2). atypSCA without typSCA occurred in 8 tumors and were associated with one relapse.

Ploidy. Tumor diploidy was associated with worse 5-year EFS (56% ± 17% compared with 96% ± 4%; *P* = .001) and OS (76% ± 15% compared with 94% ± 6%; *P* = .09; Table 2).

Figure 3 shows genomic subtypes and individual SCAs (1p and 11q losses) associated with decreased EFS and OS as identified in the three patient cohorts: LNESG1, LNESG2, and COG.

Multivariate Analyses (Cox Regression)

In a multivariate analysis of prognostic factors for EFS (ie, stage, study, age, and typSCA in different age groups), typSCA versus NCA in patients ≥ 18 months was the strongest prognostic indicator (HR, 12.97; *P* = .015), followed by stage 2 versus stage 1 (Table 3). Conversely, genomic aberrations had no power in patients < 18 months (HR, 1.02; *P* = .968).

DISCUSSION

To scrutinize the prognostic impact of neuroblastoma-typical genomic aberrations in non-MNA, localized, resectable neuroblastomas in patients < 18 months and those ≥ 18 months of age, the SIOPEN Biology Group conducted genomic analyses of tumors from patients enrolled in LNESG1, LNESG2, and COG trials who were treated with surgery alone irrespective of a tumor residuum. Evaluation of the association of genomic features with stage, age, and outcome demonstrate an age-dependent impact of tumor genomics: In patients < 18 months with stage 2 disease: (1) SCA and NCA tumors led to recurrences with similar frequencies; (2) 1p loss was the only typSCA associated with a higher relapse rate; and (3) patients with disease relapse could nearly always undergo a salvage treatment irrespective of the tumor genetics. In patients ≥ 18 months with stage 2 disease, tumors with typSCA were almost the only tumors that relapsed.

In this report, we show that NCA stage 2 tumors frequently relapse or progress in patients < 18 months, but only rarely in older patients. This may reflect a high proliferative capacity of still immature NCA tumors in the younger age group and a markedly diminished potential for relapse and dissemination in tumors with this genomic profile and activated maturation processes in the older age group.[37]

Another striking observation was the different prognostic implications of typSCA in the different age groups: loss of 1p in patients < 18 months was associated with a higher relapse rate but not with diminished OS. In patients ≥ 18 months with stage 2 disease, the presence of typSCA was associated with relapses in nearly 40% of patients and almost one-quarter died; patients with 11q loss did even worse.

Multivariate analysis supported these data demonstrating typSCA versus NCA in the ≥ 18-month age group as the strongest discriminating factor. However, there was no discriminating power of genomic aberrations, in the younger population. Stage, as expected, was the second strongest prognostic factor.

Comparable results have been shown for typSCA in unresectable neuroblastomas for the age group 12-18 months.[38] The European Infant Neuroblastoma Study revealed an excellent OS irrespective of SCA but a higher relapse rate in unresectable and stage 4S SCA tumors.[39] In patients > 18 months, Schleiermacher et al[17] reported a significantly poorer EFS in cases of non–stage 4 typSCA

tumors. Moreover, Pinto et al[40] identified a lower EFS in localized SCA tumors. In SCA marker studies, 11q deletion reached statistical significance in localized and stage 4S neuroblastomas, and some cooperative groups use 11q for stratification.[20,29,30] Because the number of patients bearing tumors with 1q and 2p gain was small, we did not draw any conclusion on the prognostic impact of these markers.

Although slightly higher, the observed frequencies for typSCA were comparable with those in most previous reports.[22,27,35,41] This may be explained by analysis of different tumor areas (according to the SIOPEN Biology guidelines).[34,35] The higher incidence of typSCA in patients in the older age group with stage 2 disease could be partly due to the lack of SCA data in Schwann cell stroma-rich tumors. These tumors occur almost exclusively in the older age group. Shimada et al[42-44] were the first to consider the need for an age-dependent interpretation of histopathologic features. After demonstration of the nonneoplastic, reactive nature of the Schwann cell in neuroblastoma, the new model of maturation could explain this age dependency, because Schwann cell stroma development and gangliocytic differentiation take considerable time.[37,45,46] Schwann cell stroma-rich tumors most likely represent "true" NCA tumors; however, the frequently high amount of normal Schwann cells hampers pangenomic analysis.[37] Because of this, there were incomplete SCA data for 32 tumors in the older age group. However, none of these patients experienced disease relapse. In contrast, a higher frequency of SCA occurs with increasing age in Schwann cell stroma-poor tumors.[39,47] With regard to tumor cell ploidy, the known association of diploidy with poorer outcome in neuroblastoma was also confirmed in this study for stage 2 tumors in patients $\geq$ 18 months of age.[36]

Postrelapse survival was comparatively poor in the older age group in the LNESG1 and COG cohorts. With the LNESG2 cohort, the respective patient subgroup contained too few patients to draw any definite conclusions. In addition, the LNESG2 trial used image-defined risk factors[31] to assess operability as well as uniform guidelines for postrelapse treatment (ie high-risk protocol for disseminated relapses (M. Beck-Popovic, unpublished data).

The results of this study reconfirm the significant prognostic impact of age at diagnosis in patients with neuroblastoma[48,49] but challenge the view of age ($<$ 18 months) as a simple surrogate marker for favorable genetics. The lack of prognostic significance for most typSCAs in patients $<$ 18 months, except for 1p loss, was unexpected, as was the similar relapse frequency of NCA tumors in this age group. Other features may be of prognostic importance, including the delay of the developmental switch of neuroblast involution and the role of low- or high-affinity nerve growth factor (NGF) receptor expression together with limited NGF supply.[50-53] Moreover, absence of *TERT* activation in most, if not all, favorable, low-stage neuroblastomas may trigger apoptosis and regression.[3] Altogether, the age factor in neuroblastoma is still not understood and may be multifactorial. It would be interesting to determine whether genetic aberrations could precede metabolic tumor cell changes and if studying other factors in this cohort could further optimize predictions of which SCA-positive patients might experience recurrence (eg, LDH, TERT, and other telomere maintenance mechanisms).[5,43,55]

In this study, we identified genomic risk factors for relapse in patients with localized, resectable, non-MNA neuroblastomas, which are different for children $<$ 18 months of age compared with those $\geq$ 18 months. Chromosome 1p loss in patients $<$ 18 months can be regarded as a significant risk factor for relapse but not survival. However, although patients $<$ 18 months with stage 2 disease could almost always receive salvage treatment after disease relapse, this was not the case for patients $\geq$ 18 months, for whom SCAs (especially 11q loss) was a risk factor for EFS and OS as well as diploidy.

For both age-dependent genomic subgroups, we recommend the prognostic impact in these subgroups be validated prospectively in large international trials. A risk classification then may be developed on the basis of genomic and clinical factors.

The decisions can range from careful observation when no other adverse markers are present to conventional chemotherapy or other treatment options in case of 11q-deleted tumors in the older patient group, for example. A trial comparing EFS and OS after a limited number of courses of postoperative chemotherapy versus close observation of older patients with SCA (11q loss) should be considered.

AFFILIATIONS

[1]Children's Cancer Research Institute, St Anna Kinderkrebsforschung, Vienna, Austria
[2]Paediatric Research Institute, Fondazione Città della Speranza, Neuroblastoma Laboratory, Padua, Italy
[3]Pediatric Oncology Research, Department of Pediatrics, University Hospital, Lausanne, Switzerland
[4]Service de Biostatistiques, Institut Curie, Paris, France
[5]Department of Pathology, Oslo University Hospital Rikshospitalet, Oslo, Norway
[6]Department of Pathology, Medical School, University of Valencia–Fundación de Investigación del Hospital Clínico Universitario de Valencia, Valencia, Spain
[7]Centro de Investigación Biomédica en Red de Cáncer, Madrid, Spain
[8]Département de Biologie et de Pathologie Médicales, Service de Pathologie Moléculaire, Institut Gustave Roussy, Villejuif, France
[9]Northern Genetics Service, Newcastle upon Tyne, United Kingdom
[10]Department of Pediatric Oncology, Emma Children's Hospital, Academic Medical Center, Amsterdam, the Netherlands

[11]Centre Léon Bérard, Laboratoire de Recherche Translationnelle, Lyon, France
[12]Unité de Génétique Somatique et Cytogénétique, Institut Curie, Paris, France
[13]Department of Pathology, Istituto G. Gaslini, Genoa, Italy
[14]INSERM U830, Laboratoire de Génétique et Biologie des Cancers, Paris, France
[15]Ca-Cytogenetic Laboratory, Pediatric Hematology Oncology Department, Schneider Children's Medical Center of Israel, Petah Tikvah, Israel
[16]Childhood Cancer Research Unit, Karolinska Institutet, Astrid Lindgren Children's Hospital, Stockholm, Sweden
[17]Biosciences Institute, Newcastle University, Newcastle upon Tyne, United Kingdom
[18]Centro de Genética Humana, Instituto Nacional de Saude doutor Ricardo Jorge, Lisbon, Portugal
[19]Department of Clinical Genetics, Institute of Biomedicine, University of Gothenburg, Sahlgrenska University Hospital, Göteborg, Sweden
[20]Département de Pédiatrie, Institut Curie, Paris, France
[21]Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium
[22]Division of Oncology, The Children's Hospital of Philadelphia, Philadelphia, PA
[23]Section for Paediatric Surgery, Division of Surgery, Rikshospitalet University Hospital, Oslo, Norway
[24]Department of Paediatric Surgery, St George's Hospital, London, UK
[25]Département de Cancérologie de l'Enfant et de l'Adolescent, Gustave Roussy, Villejuif, France
[26]Unidad de Oncologia Pediatrica Hospital Universitario La Fe, Valencia, Spain
[27]Wolfson Childhood Cancer Research Centre, Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, United Kingdom
[28]Seattle Children's Hospital and University of Washington School of Medicine, Seattle, WA
[29]Department of Pediatrics, The University of Chicago, Chicago, IL
[30]Department of Pediatrics, Medical University of Vienna, Vienna, Austria
[31]Pediatric Hematology Oncology Unit, University Hospital of Lausanne, Lausanne, Switzerland
[32]Department of Paediatric Haematology and Oncology, Giannina Gaslini Children's Hospital, Genova, Italy
[33]Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey, United Kingdom

CORRESPONDING AUTHOR

Inge M. Ambros, MD, CCRI, Children's Cancer Research Institute, St Anna Kinderkrebsforschung, Zimmermannplatz 10, 1090 Vienna, Austria; e-mail: inge.ambros@ccri.at.

EQUAL CONTRIBUTION

I.M.A. and P.F.A. contributed equally to this manuscript.

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AUTHOR CONTRIBUTIONS

Conception and design: Inge M. Ambros, Nicole Gross, Huib Caron, Tommy Martinsson, Gudrun Schleiermacher, Edward F. Attiyeh, Ruth Ladenstein, Jean Michon, Peter F. Ambros
Financial support: Inge M. Ambros, Nicole Gross, Deborah Tweddle, Peter F. Ambros
Administrative support: Inge M. Ambros, Nicole Gross, John Lunec, Dasa Janousek, Peter F. Ambros
Provision of study material or patients: Inge M. Ambros, Gian-Paolo Tonini, Nicole Gross, Jean Bénard, Huib Caron, Valérie Combaret, Jerome Couturier, Olivier Delattre, Marta Jeison, Per Kogner, John Lunec, Gudrun Schleiermacher, Alexander Valent, Nadine Van Roy, Eva Villamon, Michael D. Hogarty, Dominique Valteau-Couanet, Victoria Castel, Deborah A. Tweddle, Sue Cohn, Ruth Ladenstein, Maja Beck-Popovic, Jean Michon, Peter F. Ambros
Collection and assembly of data: Inge M. Ambros, Gian-Paolo Tonini, Nicole Gross, Klaus Beiske, Jean Bénard, Nick Bown, Huib Caron, Valérie Combaret, Jerome Couturier, Raffaella Defferrari, Olivier Delattre, Marta Jeison, Per Kogner, John Lunec, Barbara Marques, Tommy Martinsson, Katia Mazzocco, Rosa Noguera, Gudrun Schleiermacher, Alexander Valent, Nadine Van Roy, Eva Villamon, Dasa Janousek, Ingrid Pribill, Edward F. Attiyeh, Tom F. Monclair, Dominique Valteau-Couanet, Victoria Castel, Deborah A. Tweddle, Julie R. Park, Sue Cohn, Ruth Ladenstein, Maja Beck-Popovic, Peter F. Ambros
Data analysis and interpretation: Inge M. Ambros, Nicole Gross, Ulrike Pötschger, Véronique Mosseri, Klaus Beiske, Ana P. Berbegall, Nick Bown, Jerome Couturier, Marta Jeison, Per Kogner, John Lunec, Tommy Martinsson, Rosa Noguera, Gudrun Schleiermacher, Alexander Valent, Nadine Van Roy, Evgenia Glogova, Michael D. Hogarty, Keith Holmes, Victoria Castel, Deborah A. Tweddle, Julie R. Park, Sue Cohn, Ruth Ladenstein, Bruno De Bernardi, Andrew D.J. Pearson, Peter F. Ambros
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Age Dependency of the Prognostic Impact of Tumor Genomics in Localized Resectable *MYCN*-Nonamplified Neuroblastomas. Report From the SIOPEN Biology Group on the LNESG Trials and a COG Validation Group

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Huib Caron
Employment: Roche
Stock and Other Ownership Interests: Roche
Travel, Accommodations, Expenses: Roche

Per Kogner
Consulting or Advisory Role: Fennec Pharmaceuticals

John Lunec
Patents, Royalties, Other Intellectual Property: Astex Pharmaceuticals (Inst)
Travel, Accommodations, Expenses: Astex Pharmaceuticals

Gudrun Schleiermacher
Honoraria: Bristol Myers Squibb
Research Funding: Bristol Myers Squibb (Inst), Pfizer (Inst), MSDavenir (Inst), Roche (Inst)
Travel, Accommodations, Expenses: Roche

Edward F. Attiyeh
Employment: Janssen Research & Development
Stock and Other Ownership Interests: Johnson & Johnson

Dominique Valteau-Couanet
Consulting or Advisory Role: EUSA Pharma (Inst)
Research Funding: Orphelia (Inst)
Patents, Royalties, Other Intellectual Property: Royalties from Apeiron to SIOPEN (Inst)
Travel, Accommodations, Expenses: EUSA Pharma, Jazz Pharmaceuticals

Deborah A. Tweddle
Honoraria: EUSA Pharma
Travel, Accommodations, Expenses: EUSA Pharma

Sue Cohn
Stock and Other Ownership Interests: United Therapeutics (I), United Therapeutics, Merck, Stryker (I), Stryker, Amgen (I), Pfizer (I), AbbVie, Amgen, Jazz Pharmaceuticals, Eli Lilly, Sanofi, Varex Imaging, Pfizer, Accelerated Medical Diagnostics, Anthem, Cardinal Health, Novo Nordisk, Regeneron, Zimmer BioMet
Research Funding: United Therapeutics (Inst), Merck (Inst)
Open Payments Link: https://openpaymentsdata.cms.gov/physician/46569/summary

Ruth Ladenstein
Honoraria: Apeiron Biologics, Boehringer Ingelheim, EUSA Pharma
Consulting or Advisory Role: Apeiron Biologics, Boehringer Ingelheim, EUSA Pharma (Inst)
Research Funding: Apeiron Biologics (Inst), EUSA Pharma (Inst)
Patents, Royalties, Other Intellectual Property: Apeiron Biologics (Inst), EUSA Pharma (Inst)
Expert Testimony: Apeiron Biologics, EUSA Pharma
Travel, Accommodations, Expenses: Apeiron Biologics, EUSA Pharma

Jean Michon
Travel, Accommodations, Expenses: Bristol Myers Squibb, Roche

Andrew D. J. Pearson
Consulting or Advisory Role: Eli Lilly, Takeda, Merck, Celgene
Travel, Accommodations, Expenses: Eli Lilly, Takeda, Merck, Celgene

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APPENDIX

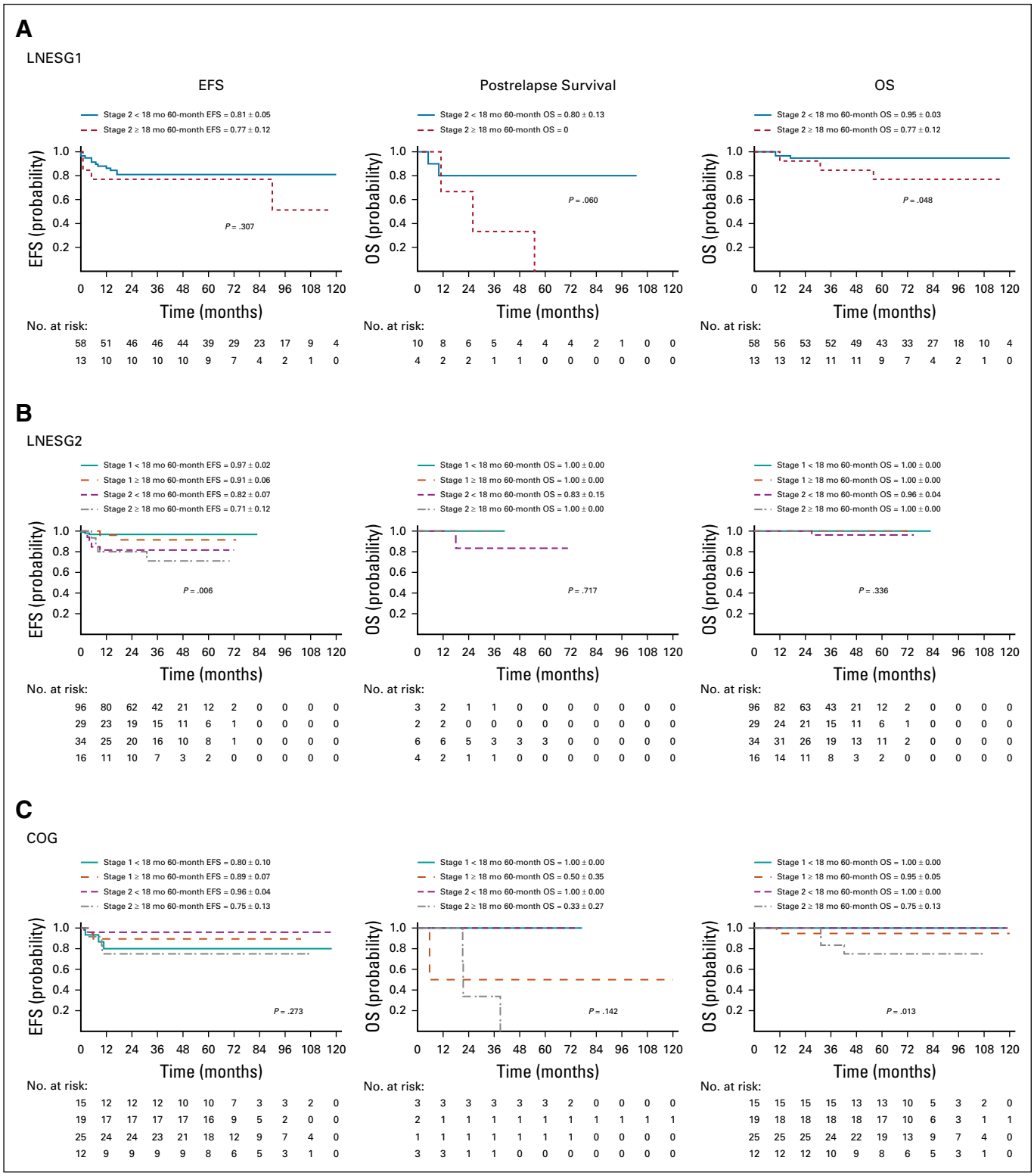

FIG A1. Event-free survival (EFS), postrelapse EFS, and overall survival (OS) according to age and stage in the single cohorts (A) LNESG1, (B) LNESG2, and (C) COG validation cohort. COG, Children's Oncology Group; LNESG, Localized Neuroblastoma European Study Group.