Pergamon

European Journal of Cancer, Vol. 33, No. 12, pp. 1917–1922, 1997

Printed in Great Britain
0959-8049/97 $17.00+0.00

PII: S0959-8049(97)00295-5

Original Paper

Loss of Chromosome 1p May Have a Prognostic Value in Localised Neuroblastoma: Results of the French NBL 90 Study

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Between March 1990 and December 1994, 316 consecutive children with localised neuroblastoma were registered in the French NBL 90 study. In addition to the assessment of a new chemotherapy regimen in unresectable neuroblastoma, we evaluated the prognostic significance of *MYCN* amplification and loss of the short arm of chromosome 1 (LOH1p). *MYCN* was found in 22/225 children (10%) and associated with unfavourable clinical features such as age at diagnosis > 1 year and large and unresectable tumours. LOH1p was observed in 9/91 patients (10%), of whom some had favourable prognostic factors such as age at diagnosis < 1 year ($n = 4$), INSS stage 1 or 2 ($n = 3$) and no *MYCN* amplification ($n = 4$). Overall survival (OS) and event-free survival (EFS) were, respectively, 56% and 22% (median follow-up: 36 months) for children with LOH1p compared with 97% and 94% for those without (log-rank = 10^{-8}). All except 1 of the 5 children with *MYCN* amplification and LOH1p relapsed and ultimately died of the disease. Among the 4 with LOH1p and no *MYCN* amplification, recurrence occurred in 3 (2 local, 1 metastatic), all alive in second remission after salvage therapy (12–19 months after the relapse). In multivariate analysis, LOH1p was the strongest prognostic indicator for subsequent relapse. LOH1p appears more discriminant than *MYCN* amplification for predicting the risk of recurrence in children with localised neuroblastoma. However, its analysis was possible in only 30% of our patients and its final impact on survival should be confirmed in larger, prospective studies in order to stratify subsequent treatment. © **1997 Elsevier Science Ltd.**

Key words: neuroblastoma, *MYCN*, chromosome 1p
Eur J Cancer, Vol. 33, No. 12, pp. 1917–1922, 1997

INTRODUCTION

NEUROBLASTOMA IS one of the most common solid tumours that affects children in the first years of life [1]. Approximately 50% of patients present with localised tumours which have a good prognosis [2, 3]. Radical surgical excision is generally considered the main condition for cure [4, 5]. Initial surgery can be performed in approximately half of these children and reported survival rates are usually high [6]. However, a small proportion of patients relapse and may not be cured by salvage therapy [7]. These children are not yet reliably identified by specific clinical and biological features. Factors such as histology, biological markers, sometimes combined with age and stage, may be clinically relevant but

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inconsistently predict subsequent outcome [8–12]. Recently described genetic factors such as tumour cell DNA, *MYCN* and chromosome 1p have not been extensively analysed in localised neuroblastomas and their prognostic significance remains unclear [13–17]. Although some cytogenetic studies have identified the loss of heterozygosity of the short arm of chromosome 1 (LOH1p) as the most consistent chromosomal aberration in neuroblastoma cells [15, 18], its clinical significance remains controversial [19–21].

In 1990, we initiated a national prospective trial (NBL 90) including all children with localised neuroblastomas diagnosed in the institutions of the French Society of Pediatric Oncology (SFOP) to evaluate the accuracy of a new therapeutic regimen in unresectable neuroblastomas and to assess the prognostic significance of *MYCN* amplification and LOH1p in such a population. We report herein the results of the SFOP–NBL 90 study.

PATIENTS AND METHODS

Patient population

The NBL 90 study included all new consecutive and untreated children aged < 1 to 16 years presenting with a localised neuroblastoma. Thirty-one institutions of the SFOP participated in the trial between March 1990 and December 1994. The primary tumour was studied with usual imaging techniques (CT scan, MRI) and MIBG scintigraphy. The clinical investigations to ascertain metastatic spread included skeletal study by MIBG (or a ^{99m}Tc scan in the absence of MIBG uptake), bone X-ray in infants and extensive bone marrow staging (at least four bone marrow aspirations and two trephine biopsies). Urinary VMA, HVA and dopamine excretion levels according to creatinine concentration and serum NSE, ferritin and LDH levels were measured. The diagnosis of neuroblastoma was made on the basis of conclusive clinical, laboratory and imaging findings and was always confirmed histologically [22]. The primary tumour was staged according to TNM [23] and INSS criteria [24].

Therapeutic protocol

Surgery. Primary surgery was indicated if excision was deemed radical and safe without any risk of tumour rupture or removal of major organs. Tumours defined as unresectable were lesions that crossed and infiltrated the midline structures, usually encasing large vessels and dumb-bell neuroblastomas.

Chemotherapy. Chemotherapy consisted of two courses of carboplatin (160 mg/m^2/d D1–D5) and etoposide (100 mg/m^2/d D1–D5)-CE, followed by two courses of vincristine (1.5 mg/m^2 D1,D5), cyclophosphamide (300 mg/m^2/d D1–D5) and doxorubicin (60 mg/m^2 D5)-CAdO. From April 1991, the dose intensity of CE was decreased because of haematological toxicity (total doses of carboplatin and etoposide were, respectively, 600 and 450 mg/m^2 per course over 3 days). Patients with unresectable neuroblastoma received two courses of each combination and radical surgical excision was then attempted. After surgery, chemotherapy was indicated in children over 1 year at diagnosis in case of residual disease and/or lymph node (LN) involvement, or in infants with *MYCN* amplification and included 2–4 alternating courses of each combination depending on primary treatment. On the whole, children received a maximum of three courses of each combination.

Radiotherapy. Irradiation of the tumour bed (25–35 Gy) was scheduled in case of a persistent macroscopic residue at the end of the treatment, but only in children over 1 year at diagnosis. After November 1992, loco-regional irradiation was recommended for children whose tumour showed *MYCN* amplification because of a high incidence of local relapses, regardless of age or the quality of surgical excision.

Analysis of genetic alterations

All samples were obtained at diagnosis and the percentage of tumour cells was determined. Results were considered reliable for *MYCN* and chromosome 1p only if tumour material showed, respectively, more than 10 and 50% of neuroblasts. High molecular weight DNA was isolated from the tumour and blood samples from the same patient using standard procedures. *MYCN* genomic content was determined by the Southern or Slot blot procedure using *MYCN* second exon probes (pNB1). *MYCN* amplification was defined when 10 copies or more per haploid genome were found. Deletion of chromosome 1p was determined by analysis of loss of heterozygosity (LOH) at loci on 1p and/or by FISH analysis. In brief, analysis of polymorphic loci was performed in most cases by PCR amplification or Southern blot analysis of *D1S80* and *D1S76* as previously described [25]. Some cases were also analysed at *D1S214*, *D1S199*, *D1S201*, *D1S190*, *D1S200* and *D1S220* as already published [26]. FISH analysis was performed with the centromere probe *D1Z1* and with the telomere probes *D1Z2* or *D1S32*. A total of 71 tumours were analysed by PCR, 17 by the Southern blot procedures, 12 were studied both by Southern blot and FISH and finally 5 tumours were explored only by FISH.

Statistical analysis

Comparisons between children who had LOH1p and those without were performed for each variable with the χ^2 test corrected for heterogeneity or the Fisher's exact test. The probabilities of survival were calculated from the time of diagnosis to relapse, or death, or last follow-up according to the Kaplan–Meier method. In the event-free survival (EFS) analysis, disease progression or relapse, and death, whatever the reasons, were considered as events. Multivariate assessment of EFS was performed by Cox's proportional hazards model and curves were compared using the log-rank test.

RESULTS

Patient characteristics

From March 1990 to December 1994, 316 consecutive children with localised neuroblastoma were registered in the study and were evaluable. The median age was 12 months and 48 were newborn. Most primary tumours were located in the abdomen (63%). Elevated urinary catecholamine excretion and high serum levels of NSE, ferritin and LDH were found in, respectively, 79%, 81%, 12% and 60% of the patients. Positive MIBG uptake was observed in 85% of the children. According to INSS criteria, there were 107 stage 1, 11 stage 2A, 42 stage 2B and 155 stage 3 patients. Among these stage 3 patients, 130 received primary chemotherapy (of whom 34 children had dumbbell tumours).

Genetic alterations and correlations with patient characteristics

MYCN oncogene content was assayed in 225 tumours and found to be amplified ($\geq$ 10 copies) in 22 (10%). The analysis could not be performed in 91 cases either because biopsy was not available ($n = 61$) or because the tumour cell content was poor ($n = 30$). Amplification of *MYCN* correlated with

unfavourable clinical features such as age at diagnosis > 1 year, abdominal primary, large and unresectable tumour, a VMA/HVA ratio < 1 and high levels of serum NSE, ferritin and LDH (data not shown). 1p status was not available in 225 cases either for the same reasons as for *MYCN* or because constitutional DNA has not yet been analysed or because of discrepancy in the results according to the technique used. A total of 9 out of 91 cases demonstrated deletion of 1p (10%). Table 1 shows the clinical characteristics of these children as compared with those without LOH1p. Although LOH1p was associated with usual adverse prognostic factors, it must be stressed that LOH1p was observed in favourable subgroups of patients such as infants ($n = 4$), INSS stage 1 or 2 ($n = 3$) and no *MYCN* amplification ($n = 4$).

Prognostic value of LOH1p

The projected overall survival (OS ± SE) and EFS rates at 5 years for the whole cohort of children with localised neuroblastoma were, respectively, 91 ± 3% and 83 ± 3% with a median follow-up of 36 months (range 12–72). An event occurred in 50 patients: 6 died of treatment related toxicity either after surgery ($n = 5$) or chemotherapy ($n = 1$). Two additional patients died of unexplained causes 18 and 26 months after diagnosis. Forty-two experienced progressive disease ($n = 7$) or subsequent relapse ($n = 35$) either local ($n = 26$), metastatic ($n = 5$) or combined ($n = 4$) at a median time of 7 months postdiagnosis (range 2–27). For children with LOH1p, OS and EFS were, respectively, 56% and 22 ± 26%, as compared with 97% and 94 ± 5% for those without ($P = 10^{-8}$) (Figure 1). In the univariate analysis, variables such as the size of the primary, histological LN involvement, levels of NSE and LDH had significant prognostic value, as well as *MYCN* amplification and LOH1p, which were associated with an adverse outcome (Table 2). However, the calculation of hazard ratios showed that LOH1p and *MYCN* amplification were the strongest factors to predict subsequent outcome. Multivariate analysis was further performed with the five variables found to be significant in the univariate analysis in 73 patients to assess particularly the respective prognostic value of LOH1p and *MYCN* amplification. The simultaneous evaluation of these two genetic factors and the three other clinical characteristics with the Cox model showed that LOH1p remained the unique and the most powerful indicator of subsequent relapse. When combined with LOH1p, *MYCN* amplification was less relevant to predict outcome. Indeed, LOH1p was observed in 4 patients without *MYCN* amplification, of whom 3 experienced subsequent relapse either local ($n = 2$) or metastatic ($n = 1$) (Figure 2). Moreover, some of these children presented with favourable features at diagnosis such as age < 1 year ($n = 3$), primary radical excision and INSS stage 1 or 2 ($n = 2$). All are alive in second remission, but postrelapse follow-up is still short (12–19 months). All patients but one with both LOH1p and *MYCN* amplification suffered from recurrence and ultimately died of the disease. The reason why *MYCN* amplification was observed in 1 patient without LOH1p remains unclear and is still under investigation.

Table 1. *Patient characteristics* (n = 91)

	LOH1p ($n = 9$) No (%)	No LOH1p ($n = 82$) No (%)	*P* value
Sex male	2 (22)	45 (55)	NS
Age < 12 months	4 (44)	46 (56)	NS
Abdominal primary	6 (67)	51 (62)	NS
Size of primary			
≤ 10 cm (T1, T2)	3 (33)	73 (89)	0.0003
> 10 cm (T3)	6 (67)	9 (11)	
INSS stage			
1	1 (11)	30 (37)	0.02
2A	1 (11)	3 (4)	
2B	1 (11)	18 (22)	
3	6 (67)	30 (37)	
Initial histology			
Neuroblastoma	8/8 (100)	51/73 (70)	NS
Positive MIBG uptake	8 (89)	69/76 (91)	NS
Urinary catecholamine excretion			
Abnormal	8 (89)	60/73 (82)	NS
VMA/HVA < 1	7 (87)	36/73 (49)	0.03
Dopamine			
< 2000	6/8 (75)	39/59 (66)	
> 2000–< 3000	0/8	6/59 (10)	
> 3000	2/8 (25)	14/59 (24)	NS
Elevated NSE	6/6 (100)	43/56 (77)	NS
Elevated Ferritin	0/6 (0)	8/60 (13)	NS
Elevated LDH	3/3 (100)	26/32 (81)	NS
MYCN amplification	5 (56)	1/73 (1)	0.0000001
Initial resectability	3 (33)	59 (72)	0.02
Postsurgical macroscopic residual disease	2/7 (29)	18/81 (22)	NS
Histological invasion of lymph nodes	6/7 (86)	36/75 (48)	NS
Response to primary chemotherapy	4/5 (80)	15/24 (62)	NS
CR at the end of therapy	5 (56)	74/81 (91)	NS

DISCUSSION

To our knowledge, this is the first prospective study focusing on children with localised neuroblastoma and to assess the clinical relevance of genetic alterations in such a population. Biased selection was avoided by registering all consecutive cases of localised neuroblastoma observed in all referring institutions of the Société Française d'Oncologie Pédiatrique. All patients were studied according to INSS recommendations, with particular recourse to MIBG scintigraphy and extensive bone marrow staging. OS and EFS of the whole cohort are good and these results should be emphasised as this series includes unresectable neuroblastomas which are usually reported with metastatic neuroblastomas. Apart from the clinical prognostic factors, genetic alterations have been rarely assessed in localised neuroblastomas. *MYCN* amplification is considered as one of the

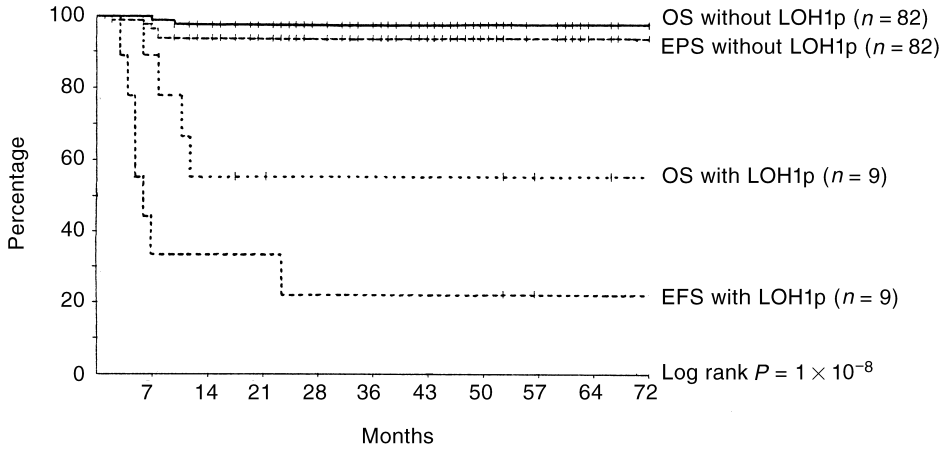

Figure 1. Overall survival (OS) and event-free survival (EFS) according to 1p status ($n = 91$).

Table 2. Prognostic factors

	No. event/No. patient (EFS)	Univariate analysis *P* value (log-rank)	Hazard ratio (95% CI)
Sex			
Male versus female	26/167 (84) versus 24/149 (84)	NS	
Age			
0–12 months versus > 12 months	20/152 (87) versus 30/164 (82)	NS	
Site of primary tumour			
Abdominal versus non-abdominal	34/191 (82) versus 16/125 (86)	NS	
Size of primary tumour			
≤ 10 cm versus > 10 cm	27/259 (89) versus 23/55 (59)	10^{-7}	4.2 (2.4–7.3)
INSS stage			
1-2A-2B versus 3	17/160 (89) versus 5/25 (80)	NS	
MIBG uptake			
Positive versus negative	38/245 (85) versus 5/40 (78)	NS	
Initial histology			
Neuroblastoma versus ganglioneuroblastoma	31/175 (82) versus 7/84 (91)	NS	
Urinary catecholamines			
VMA/HVA ≥ 1 versus VMA/HVA < 1	17/112 (86) versus 32/178 (82)	NS	
Dopamine < 2000 versus > 2000	20/163 (88) versus 23/95 (74)	6×10^{-3}	
NSE normal versus > 2N	2/41 (93) versus 28/114 (76)	2×10^{-3}	
Ferritin normal versus > 2N	23/165 (86) versus 4/15 (78)	NS	
LDH < 2N versus > 2N	5/82 (92) versus 10/22 (62)	6×10^{-5}	
MYCN amplification			
< 10 copies versus ≥ 10 copies	20/203 (89) versus 14/22 (31)	8×10^{-8}	8.9 (4.5–17.7)
LOH1p* no versus yes	5/82 (94) versus 7/9 (22)	10^{-8}	21.3 (6.7–68.2)
Initial treatment			
Surgery versus chemotherapy	22/186 (88) versus 28/130 (77)	4×10^{-2}	
Results of surgery			
CR versus VGPR + PR	27/245 (88) versus 19/66 (77)	3×10^{-4}	2.7 (1.5–5.1)
Histological lymph node invasion			
No versus yes	14/178 (92) versus 29/121 (76)	9×10^{-5}	3.5 (1.8–6.8)
*Multivariate analysis			
LOH1p		$< 10^{-4}$	20 (4.4–88.9)

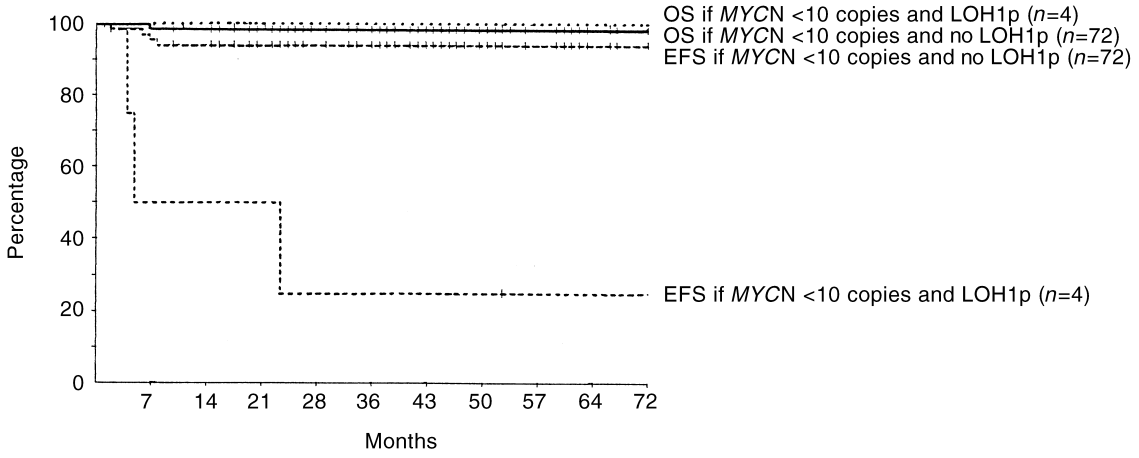

Figure 2. Overall survival (OS) and event-free survival (EFS) according to *MYCN* and 1p status.

most powerful indicators of unfavourable outcome [14], and we recently confirmed its negative influence in localised neuroblastomas [27]. However, some patients without *MYCN* amplification may still experience relapses, even metastatic. In our series, LOH1p was observed in 10% of the evaluable patients with localised neuroblastoma, which is consistent with other series, although the number of cases with localised disease is low or even not reported [19–21]. By far the most relevant finding in this study was the extremely powerful significance of LOH1p. Indeed, the main clinical value of LOH1p as a prognostic factor lies in its ability to predict an adverse event in patients with so-called favourable features such as age < 1 year, small and resectable tumours. Furthermore, analysis of chromosome 1 yielded additional prognostic information in patients with no *MYCN* amplification. Patients with LOH1p were at a higher risk of relapse, including metastatic recurrences, than those who did not have LOH1P (EFS = 22% versus 94%). This difference remained significant in the subset of patients ($n = 76$) with no *MYCN* amplification (EFS = 25% versus 94%). Conversely, children with neither LOH1p nor *MYCN* amplification had an excellent outcome since their 3-year OS was 99%. The prognostic significance of LOH1p is controversial. Two studies reported that 1p deletion was not a reliable marker for prognosis, but more than half of their cases presented with metastatic disease at diagnosis [19, 20]. Furthermore, some authors suggest that there may be two close tumour suppressor genes located on chromosome 1p with biologically distinct subtypes of neuroblastoma [28] and these different genes may be alternatively identified depending on the technique used. In contrast, some authors reported the negative influence of LOH1p on outcome in patients with low-stage disease, including localised neuroblastomas [21, 29] sometimes as the second prognostic indicator after *MYCN* amplification [30].

Our data confirm the excellent outcome of localised neuroblastomas. We report the first study assessing the prognostic significance of LOH1p in localised neuroblastomas. LOH1p appears more discriminant than *MYCN* amplification in predicting the risk of recurrence in children with localised neuroblastoma, including patients with so-called favourable clinical features. However, these results concern only 30% of the tumours of our cohort and their final impact on survival should be confirmed in larger studies in order to stratify subsequent treatment.

1. Bernstein ML, Leclerc JM, Bunin G, *et al.* A population–based study of neuroblastoma incidence, survival, and mortality in North America. *J Clin Oncol* 1992, **10**, 323–329.
2. Rosen EM, Cassady JR, Frantz CN, *et al.* Neuroblastoma: The Joint Center for Radiation Therapy/Dana Farber Cancer Institute/Children's Hospital experience. *J Clin Oncol* 1984, **2**, 719–732.
3. Berthold F, Brandeis WE, Lampert F. Neuroblastoma: diagnostic advances and therapeutic results in 370 patients. *Monogr Paediatr* 1986, **18**, 206–223.
4. Evans AE, Albo V, D'Angio GJ, *et al.* Factors influencing survival of children with non metastatic neuroblastoma. *Cancer* 1976, **38**, 661–666.
5. Le Tourneau JN, Bernard JL, Hendren WH, Carcassonne M. Evaluation of the role of surgery in 130 patients with neuroblastoma. *J Pediatr Surg* 1985, **20**, 244–249.
6. De Bernardi B, Rogers D, Carli M, *et al.* Localized neuroblastoma. Surgical and pathological staging. *Cancer* 1987, **60**, 1066–1072.
7. Evans AE, D'Angio GH, Koop C, *et al.* The role of multimodal therapy in patients with local and regional neuroblastoma. *J Pediatr Surg* 1984, **19**, 77–80.
8. Joshi VV, Cantor A, Altshuler G, *et al.* Recommendations for modification of terminology of neuroblastic tumors and prognostic significance of Shimada classification: a clinicopathologic study of 213 cases from the Pediatric Oncology Group. *Cancer* 1992, **69**, 2183–2196.
9. Zelter PM, Marangos PJ, Evans AE, Schneider SL. Serum neuron specific enolase in children with neuroblastoma. Relationship to stage and disease course. *Cancer* 1986, **57**, 1230–1234.
10. Hann HWL, Levy HM, Evans AE. Serum ferritin as a guide to therapy in neuroblastoma. *Cancer Res* 1980, **40**, 1411–1413.
11. Shuster JH, McWilliams NB, Castelberry R, *et al.* Serum lactate dehydrogenase in childhood neuroblastoma. *Am J Clin Oncol* 1992, **15**, 295–303.
12. Evans AE, D'Angio GJ, Propert K, Anderson J, Hann HWL. Prognostic factors in Neuroblastoma. *Cancer* 1987, **59**, 1853–1859.
13. Look AT, Hayes FA, Nitschke R, McWilliams, Green AA. Cellular DNA content as predictor of response to chemotherapy in infants with unresectable neuroblastoma. *N Engl J Med* 1984, **311**, 231–235.
14. Seeger RC, Brodeur GM, Sather H, *et al.* Association of multiple copies of N-*Myc* oncogene with rapid progression of neuroblastomas. *N Engl J Med* 1985, **318**, 111–116.
15. Fong CT, White PS, Peterson K, *et al.* Loss of heterozyosity for chromosome 1 or 14 defines subsets of advanced neuroblastomas. *Cancer Res* 1992, **52**, 1780–1785.
16. Brodeur GM, Azar C, Brother M, *et al.* Neuroblastoma: effect of genetic factors on prognosis and treatment. *Cancer* 1992, **70**, 1685–1694.
17. Joshi VV, Cantor AB, Brodeur GM, *et al.* Correlations between morphologic and other prognostic markers of neuroblastoma. *Cancer* 1993, **71**, 3173–3181.

18. Gilbert F, Balaban G, Moorhead P, Bianchi D, Schlesinger H. Abnormalities of chromosome 1 in human neuroblastoma tumors and cell lines. *Cancer Genet Cytogenet* 1982, **7**, 33–42.
19. Michon J, Delattre O, Zucker JM, *et al.* Prospective evaluation of Nmyc amplification and deletion of the short arm of chromosome 1 in neuroblastoma tumours: a single institution study. *6th Symp. Advances in Neuroblastoma Research*,13–15.05.93, Philadelphia. Philadelphia, 1993.
20. Gehring M, Berthold F, Edler L, Schwab M, Amler LC. The 1p deletion is not a reliable marker for the prognosis of patients with neuroblastoma. *Cancer Res* 1995, **55**, 5366–5369.
21. Caron H, Van Sluis P, De Kraker J, *et al.* Allelic loss of chromosome 1p as a predictor of unfavorable outcome in patients with neuroblastoma. *N Engl J Med* 1996, **334**, 225–230.
22. Mäkinen J. Microscopic patterns as a guide to prognosis of neuroblastoma in childhood. *Cancer* 1972, **29**, 1637–1646.
23. American Joint Committee. Neuroblastoma. In Beahrs OH, Myers MH, eds. *Manual of Staging of Cancer.* Philadelphia, Lippincott, 1983, 237–239.
24. Brodeur GM, Pritchard J, Berthold F, *et al.* Revisions of the International Criteria for Neuroblastoma Diagnosis, Staging and Response to Treatment. *J Clin Oncol* 1993, **11**, 1466–1477.
25. Peter M, Michon J, Vielh P, *et al.* PCR assay for chromosome 1p deletion in small neuroblastoma samples. *Int J Cancer* 1992, **52**, 544–548.
26. Schleiermacher G, Peter M, Michon J, *et al.* A multiplex PCR assay for routine evaluation of deletion of the short arm of chromosome 1 in neuroblastoma. *Eur J Cancer* 1995, **31A**, 535–538.
27. Rubie H, Hartmann O, Michon J, *et al.* N-Myc gene amplification is a major prognostic factor in localized neuroblastoma: results of the French NBL 90 study. *J Clin Oncol* 1997, **15**, 1171–1182.
28. Cheng NC, Van Roy N, Chan A, *et al.* Deletion mapping in neuroblastoma cell lines suggests two distinct tumour suppressor genes in the 1p 35–36 region, only one of which is associated with N-*Myc* amplification. *Oncogene* 1995, **10**, 291–297.
29. Ambros P, Ambros IM, Strehl S, *et al.* Regression and progression in neublastoma: does genetics predict tumor behaviour? *Eur J Cancer* 1995, **31A**, 510–516.
30. Christiansen H, Sahin K, Berthold F, *et al.* Comparison of DNA Aneuploïdy, Chromosome 1p abnormalities, MYCN amplification and CD44 expression as prognostic factors in neuroblastoma. *Eur J Cancer* 1995, **31A**, 541–544.

Acknowledgements—This work was supported by the Association pour la Recherche contre le Cancer.