("confounding") of age when histology is used in a risk-group schema that includes age. To determine which histologic features were independently associated with outcome, tumor grade (differentiating *v* poorly differentiated or undifferentiated), MKI (low or intermediate *v* high), histologic category (GN-maturing or GNB-intermixed *v* GNB-nodular or NB), and age (< 547 *v* ≥ 547 days) were analyzed with EFS tree regression.[17-19,21]

Methods to Reduce the Number of Prognostic Variables

The 35 potentially prognostic factors were consolidated to 13 for analysis. Only factors where data were available for more than 5% of the 8,800 patients were included. Because Shimada and INPC are similar, histology data were consolidated into a single system. INPC was the default, but Shimada diagnosis, grade of tumor differentiation, or MKI were used if the corresponding INPC value was unknown. INSS was selected as the staging criteria. In situations where INSS and Evans definitions were the same (ie, INSS stage 1 = Evans stage I), Evans stage was used if INSS was unknown. Unbalanced 11q LOH and 11q aberrations data were combined into a single variable: "11q aberration." Similarly, 1p LOH and 1p aberrations were combined into the variable "1p aberration." 17q gain data were available for less than 5% of the patients, so 17q was not further analyzed. Using univariate analyses, six primary tumor sites were consolidated into one binary variable (adrenal *v* nonadrenal), as were eight metastatic sites (presence of metastases *v* no metastases).

The INRG database included a crude categoric variable for initial treatment. However, no statistical adjustment for treatment was performed. Because treatment has been assigned for many years using prognostic factors, treatment group is confounded with the prognostic factors,

Table 2. Clinical Characteristics of the International Neuroblastoma Risk Group Analytic Cohort (N = 8,800)

Factor	EFS		Patients		5-Year EFS (%)			5-Year OS (%)		
	Hazard Ratio	95% CI	No.	%	Rate	SE	Log-Rank *P*	Rate	SE	Log-Rank *P*
Age, days										
< 365	3.6	3.3 to 4.0	3,734	42	84	1		91	1	
≥ 365			5,066	58	49	1	< .0001	55	1	< .0001
Age, days										
< 547	3.7	3.4 to 4.0	4,773	54	82	1		88	1	
≥ 547			4,027	46	42	1	< .0001	49	1	< .0001
Year of enrollment/diagnosis										
≥ 1996	1.4	1.2 to 1.4	4,493	51	69	1		76	1	
< 1996			4,307	49	59	1	< .0001	66	1	< .0001
Initial treatment										
Observation, surgery, or standard chemotherapy	4.1	3.8 to 4.4	4,615	68	79	1		86	1	
Intensive multimodality			2,170	32	34	1	< .0001	41	1	< .0001
INSS stage										
1, 2, 3, 4S	5.2	4.8 to 5.7	5,131	60	83	1		91	1	
4			3,425	40	35	1	< .0001	42	1	< .0001
Evans stage										
I, II, III, IVS	6.6	5.8 to 7.6	2,022	63	86	1		91	1	
IV			1,177	37	31	2	< .0001	36	2	< .0001
Serum ferritin (ng/mL)										
< 92	3.6	3.2 to 4.0	2,170	50	81	1		87	1	
≥ 92			2,175	50	46	1	< .0001	52	1	< .0001
LDH (U/L)										
< 587	2.4	2.2 to 2.7	2,586	50	77	1		85	1	
≥ 587			2,592	50	53	1	< .0001	58	1	< .0001
Histologic classification (INPC, Shimada if INPC missing)										
Favorable	6.6	5.7 to 7.5	2,724	64	89	1		95	1	
Unfavorable			1,536	36	40	2	< .0001	49	2	< .0001
Diagnostic category (INPC, Shimada if INPC missing)										
1 = NB, stroma-poor			3,657	90	64	1		71 ±	1	
2 = GNB, intermixed, stroma-rich			144	3	95	3		96	2	
3 = GNB, well diff., stroma-rich			38	1	80	9	< .0001	79	9	< .0001
4 = GNB, nodular (composite)			232	6	53	5		68	5	
(2 and 3) *v* (1 and 4)	4.7	2.8 to 7.8								
Grade of NB differentiation (INPC, Shimada if INPC missing)										
Differentiating	2.5	2.0 to 3.3	518	16	83	2		89	2	
Undifferentiated			2,759	84	63	1	< .0001	72	1	< .0001
MKI (INPC, Shimada if INPC missing)										
Low, intermediate	3.2	2.8 to 3.8	2,690	87	74	1		82	1	
High			393	13	37	4	< .0001	44	4	< .0001

NOTE. Hazard ratios denote increased risk of an event for the second row within a given category compared with the first row.
Abbreviations: INPC, International Neuroblastoma Pathology Classification; EFS, event-free survival; OS, overall survival; NB, Neuroblastoma; GNB, Ganglioneuroblastoma; MKI, Mitosis Karyorrhexis Index.

resulting in reduced ability to detect the effect of a prognostic factor if adjustment for treatment is made. Therefore, instead of statistically adjusting for treatment, post hoc interpretation and the delineation of pretreatment groups were based on knowledge of how groups of patients had been treated historically.

Methods to Identify Prognostically Distinct Subgroups

The methodologic goal was to identify subgroups that were both statistically and clinically significantly different from one another, such that resulting subgroups of patients would be as homogenous as possible in terms of biology and outcome. The prognostic significance of the 13 factors was tested in the overall cohort, and the one with the highest χ^2 value was retained to create two subgroups or "nodes." The remaining factors were then tested within each node. This process was repeated within each node until the sample size was too small to proceed, or until no further statistically significant variables were found. In some nodes, the number of patients with known values for all factors being tested became too small for multivariate analysis. In this situation, factors were tested in a pairwise fashion in the model. The winner for each comparison was recorded, and the factor with the most "wins" was selected to create the next branch. Although not optimal, this approach was deemed necessary to overcome the problem of missing data.

RESULTS

INRG Cohort

The proportion of patients in the INRG analytic cohort of 8,800 was fairly evenly distributed between North America (48%) and Europe (47%), plus patients from Japan (5%) (Table 1). Tables 2 and 3 and Appendix Table A2 (online only) summarize the clinical and biologic characteristics of the cohort. The overall 5-year EFS and OS rates were 63% ± 1% and 70% ± 1%, respectively, with median follow-up of 5.2 years in 5,819 patients alive without an event. The assumption of proportional hazards was not violated for either EFS or OS except for 17q gain and skin metastases which were of no consequence because they were not among the final 13 risk factors evaluated. Also, at each split of the survival regression tree, the assumption of proportional hazards was upheld for EFS and OS.

Stage

The EFS tree regression analysis was performed on the basis of INSS stage. As described in Monclair et al,[14] an analysis of SIOPEN data (n = 474) found both INSS stage and INRGSS highly prognostic of EFS, and validated the German study.[22] This retrospective analysis supports the translation of EFS tree regression results (in terms of INSS stage) into the INRG Classification system (in terms of INRGSS): INSS 1 → INRGSS L1; INSS 2, 3 → INRGSS L2; INSS 4 → INRGSS M; and INSS 4S → INRGSS MS.

Age

The predictive ability of age was shown to be continuous in nature in the analysis of COG patients (n = 3,666) and within the balance of INRG patients. As recognized by the Task Force, it would be optimal to evaluate age as a continuous variable for risk stratification because outcome gradually worsens with increasing age. However, using two age groups was considered more feasible for these analyses. The analysis of non-COG patients within the INRG cohort confirmed the findings of London et al,[20] with support for an optimal "cutoff" between 15 and 19 months. For practical reasons, the Task Force's consensus was an age cutoff of 18 months (547 days) for the INRG classification system. Although the cutoff could be anywhere in this range, once selected, this age cutoff must be consistently applied as the exact number of days. However, for patients with diploid, stage M, *MYCN* nonamplified tumors, the Task Force elected to use the more conservative age cutoff of 12 months (365 days).

LDH and Ferritin

The median value to dichotomize LDH was 587 U/L, and for ferritin was 92 ng/mL.

Tumor Histology

In the EFS tree analysis testing histologic category, grade of tumor differentiation, MKI, and age, we found evidence of independent prognostic ability of each factor. This was tested in half the patients

Table 3. Genetic Characteristics of the International Neuroblastoma Risk Group Analytic Cohort (N = 8,800)

Factor	EFS		Patients		5-Year EFS (%)			5-Year OS (%)		
	Hazard Ratio	95% CI	No.	%	Rate	SE	Log-Rank *P*	Rate	SE	Log-Rank *P*
MYCN status										
Not amplified	4.1	3.8 to 4.5	5,947	84	74	1		82	1	
Amplified			1,155	16	28	2	< .0001	34	2	< .0001
Ploidy										
> 1 (hyperdiploid)	2.3	2.0 to 2.6	2,611	71	76	1		82	1	
≤ 1 (diploid, hypodiploid)			1,086	29	55	2	< .0001	60	2	< .0001
11q										
Normal	2.3	1.9 to 2.9	844	79	68	3		79	2	
Aberration			220	21	35	5	< .0001	57	5	< .0001
1p										
Normal	3.2	2.8 to 3.8	1,659	77	74	2		83	1	
Aberration			493	23	38	3	< .0001	48	3	< .0001
17q gain										
No gain	1.7	1.3 to 2.3	187	52	63	4		74	4	
Gain			175	48	41	5	.0006	55	5	.0009

NOTE. Hazard ratios denote increased risk of an event for the second row within a given category compared with the first row.
Abbreviations: INPC, International Neuroblastoma Pathology Classification; EFS, event-free survival; OS, overall survival; LOH, loss of heterozygosity.

(randomly selected) and the results confirmed in the other half. Excellent outcome was seen for patients with GN-maturing and GNB-intermixed tumors. For patients with GNB-nodular and NB tumors, age (younger than 18 $v \geq$ 18 months) was the most statistically significant factor. Within patients younger than 18 months with GNB-nodular and NB tumors, high MKI was associated with significantly lower EFS than low/intermediate MKI. Within patients 18 months of age or older with GNB-nodular and NB tumors, undifferentiated or poorly differentiated grade was associated with significantly lower EFS than differentiating grade. To prevent confounding of the effect of age, we analyzed histologic features (histologic category, MKI, and grade of differentiation) in lieu of the INPC.

Primary Site and Metastases

Adrenal primary tumor site had statistically significantly worse EFS than all other primary sites combined. For metastases, the most significant split was the presence versus absence of metastases.

EFS Tree Regression Analyses

The presence of classic metastases was the most significant prognostic factor in the EFS tree regression analysis of the overall cohort. The EFS and OS of INSS non–stage 4 (including 4S) patients were 83% ± 1% and 91% ± 1%, respectively, and 35% ± 1% and 42% ± 1% for children with stage 4 disease (Fig 1A).

Subclassification of Non–Stage 4 Patients

Within the patients with non–stage 4 disease (INSS stage 1, 2, 3, and 4S), histologic category (ie, GN-maturing and GNB-intermixed versus GNB-nodular and NB) was the most powerful prognostic factor (EFS: 97% ± 2% and 83% ±1, respectively). Of the 162 non–stage 4 INSS stage patients with GN-maturing or GNB-intermixed, only two had *MYCN* amplification, and both were alive without event at the time of this analysis. Because these tumors have a distinct clinical nature, the cohort of GN-maturing and GNB-intermixed was regarded as a terminal node. Within non–stage 4 GNB-nodular and NB patients, *MYCN* status was the most powerful prognostic factor (Fig 1A). Patients with *MYCN*-nonamplified tumors had EFS of 87% ± 1% and OS of 95% ± 1%, and 46% ± 4% and 53% ± 4% for patients with *MYCN*-amplified tumors. Within the *MYCN*-nonamplified cohort, patients with stage 1 disease had significantly better outcome than those with stages 2,3,4S (EFS: 93% ± 1% v 82% ± 1%; OS: 98% ± 1% v 92% ± 1%; Fig 1B). EFS for stage 1 patients with normal chromosome 1p was statistically better compared with those with 1p aberration (94% ± 2% v 78% ± 10%). However, OS was excellent regardless of the status of chromosome 1p (normal 1p: 99% ± 1%; 1p aberration: 100%). Therefore, 1p status was not included as a criterion in the INRG classification system and stage 1 was a terminal node.

Although survival rates for patients with stages 2, 3 disease (EFS: 82% ± 1%; OS: 92% ± 1%) and stage 4S patients (EFS: 82% ± 2%; OS: 91% ± 2%) were not statistically significantly different, treatment intensity differed. Because there are different treatment approaches in this group (4S disease is commonly observed whereas treatment for stage 2 and 3 tumors is surgery with or without chemotherapy), stage 2, 3 patients were split from stage 4S patients for further survival tree analyses. Within stage 2, 3 patients, those younger than 18 months old had statistically higher EFS than those 18 months of age or older (88% ± 1% v 69% ± 3%). In *MYCN* nonamplified stage 2, 3 patients younger than 18 months old, 11q aberration was the most highly prognostic of the biomarkers evaluated, with lower EFS (60% ± 20%) and OS (84% ± 14%) than normal 11q (EFS: 83% ± 5%; OS: 98% ± 2%; Fig 1B).

In patients with *MYCN*-nonamplified stage 2, 3 tumors who were 18 months of age or older, 11q aberration was the most statistically significant factor, but grade of tumor differentiation was also highly significant and identified additional poor-prognosis patients without evidence of 11q aberration (Fig 1B). The Task Force therefore decided to combine 11q aberration with grade into a single prognostic factor, categorizing patients who had either 11q aberration and/or undifferentiated (or poorly differentiated) histology (EFS: 61% ± 11%; OS: 73% ± 11%) versus those who did not have either one of the poor-outcome features (EFS: 80% ± 16%; OS: 100%).

Within the patients with *MYCN*-nonamplified stage 4S tumors, 11q aberration was the most highly prognostic factor (11q aberration—EFS: 38% ± 30%, OS: 63% ± 38%; normal 11q—EFS: 87% ± 7%, OS: 97% ± 4%). The number of patients within this cohort is small, and additional evaluation will be needed to further evaluate the impact of 11q aberration in this subset of patients.

MYCN-amplification was detected in only 8% of patients with stage 1 to stage 4S disease (Fig 1C). Although EFS rates for stage 1 patients were not statistically significant different from those of stage 2, 3, and 4S patients, less intensive treatment was administered to patients with *MYCN*-amplified stage 1 tumors. Because of the difference in treatment strategies, further survival tree analyses were performed separately in stage 1 patients versus stage 2, 3, and 4S patients. LDH was most highly prognostic for patients with *MYCN*-amplified stage 1 tumors (< 587 U/L—EFS: 55% ± 15%, OS: 85% ± 10% $v \geq$ 587 U/L—EFS: 40% ± 22%, OS: 58% ± 22%) and within the stage 2, 3, and 4S subset (< 587 U/L—EFS: 67% ± 9%, OS: 72% ± 8% $v \geq$ 587 U/L—EFS: 43% ± 5%, OS: 47% ± 5%). LDH is known to reflect tumor burden, and of the 169 *MYCN*-amplified stage 2, 3, and 4S patients with elevated LDH, 72% were stage 3. In view of the small number of patients in this cohort and the nonspecific nature of LDH, the Task Force decided not to include LDH in the classification system.

Subclassification of Patients With Stage 4 Disease

Age was the most powerful prognostic variable within 3,425 patients with stage 4 disease (Fig 1D). Children younger than 18 months had EFS and OS rates of 63% ± 2% and 68% ± 2%, respectively. Children 18 months of age or older had EFS and OS rates of 23% ± 1% and 31% ± 1%, respectively. Although serum ferritin (< $v \geq$ 92 ng/mL) was shown to be prognostic in the cohort of patients age 18 months and older by the EFS tree regression, outcome was poor in both cohorts, with EFS rates of 43% ± 4% and 20% ± 2%, respectively. Further statistically significant splits for *MYCN* status were identified within both ferritin cohorts (< $v \geq$ 92 ng/mL), but EFS and OS were poor in all of these subsets. Thus, serum ferritin did not add clinically relevant information in this cohort of patients with poor prognosis and was not included in the INRG classification schema. Within patients younger than 18 months with stage 4 disease, *MYCN* status was the most powerful prognostic factor. EFS was 83% ± 2% for children younger than 18 months with stage 4 disease lacking *MYCN* amplification versus 26% ± 4% for those with *MYCN*-amplified tumors. Within *MYCN*-nonamplified patients younger than 18 months with stage 4 disease, ploidy had prognostic significance. Patients with a DNA index greater than 1.0 had EFS of 85% ±

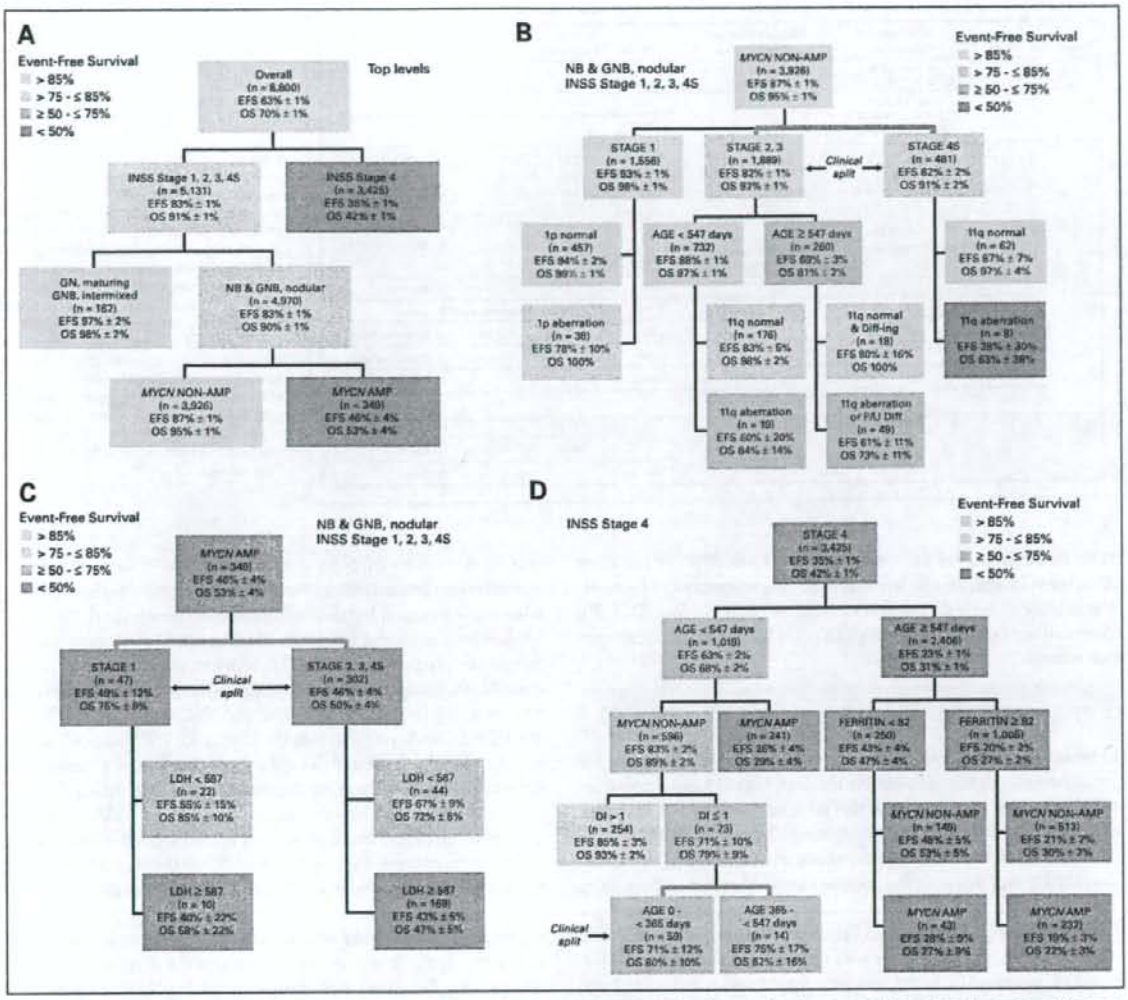

Fig 1. EFS tree regression analysis of INRG analytic cohort. Unless otherwise noted, a split or branch occurs for the most highly statistically significant factor as identified using a Cox proportional hazards regression model. (A) Top levels of the overall tree. (B) Subtree for NB and GNB-nodular, non–stage 4 *MYCN* NON-AMP patients. The split of stage 2, 3 from stage 4S patients was a clinical decision and not the result of statistical significance. (C) Subtree for NB and GNB-nodular, non–stage 4 *MYCN* AMP patients. The split of stage 1 from stage 2, 3, 4S patients was a clinical decision and not the result of statistical significance. (D) Subtree for INSS stage 4 patients. EFS, event-free survival; OS, overall survival; DI, DNA index; AMP, amplified; NON-AMP, nonamplified; INRG, International Neuroblastoma Risk Group; NB, neuroblastoma; GNB, ganglioneuroblastoma; GN, ganglioneuroma; INSS, International Neuroblastoma Staging System; LDH, lactate dehydrogenase.

3%, whereas EFS was 71% ± 10% for DNA index 1.0 or less. Although EFS for patients with stage 4 tumors younger than 12 months were not statistically significantly different from those 12 months or older to younger than 18 months, substantially higher-intensity treatment regimens were administered to patients who were 12 to younger than 18 months of age. On the basis of ploidy data and the excellent outcome of young children with stage 4 disease with favorable biologic features, several cooperative groups have developed clinical trials testing reduction in treatment for this cohort. In patients with diploid, *MYCN*-nonamplified stage 4 tumors, clinical justification was used to split patients younger than 12 months from 12 months and older to younger than 18 months of age, as the international consensus is that the intensity of therapy should not be reduced in this later group.

INRG Classification System

In summary, the consensus INRG classification schema includes the criteria INRG stage, age, histologic category, grade of tumor differentiation, *MYCN* status, presence/absence of 11q aberrations, and tumor cell ploidy. Sixteen statistically and/or clinically different pretreatment groups of patients (lettered A through R) were identified using these criteria (Fig 2). The proportion of patients grouped using EFS cut points for 5-year EFS of more than

INRG Stage	Age (months)	Histologic Category	Grade of Tumor Differentiation	*MYCN*	11q Aberration	Ploidy	Pretreatment Risk Group
L1/L2		GN maturing; GNB intermixed					A Very low
L1		Any, except GN maturing or GNB intermixed		NA			B Very low
				Amp			K High
L2	< 18	Any, except GN maturing or GNB intermixed		NA	No		D Low
					Yes		G Intermediate
	≥ 18	GNB nodular; neuroblastoma	Differentiating	NA	No		E Low
					Yes		H Intermediate
			Poorly differentiated or undifferentiated	NA			
				Amp			N High
M	< 18			NA		Hyperdiploid	F Low
	< 12			NA		Diploid	I Intermediate
	12 to < 18			NA		Diploid	J Intermediate
	< 18			Amp			O High
	≥ 18						P High
MS	< 18			NA	No		C Very low
					Yes		O High
				Amp			R High

Fig 2. International Neuroblastoma Risk Group (INRG) Consensus Pretreatment Classification schema. Pretreatment risk group H has two entries. 12 months = 365 days; 18 months = 547 days; blank field = "any"; diploid (DNA index ≤ 1.0); hyperdiploid (DNA index > 1.0 and includes near-triploid and near-tetraploid tumors); very low risk (5-year EFS > 85%); low risk (5-year EFS > 75% to ≤ 85%); intermediate risk (5-year EFS ≥ 50% to ≤ 75%); high risk (5-year EFS < 50%). GN, ganglioneuroma; GNB, ganglioneuroblastoma; Amp, amplified; NA, not amplified; L1, localized tumor confined to one body compartment and with absence of image-defined risk factors (IDRFs); L2, locoregional tumor with presence of one or more IDRFs; M, distant metastatic disease (except stage MS); MS, metastatic disease confined to skin, liver and/or bone marrow in children < 18 months of age (for staging details see text and Monclair et al[14]); EFS, event-free survival.

85%, more than 75% to ≤ 85%, ≥ 50% to ≤ 75%, or less than 50%, were 28.2%, 26.8%, 9.0%, and 36.1%, respectively (Table 4). The categories were designated as very low (A, B, C), low (D, E, F), intermediate (G, H, I, J), or high (K, N, O, P, Q, R) pretreatment risk subsets.

DISCUSSION

In recent years, the need to develop an international consensus for pretreatment risk stratification for children with NB has become increasingly apparent. To achieve this goal, an international task force established the INRG classification system. The prognostic effect of 13 variables in an 8,800-patient cohort was analyzed, with EFS, not OS, as the primary end point for the reasons identified earlier in this article. The INRG classification system includes the seven factors that were highly statistically significant and also considered clinically relevant. Similar to other series, patients with widely disseminated stage 4 disease had significantly worse outcome than those with locoregional disease or stage 4S NB.[9,23] As described in the article by Monclair et al,[14] a new pretreatment staging system was designed for the INRG classification system. In the INRGSS, extent of locoregional disease is determined by the absence or presence of image-defined risk factors (L1 and L2, respectively). Stage M will be used for disseminated disease, analogous to INSS stage 4. Similar to INSS stage 4S tumors, metastases are limited to skin, liver, and bone marrow without cortical bone involvement in INRGSS MS disease. However, the definition of MS has been expanded to include toddlers age 12 to younger than 18 months and large "unresectable" primary tumors (L1 or L2). As discussed in the companion article by Monclair et al,[14] the inclusion of L2 tumors is based on the excellent outcome of all 30 children enrolled on the SIOPEN 99.2 trial who met the criteria for INSS stage 4S disease and, in addition, had midline infiltration of the primary tumor, after treatment with a few cycles of chemotherapy or observation alone (B. De Bernardi, personal communication, February 2008). Although there is some concordance of patients between the INRGSS and the INSS staging systems, the two systems differ in the sense that the INSS staging system contains inherent confounding of surgical treatment versus extent of tumor, whereas INRGSS removes that confounding because it is assigned before surgery. The important similarity of the two systems is that INRGSS retains the prognostic value of staging that has been well documented for INSS staging, with statistically significantly higher EFS for L1 compared with L2. There is statistical justification for use of INRG staging for assigning patients to pretreatment groups, although prospective evaluation of the risk grouping based on the INRGSS staging system will be mandatory.

Table 4. Proportion of Patients When Arbitrary EFS Cut Points Are Applied to Cluster Rows of the International Neuroblastoma Risk Group Consensus Stratification (for illustrative purposes)

Pretreatment Risk Group	%	
	5-Year EFS	Proportion of Patients
Very low	> 85	28.2
Low	> 75 to ≤ 85	26.8
Intermediate	≥ 50 to ≤ 75	9.0
High	< 50	36.1

Abbreviation: EFS, event-free survival.

The analysis of the INRG data confirmed that the predictive ability of age is continuous in nature for NB. By convention, virtually all cooperative groups have used the 12-month cutoff to determine risk.[1] Similar to a previous study of COG patients,[20] our analysis of the INRG cohort indicated that the optimal age cutoff is between 15 and 19 months. Children age 12 to younger than 18 months with hyperdiploid stage 4 disease who lack *MYCN* amplification have excellent outcome when treated with intensive therapy on high-risk clinical trials.[24,25] These results suggest that therapy may be reduced safely in a subset of young children with stage 4 disease, and clinical trials testing this question have recently been developed. An age cutoff of 18 months (547 days) was, therefore, selected for the INRG classification system for all children except those with diploid, stage M tumors

without amplification of *MYCN* for whom the more conservative, 12-month cutoff will be maintained.

Tumor histology is another well established prognostic variable in NB.[12,13] To avoid confounding of age and INPC, we tested histologic category, MKI, grade of tumor differentiation, and age in the EFS tree regression analyses in lieu of INPC. We found that histologic category and tumor differentiation were statistically significantly associated with EFS. Consistent with the inferior prognosis that has been reported in patients with Shimada unfavorable histology INSS stage 3 tumors that lack *MYCN* amplification,[26] we found that outcome was worse for patients age 18 months and older with *MYCN*-nonamplified stage 2, 3 poorly differentiated or undifferentiated tumors compared with those with differentiating tumors.

To accurately stratify patients with locoregional tumors using the INRG classification system, sufficient samples of tumor tissue will be required for genetic/expression studies and for histologic category determination. In addition, there is a need for wide-scale education of pediatric pathologists to ensure that different histopathologic grades are uniformly and reproducibly recognized. The challenges of distinguishing GNB-intermixed from GNB-nodular are significant when the entire tumor is not resected. Surgical biopsy needs to be guided by the radiological appearances of the tumor, with any heterogeneous areas targeted. Adequate tissue samples are mandatory to evaluate histologic grade of differentiation in locoregional NBs that lack *MYCN* amplification in children 18 months of age or older. In most cases, multiple "true-cut" cores will yield sufficient tissue to determine tumor grade of differentiation, but fine-needle aspirates are not likely to provide adequate quantities of tissue for histologic analysis and are not appropriate. In metastatic tumors, fine-needle aspirates may provide adequate information for genetic analysis.

A number of genetic aberrations have been identified in NB tumors that are strongly associated with outcome. Our analysis confirmed the unfavorable prognostic significance of *MYCN* amplification, and in the INRG classification system, *MYCN* status is used to stratify patients into different pretreatment risk groups. We also found that 11q aberration was associated with worse outcome in patients with L2 or MS tumors that lack *MYCN* amplification. Similar to previous studies,[25,27-29] the prognostic value of DNA ploidy was demonstrated in children younger than 18 months of age with stage 4 disease and normal *MYCN* copy number. Recommendations regarding standardized methods for evaluating *MYCN* copy number, tumor cell ploidy, and other genetic aberrations in NB tumors will be reported in a future article.

Recent studies suggest that low-risk tumors may be best defined by the absence of *MYCN* gene amplification and any structural genetic abnormalities, (including either 11q and/or 1p aberrations and/or 17q gain).[30,31] The Task Force agreed that it would be optimal to evaluate genetic aberrations in NB tumors using genome-wide methods. However, because this type of analysis is not routinely performed by the large cooperative groups, incorporation of more global genetic data in the current INRG was not considered feasible at the present time. The immediate challenges are (1) to ensure that adequate tumor material is available for prospective "comprehensive" genetic investigations on every patient and (2) to identify technologies that are not cost prohibitive and will yield rapid and reproducible results. It is anticipated that the future INRG classification system will rely on the genetic profile of NB tumors, rather than the presence or absence of individual genetic abnormalities.

A limitation of this analysis is that there was no statistical adjustment for treatment, and therefore, patients in any of the 16 lettered rows may have received very different therapy. It is intended to extend the INRG database prospectively, and it will be critical to collect data on details of therapy.

In conclusion, the INRG classification system will ensure that children diagnosed with NB in any country are stratified into homogenous pretreatment groups. We strongly recommend that cooperative groups begin using this risk schema now. The very low-, low-, intermediate-, and high-risk categories were defined according to EFS cutoffs. These four categories were included in the classification schema to assist treating physicians in evaluating the prognostic impact of the combination of factors in each of the 16 lettered rows in the INRG classification system. Although these risk categories could have been defined differently, we selected EFS cutoff values that are commonly used for treatment stratification at the present time. For example, at most centers around the world, patients with features that are associated with estimated EFS rates of less than 50% are treated with intensive, multimodality strategies, whereas those predicted to have more than 85% EFS receive minimal therapy. We anticipate that risk group stratification will be further refined as treatment for high-risk disease improves and genome-wide DNA and expression analysis of tumors becomes more routine. It must be emphasized that we are not recommending that treatment be assigned according to these four broad risk-group categories. Rather, the key to reaping the benefits of this system will be the assignment of patients in one of the 16 pretreatment lettered designations in the INRG classification system to a single treatment group without splitting that row in different treatment subgroups. We anticipate that eligibility criteria for treatment protocols will likely include several of the 16 INRG pretreatment designations, and that the combinations of the 16 pretreatment groups that will be included in clinical trials studies conducted by each of the cooperative groups may be different. Therefore, it will be critical to individually report the outcome of patients assigned to each of the 16 pretreatment designations. This approach will greatly facilitate the comparison of risk-based clinical trials conducted in different regions of the world, provide a platform to ask randomized surgical questions, and lead to the development of international collaborative studies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

AUTHOR CONTRIBUTIONS

Conception and design: Susan L. Cohn, Andrew D.J. Pearson, Wendy B. London, Tom Monclair, Garrett M. Brodeur, Katherine K. Matthay
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Administrative support: Susan L. Cohn, Andrew D.J. Pearson, Wendy B. London
Provision of study materials or patients: Susan L. Cohn, Andrew D.J. Pearson, Wendy B. London, Tom Monclair, Peter F. Ambros, Garrett M.

Brodeur, Barbara Hero, Tomoko Iehara, Thorsten Simon, Alberto Garaventa, Victoria Castel

Collection and assembly of data: Susan L. Cohn, Andrew D.J. Pearson, Wendy B. London, Tom Monclair, Peter F. Ambros, Barbara Hero, Tomoko Iehara, David Machin, Veronique Mosseri, Thorsten Simon

Data analysis and interpretation: Susan L. Cohn, Andrew D.J. Pearson, Wendy B. London, Andreas Faldum, David Machin

Manuscript writing: Susan L. Cohn, Andrew D.J. Pearson, Wendy B. London, Tom Monclair, Garrett M. Brodeur, Katherine K. Matthay

Final approval of manuscript: Susan L. Cohn, Andrew D.J. Pearson, Wendy B. London, Tom Monclair, Peter F. Ambros, Garrett M. Brodeur, Andreas Faldum, Barbara Hero, Tomoko Iehara, David Machin, Veronique Mosseri, Thorsten Simon, Alberto Garaventa, Victoria Castel, Katherine K. Matthay

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INRG Task Force members: Susan L. Cohn, Andrew D.J. Pearson, Wendy B. London, Emanuele S.G. d'Amore, Andreas Faldum, Barbara Hero, Tomoko Iehara, David Machin, Veronique Mosseri, Michel Peuchmaur, Hiroyuki Shimada, Peter F. Ambros, Inge M. Ambros, Garrett M. Brodeur, Jerome Couturier, Michelle Haber, Javed Khan, John M. Maris, Akira Nakagawara, Gudrun Schleiermacher, Frank Speleman, Ruediger Spitz, Nadine Van Roy, Katherine K. Matthay, Klaus Beiske, Sue Burchill, Irene Cheung, Francesco Giammarile, Eiso Hiyama, Jean Michon, Robert C. Seeger, Barry Shulkin, Tom Monclair, Hervé Brisse, Giovanni Cecchetto, Keith S.J. Holmes, Michio Kaneko, Jed G. Nuchtern, Dietrich von Schweinitz, Frank Berthold, Victoria Castel, Robert P. Castleberry, Nai-Kong Cheung, Bruno De Bernardi, Helen Irving, Ruth Ladenstein, C. Patrick Reynolds, Jinhua Zhang, Julie R. Park, Roswitha Schumacher-Kuckelkorn, Thorsten Simon, Hidetaka Niizuma, Toby Trahair, Jennifer Forbeck, and John T. Kemshead. Participants in the INRG Meeting (September 17-19, 2005, Whistler, Vancouver, British Columbia, Canada), locations and group names, and roles are listed in Appendix Table A2, online only.

特集　小児病理―最近のトピックス―

小児固形腫瘍の病理 (2)神経芽腫群腫瘍・腎腫瘍・胚細胞性腫瘍

中川温子[*1]
大喜多 肇[*2]

Ⅰ．神経芽腫群腫瘍の組織像と生物学的特性

International Neuroblastoma Pathology Classification (INPC：国際神経芽腫病理分類) は，神経芽腫の生物学的特性に基づいた病理組織学的分類であり，強力な予後予測因子である (**表1**)[1,2]．本稿では，INPC における favorable histology (FH) 群と unfavorable histology (UH) 群，および ganglioneuroblastoma, nodular subtype (GNBn) の組織像と分子生物学的特性について最近明らかになりつつあるゲノムアレイ情報を含めて概説する．

1. Favorable Histology (FH) 群

FH 群とは，poorly differentiated neuroblastoma (poorly diff. NBL) から differentiating NBL, ganglioneuroblastoma, intermixed, ganglioneuroma に至る年齢相応の分化・成熟能をもつ腫瘍群である．生物学的には，high-affinity nerve growth factor receptor (TrkA) の発現が高く，DNA index は near-triploid (hyperdiploid) を示し，*MYCN* 遺伝子の増幅や 1p 欠失は認められないという特徴をもつ[3-5]．TrkA の発現が高く，神経節細胞への分化能をもっていても，18ヵ月未満ではほとんどの腫瘍が poorly diff. NBL の像を呈しており，組織学的に分化した像を呈するまでには一定の時間を必要とする (**図1**)[5]．リンパ節転移などの転移巣においても年齢に伴って分化・成熟が認められる．genomic DNA profile では，whole chromosome gain and loss を示す[6]．臨床的に予後良好とされる乳児神経芽腫の大部分はこの群に含まれ，マススクリーニング発見例を経過観察した症例では，患児の年齢に相当した組織学的な分化・成熟が観察された[7]．netrin 受容体である *UNC5H4* は p53 のターゲット遺伝子であり，p53 を介した細胞の生死をスイッチングする機能をもつ[8]．p53 mutation は，治療前 NBL には認められない．*UNC5H4* は FH 群において発現が高く，NBL の自然退縮機構において重要な役割を果たしていると考えられる (unpublished data)．FH 群における5年無病生存率は 90±1%，5年生存率は 97±1% と UH 群 (5年無病生存率 39±3%，5年生存率 48±3%) に比較して格段に予後良好である[9]．FH 群には，自然退縮するものや分化・成熟していくものがあるが，後者の場合，化学療法に反応せずかえって腫瘍が再増大する症例もみられる[10]．治療効果の判定や治療方針の決定にあたっては FH 群の生物学的特性を十分に理解することが重要である．

2. Unfavorable Histology (UH) 群

この群は，組織学的には，分化・成熟傾向が全く認められない undifferentiated NBL および年齢相当の分化・成熟を示さない NBL が含まれ，*MYCN* 増幅腫瘍と *MYCN* 非増幅腫瘍とに大別される．

MYCN 増幅腫瘍は，*MYCN* 増幅により，TrkA の発現低下による細胞分化の停止，細胞増殖の促進とアポトーシスの増加が起こるため，**図2**に示すような特徴的な組織像を呈する[4,11]．DNA index は near-diploid を示し，genomic DNA profile では，1p deletion (1p36LOH)，17q gain を示し，他の DNA コピー数の異常は稀である[6]．UH 群 *MYCN* 増幅腫瘍における5年無病生存率は 25±5%，5年生存率は 29±5% で，UH 群 *MYCN* 非増幅腫瘍 (5年無病生存率 46±4%，5年生存率は 57±4%) に比較し，予後不良である[9]．

UH 群の約 2/3 は *MYCN* 非増幅腫瘍であり，組織学的に年齢相当の分化・成熟を示さない腫瘍であるが，生物学的特性は十分に解明されていない．TrkA 発現は高いものから低いものまで様々であるが，TrkA の発現が高くても組織学的には年齢相当の分化・成熟が認められない[5]．genomic DNA profile では，3p-，4p-，11q-，1q+，2p+，12q+，17q+ などの多数の異

[*1] 国立成育医療センター病理診断科
[*2] 同 研究所発生・分化研究部

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表1 International Neuroblastoma Pathology Classification (INPC)

年齢	Favorable histology (FH)	Unfavorable histology (UH)
全て	Ganglioneuroma Maturing Mature	
	Ganglioneuroblastoma, intermixed	
	Ganglioneuroblastoma, nodular Favorable subset	Ganglioneuroblastoma, nodular Unfavorable subset
		Neuroblastoma, undifferentiated any MKI
＜1.5歳	Neuroblastoma, poorly diff. low/intermediate MKI	Neuroblastoma, poorly diff. high MKI
	Neuroblastoma, differentiating low/intermediate MKI	Neuroblastoma, differentiating high MKI
1.5～5歳		Neuroblastoma, poorly diff. any MKI
	Neuroblastoma, differentiating low MKI	Neuroblastoma, differentiating intermediate/high MKI
5歳以上		Neuroblastoma, poorly diff./differentiating any MKI

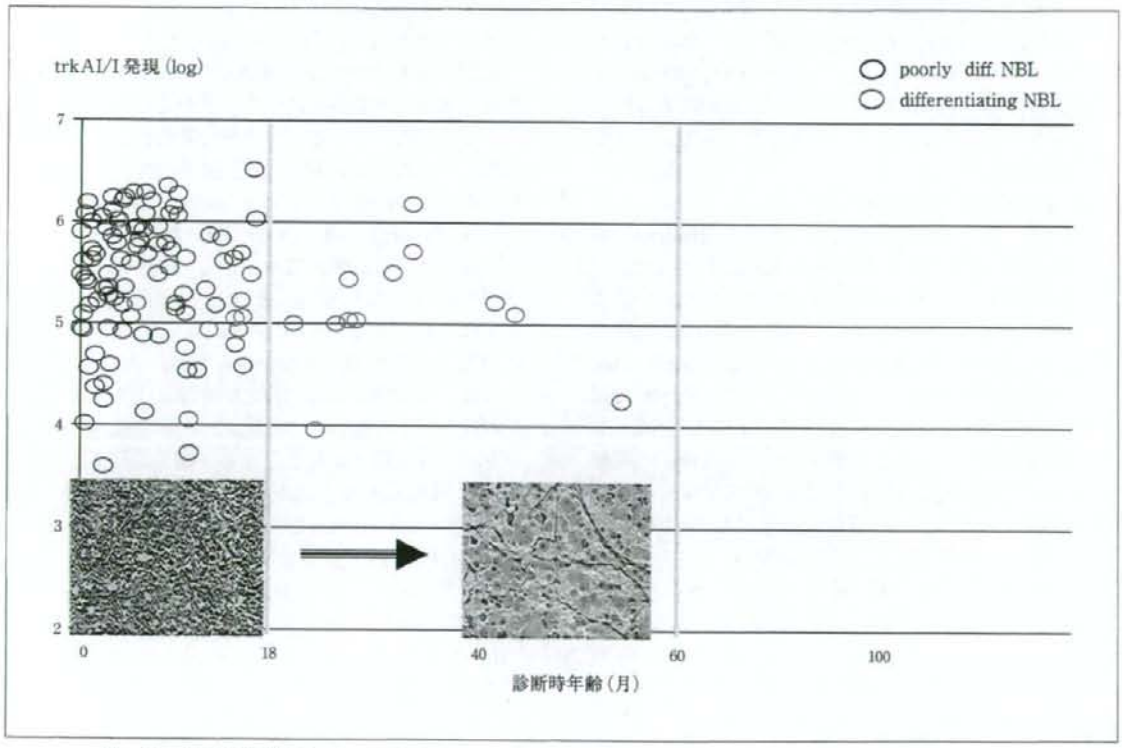

図1 TrkA発現と組織像 (poorly diff. NBL, differentiating NBL) 18ヵ月未満では，TrkAが高発現であっても組織学的には低分化である．18ヵ月以上になるとTrkA高発現腫瘍はより分化したdifferentiating NBLの像を呈する．

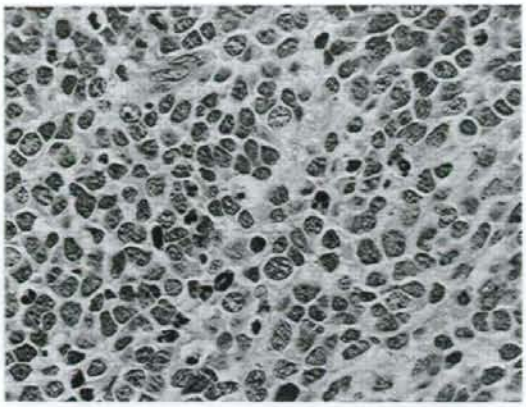

図2　unfavorable histology (UH) 群*MYCN*増幅腫瘍 (2歳2ヵ月男児の副腎腫瘍)　neuroblastoma, poorly diff. subtype, high MKIの像である.

常を示す群とDNAコピー数の異常が認められない群がみられる[6]. 最近, *MYCN*非増幅腫瘍においてunbalanced 11q LOH (unb 11qLOH) および1p36LOHが独立した予後不良因子であることが報告された[12]. unb 11qLOHを示す腫瘍では, 大型の多形性の強い腫瘍細胞が約70%の症例で観察されるという報告がある[13]. unb 11qLOHを示す腫瘍はUH群*MYCN*非増幅腫瘍の40%程度にすぎず, この群における分子生物学的予後因子は未だ確定していない[13].

3. Ganglioneuroblastoma, nodular subtype (GNBn)

GNBnは複数のクローンから構成されるcomposite tumorで, 肉眼的には暗赤色の出血を伴うNBL (stroma-poor tumor) の結節が, 白色調のGNB, intermixed subtypeあるいはganglioneuroma (stroma-rich/stroma-dominant tumor) の中に認められる. GNBnの中のNBL成分を通常のNBLと同様に, 年齢, 分化・成熟度, MKI (mitosis karyorrhexis index) を指標として分類することにより, GNBnは予後良好なfavorable subsetと予後不良なunfavorable subsetに分けられる[14]. favorable subsetのstroma-poor tumorとstroma-rich/stroma-dominant tumorとは, 組織像は異なるものの, どちらも年齢相応の分化・成熟をしていくFH群と同じ生物学的特性をもつ. 一方, unfavorable subsetのGNBnにおいては, stroma-rich/stroma-dominant tumorはFH群と同様のnon-aggressive cloneであるが, stroma-poor tumorはUH群, aggressive cloneである. 原発巣におけるstroma-rich/stroma-dominant tumorとstroma-poor tumorの占める割合は様々で, stroma-rich/stroma-dominant tumorがほとんどを占める場合には, 生検の際にサンプリングエラーのため, 正確な組織学的分類や生物学的特性 (*MYCN*増幅など) が判定できないことがある. 例えばstage 4で, 原発巣の部分切除または生検による組織がganglioneuromaの像を示す場合には, GNBnが疑われるので, 転移巣 (骨髄など) の組織学的検索が必要となる.

Ⅱ. 腎腫瘍

乳幼児に好発する腎腫瘍のなかで腎芽腫とその前駆病変とされている遺腎組織遺残, 腎明細胞肉腫, 腎ラブドイド腫瘍について, 最近のトピックスを中心に解説する. 小児の腎腫瘍では, metanephric stromal tumor, metanephric adenofibroma, anaplastic sarcoma of the kidneyなどの新たな概念の腫瘍が報告され, 骨・軟部肉腫として知られるEwing肉腫ファミリー腫瘍や滑膜肉腫が腎にも発生することが遺伝子解析の進歩により明確になってきている.

1. 腎芽腫 nephroblastoma (Wilms tumor)

腎芽腫 (Wilms腫瘍) は, 胎児期の腎組織を模倣した組織構築を示す代表的な胎児性腫瘍である. 乳幼児期の腎腫瘍では最も頻度が高いが, 我が国での発生数は, 1年間に100例未満と推測される. 組織学的に, 後腎芽組織に類似した形態を示す後腎芽細胞, 上皮細胞成分, 間葉成分が種々の割合で混在している. 後腎芽細胞は, 胎生期の後腎組織の未熟細胞に類似した形態を示す, 小型・楕円形でクロマチンの濃染する核を有する細胞で, びまん性あるいは結節状に増殖する. 上皮成分は, ロゼット様の構造や種々の程度に分化した腺管が多いが, 時に扁平上皮や粘液を有する円柱上皮もみられる. 間葉成分は, 線維芽細胞様の成分, 横紋筋成分が多いが, 骨, 軟骨, 脂肪などがみられることもある. これらの3成分全てが認められる腫瘍 (triphasic) が最も特徴的であるが, 2種類の成分から成るbiphasicあるいは1種類のみから成るmonophasicの腫瘍もある.

アメリカではNWTS (National Wilms Tumor Study Group) を中心に治療法が開発され (現在はCOG: Children's Oncology Group), 最初に腎摘しその後化学療法を行うプロトコールを長く提唱してきた. 腫瘍の組織型をfavorable histology (FH) とunfavorable histology (UH) (focalまたはdiffuse anaplasiaを伴う

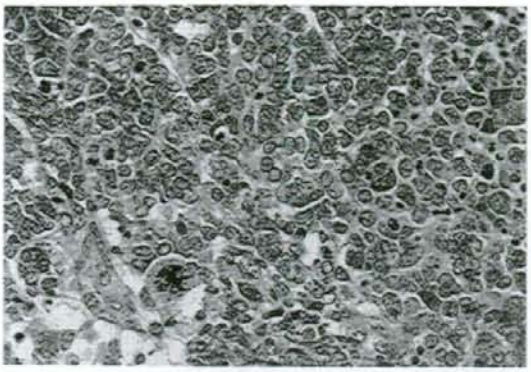

図3 anaplasiaを伴うWilms腫瘍 anaplasiaでは，多極性の核分裂像がみられる．

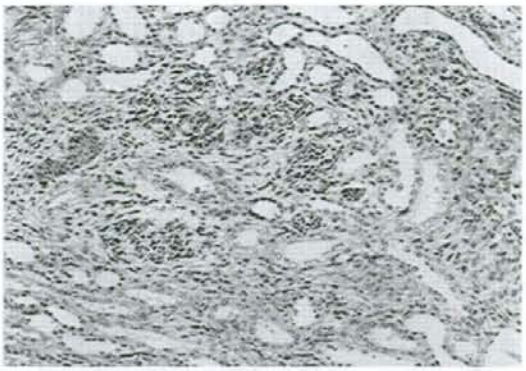

図4 葉内腎芽腫症 後腎芽細胞が，腎組織内に増生している．

腫瘍）に分類し治療方針を決定してきた．anaplasiaは，巨大な核（核の径が他の腫瘍細胞の3倍以上）と多極性核分裂像によって特徴づけられる（図3）．当初，focal anaplasiaはanaplasiaが顕微鏡的に10%以下の場合を指していたが，現在では，anaplasiaを示す領域が，周囲との境界が明瞭で，腎実質に限局し，かつ，anaplasiaがない領域で著しい核の多形性やクロマチン増量がないことと定義されている．focal anaplasia以外はdiffuse anaplasiaである．anaplasiaを有する腫瘍は，化学療法抵抗性とされており，特にdiffuse anaplasiaの場合，強い予後不良因子となる．一方，ヨーロッパでは，SIOP（International Society of Paediatric Oncology）により治療法が開発されてきた．SIOPは，術前に化学療法を行ってから腎摘する治療法を提唱しており，治療後の組織像により治療法を決定している[15]．特にSIOPの提唱する分類では，化学療法により腫瘍細胞が完全に壊死におちいっている場合は，low risk tumoursとされる．通常，後腎芽細胞成分は化学療法に感受性があるが，化学療法後の腎摘標本にて後腎芽細胞成分が優位な場合は，治療抵抗性があると考えられ，high risk tumoursとされる．また，diffuse anaplasiaを伴う症例も，high risk tumoursとされている．

NWTSとSIOPの治療法は，ほぼ同様の成績であるが，NWTSの場合，確実な病理組織診断により治療法を決定できる利点があり，一方，SIOPの場合，化学療法による腫瘍縮小のため腎摘の切除可能率の向上や切除が容易になるなどの利点が挙げられる．我が国の治療研究グループであるJWiTS（Japan Wilms Tumor Study Group）では，NWTS同様，最初に腎摘するプロトコールを採用しているが，施設によっては，SIOP同様，化学療法後に腎摘している．我が国では，小児腫瘍組織分類図譜第一篇小児泌尿器腫瘍における分類が提示されていたが，2008年2月に小児腫瘍組織分類委員会編集の新たなアトラスが出版され，腫瘍内の各構成成分の優位性（2/3以上を占める構成成分）による新たな分類が示された[16]．

2. 造腎組織遺残nephrogenic rest，腎芽腫症nephroblastomatosis

造腎組織遺残は，胎児期の後腎組織の異常な遺残と考えられ，腎芽腫の発生母地と想定されている．腎芽腫症は，造腎組織遺残が多発性，またはびまん性に存在する病変である．造腎組織遺残は，その発生部位により葉内造腎組織遺残intralobar nephrogenic rest（ILNR），辺葉造腎組織遺残perilobar nephrogenic rest（PLNR）に分類される．造腎組織遺残は腎芽腫患者の25%程度にみられるとされ，本邦ではILNRが多くPLNRは稀である．造腎組織遺残同定のためには非腫瘍部や腫瘍辺縁部からの標本作製が重要である．

ILNRは，腎臓の葉内，すなわち腎髄質の錐体とそれを取り囲む皮質から構成される腎葉の内部，時に腎盂周囲に存在する．境界不明瞭な病変で，後腎芽細胞，間葉成分，上皮成分が種々の割合で混在する．腎組織（糸球体や尿細管）と造腎組織遺残の成分が入り混じるように存在することが多い（図4）．一方，PLNRは腎葉の辺縁部に存在する．比較的境界明瞭で後腎芽細胞，上皮成分をみることが多い．造腎組織遺残は肉眼的に容易に認識できるhyperplastic restから，退縮す

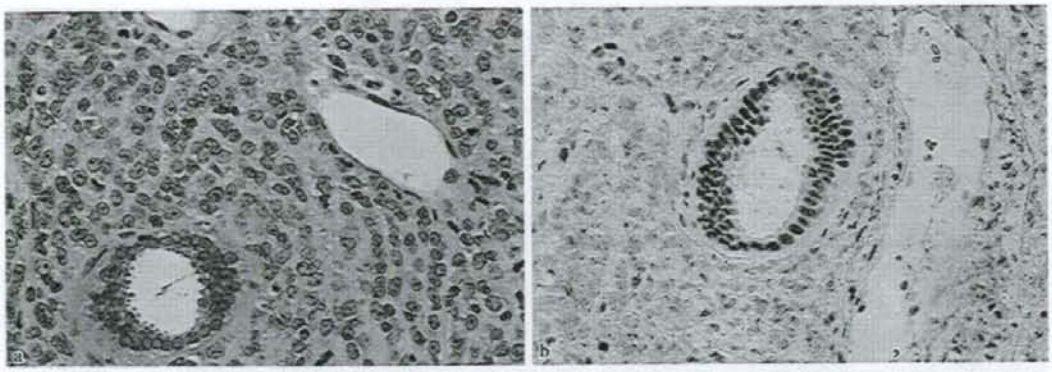

図5 腎ラブドイド腫瘍 a：HE染色．大型核を有する腫瘍細胞が浸潤性に増殖している．ラブドイド細胞が目立たない領域も多い．b：BAF47染色．腫瘍細胞の核は，BAF47陰性である．

るrestまで存在する．hyperplastic restでは腎芽腫とほぼ同様の細胞の増殖がみられるため，生検では，腎芽腫との鑑別は不可能なこともある．

3. 腎明細胞肉腫clear cell sarcoma of the kidney (CCSK)

腎明細胞肉腫は腎ラブドイド腫瘍と共に，旧小児腫瘍組織分類委員会分類において腎芽腫，不全型として分類されていたが，新分類では腎芽腫から除外され，腎明細胞肉腫，腎ラブドイド腫瘍として別個の腫瘍として分類されている．

本腫瘍は，3歳前後に発生し，骨転移の頻度が高いことが特徴的である．被膜をもたないが，肉眼的に周囲との境界明瞭な白色充実性の腫瘍である．組織学的には，樹枝状の血管あるいは血管線維性間質を伴って腫瘍細胞が索状，胞巣状に増殖するパターンが特徴的である．腫瘍細胞の核は，類円形で繊細なクロマチンを有し，核小体は目立たない．定型的な症例では比較的容易に診断できるが，硬化，間質の粘液腫状変化，上皮様形態など，様々なパターンをとることがあり，診断の難しい症例も多い．免疫組織化学的に，ビメンチン陽性となるものの，CD34，S-100，デスミン，MIC2，サイトケラチン，EMAは陰性と報告されている[17]．もともと予後不良であったが，NWTSの報告によると化学療法剤にドキソルビシンを加えることにより，生存率が大幅に向上した．

4. 腎ラブドイド腫瘍rhabdoid tumor of the kidney (RTK)

腎ラブドイド腫瘍は，乳児に好発する高悪性度の腫瘍である．もともと横紋筋肉腫との形態的な類似点からラブドイド(横紋筋肉腫様)という名称がつけられたが，横紋筋に由来する腫瘍とする証拠はない．軟部にも発生することが知られており，また脳にも同様の腫瘍が発生する(atypical teratoid/rhabdoid tumor：AT/RT)．ほとんどは3歳までに発症する．

肉眼的には，白色調の軟らかい腫瘍で，出血・壊死を伴い周囲に浸潤性に増殖する．組織学的には，明瞭な核小体と大型核を有する腫瘍細胞が，びまん性に増殖する(**図5a**)．しばしば，細胞質に好酸性の封入体を認める(ラブドイド細胞)が，必ずしも腫瘍全体に出現するわけではなく，部分的にまとまって出現することが多い．一方で紡錘形の腫瘍細胞が主体を成す部もある．核は大型で，大型の核小体を有し，水泡状のクロマチンを有する．

免疫組織化学的には，ビメンチン陽性であり，サイトケラチン，EMAが一部の細胞に陽性になる．染色体22qの異常とその領域に存在する*SMARCB1* (*hSNF5/INI1/BAF47*)遺伝子の欠失や点変異等による不活化が報告されている．また，中枢神経系と腎臓に腫瘍を発症する患児にて本遺伝子の生殖細胞系列での変異も報告されている．BAF47抗体を用いた免疫染色は，ほとんどの細胞に陽性であるが，RTKでは陰性となることが報告されている(**図5b**)[18]．AT/RTでも同様の報告がなされており，RTKやAT/RTの鑑別診断に参考にすることができる．しかしながら，類上皮肉腫，renal medullary carcinomaなどRTK以外の腫瘍でもその発現消失が報告されており，特異度についてさらなる検討が必要である．

Ⅲ．胚細胞性腫瘍における *c-kit* 遺伝子変異

胚細胞性腫瘍は，原始生殖細胞が起源と考えられており，卵巣，精巣以外に身体の正中線上に近い部位，すなわち頭蓋内，頸部，縦隔，後腹膜，仙尾部などを好発部位とする．原始生殖細胞は胎生4週までに卵黄嚢に観察され，受容体であるc-kit（KIT）を発現していく．リガンドであるstem cell factor（SCF）は卵黄嚢から生殖隆起にかけて濃度勾配を示しながら発現するため，相互の作用により原始生殖細胞は胎生6週の生殖隆起へと遊走し，その後性腺へと分化する．生殖細胞のほか，Cajal細胞，メラノサイト，赤芽球，肥満細胞の分化・増殖においてSCF-KITシステムは必須である．

KITは *c-kit* 遺伝子にコードされる受容体型チロシンキナーゼであり，細胞外領域，細胞膜貫通領域，細胞内領域（傍細胞膜領域とチロシンキナーゼ領域Ⅰ，Ⅱ）より構成される．*c-kit* 遺伝子の突然変異に起因する腫瘍には，gastrointestinal stromal tumor（GIST），肥満細胞症/肥満細胞性白血病，セミノーマなどの胚細胞性腫瘍，急性骨髄性白血病などがある．GISTにみられる突然変異は傍細胞膜領域（エクソン11）に集中しており，肥満細胞症/肥満細胞性白血病ではチロシンキナーゼ領域Ⅱ（エクソン17）の点突然変異が認められる．一方胚細胞性腫瘍では，1999年にTianらがseminoma/dysgerminoma 2例にエクソン17（codon 816）の点突然変異を報告した[19]．*c-kit* 遺伝子の突然変異は，seminoma/dysgerminoma/germinomaの25～38％に認められるが，その他の胚細胞性腫瘍（yolk sac tumor，gonadoblastoma，immature teratomaなど）には認められない[20-23]．免疫組織化学的にKIT発現は大部分のseminoma/dysgerminomaに認められ，突然変異の認められない腫瘍でも，細胞膜に陽性となる．yolk sac tumorでも時にKIT発現がみられるが，細胞質に陽性となる[19,20]．GISTでは，チロシンキナーゼ阻害剤であるイマチニブが有効であるとされているが，seminoma/dysgerminoma/germinomaに認められるエクソン17の点突然変異のうち，D816V，D816H mutantはイマチニブ抵抗性であることが報告され[20]，その臨床応用に際してはさらなる検討が望まれる．

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平成20年度　総括・分担研究報告書

研究代表者　池田　均

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