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Malignant peripheral neuroectodermal tumours of bone other than Askin's neoplasm: characterization of 14 new cases with immunohistochemistry and electron microscopy*

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Summary. Peripheral neuroectodermal tumours (PNET) of bone are rare and mimick those seen in soft tissue (peripheral neuroepithelioma of soft tissue). Their differential diagnosis from Ewing's sarcoma (Es) is extremely difficult by optical means. Here we report 14 new cases of PNET of bone (other than Askin's neoplasm) located primarily in the limbs, pelvic girdle and scapula. Clinically and radiologically they displayed Ewing's sarcoma-like features: mean age was 14.4 years, male/female ratio being 3:11. Metastasis was present in 6 cases at diagnosis (5 with bone metastasis). Prognosis was poor; thirteen patients died; only one with a metatarsal located tumour is alive and free of disease. The mean survival rate was 25 months following diagnosis and treatment with radio- and multimodal chemotherapy. Histologically the 14 cases displayed Homer-Wright rosettes and pseudorosette-like structures, as well as a fibrillary background and lobular pattern. Immunohistochemistry revealed positivity in a number of neural markers when using paraffin-embedded material: NSE, B-2-microglobuline, HNK-1 (leu-7) and E-36 antibodies. At EM level the cell cytoplasms evidenced dense-core granules with neurosecretion, neurotubules and intermediate filaments like those seen in peripheral neuroepithelioma.

Key words: Neuroectodermal bone tumours – Immunochemistry – Electron microscopy

Introduction

Peripheral neuroectodermal tumours (PNET) of bone are rare but resemble those seen in soft tissue (peripheral neuroepithelioma) (Seemayer et al. 1975; Nesbitt and Vidone 1976; Bolen and Thorning 1980; Harper et al. 1981; Hashimoto et al. 1983; Voss et al. 1984). Most PNET have been described in the thoracopulmonary region in children and young adults (Askin's tumour), following the first description of this Ewing-like tumour entity by Askin et al. in 1979. Since then a number of new cases has been reported, displaying some evidence of neuroectodermal differentiation at immunohistochemical and EM levels (Das et al. 1982; Gonzalez-Crussi et al. 1984; Schmidt et al. 1985; Linnoila et al. 1986).

Atypical variants of Ewing's sarcoma (Es) have been illustrated by our group (Llombart-Bosch et al. 1978; Llombart-Bosch et al. 1982) and by Schmidt et al. (1982) within the context of Es with neuroblast-like features. More recently we reported five cases of atypical Es with neural differentiation (Llombart-Bosch et al. 1987) resembling those previously seen by Jaffe et al. (1984) and called "peripheral neuroectodermal sarcoma of bone". Moreover, neural expression has also been found in several cases of "bona fide" Es when using fresh tissue from primary tumours (Donner et al. 1985; Llombart-Bosch et al. 1986; Moll et al. 1987) and in tissue cultures (Cavazzana et al. 1987) of established Es cell lines. Therefore the question arose: are all Es of bone actually PNET, or should this diagnosis be restricted only to atypical Es displaying morphological neural attributes?

In this report 14 new cases of PNET of bone are presented. These cases were selected because they displayed diverse atypical characteristics for an Es histologically, such as irregular cell size and contour, lobulated pattern and pseudorosettes. Since none of these tumours was located in the thoracopulmonary region, the diagnosis of Askin's tumour was not considered.

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Table 1. Immunohistochemical analysis of small round blue cell sarcomas antibodies tested

Antibodies against	Source	Method	Dilution
Neuron specific enolase (P)	Rabbit (Dako, Denmark)	PAP	1/200
Neuron specific enolase (M. Gamma)	Mouse (Innogenetics, Belgium)	ABC	1/500[a]
Protein S-100 (P)	Rabbit (Dako, Denmark)	PAP	1/200
GFAP (P)	Rabbit (Dako, Denmark)	PAP	1/500
Neurofilament prot 68 kd (M)	Mouse (Sambio, Netherlands)	ABC	Undiluted
HNK-1 (leu-7)(M)	Mouse (Dr. Lipinski)[c]	ABC	1/5
Vimentin (M)	Mouse (Dako, Denmark)	ABC	1/30
α-1 antichimotrypsin (P)	Rabbit (Dako, Denmark)	PAP	1/500
Myoglobin (P)	Rabbit (Dako, Denmark)	PAP	1/150
Ulex europaeus-1 (P)[b]	Rabbit (Dako, Denmark)	Indirect method	1/1000
β-2 microglobulin (P)	Rabbit (Accurate Chem Co., Denmark)	PAP	1/200
LON E-36 (M)	Mouse (Dr. Munro-Neville)[c]	ABC	1/5

[a] Incubation overnight at 4° C

[b] The lectin from ulex europaeus-1 (UEA-1) was obtained from the Sigma Chem. Co. (St. Louis, USA) and was employed at 2 mc gr/ml diluted in PBS

[c] Our kind thanks to Dr. Lipinski (Villejuif, France) and also to Dr. Munro-Neville (Sutton, UK) for providing us with the indicated immunosera

P = polyclonal antibody; MO = monoclonal antibody

Materials and methods

The present cases were selected from 315 small round blue cell sarcomas of bone, 261 of which were previously published with regard to several histological prognostic features determined by routine microscopical techniques (Llombart-Bosch et al. 1986). In that study, 40 cases were diagnosed as atypical Es (large cell and endothelial-like variants) and 54 more cases were found to possess rosette-like figures. From the latter we have selected this group of 14 Es-like neoplasms, on which either immunohistochemical and/or EM techniques could be performed. Being a retrospective study, only paraffin-embedded tissue was used for immunohistochemistry. The clinical records and follow-up were available in all these cases.

For light microscopy a representative number of slides was stained with hematoxylin-eosin, hematein-erythrosin-safranin (HES), periodic acid-Schiff (PAS), Best's carmine for glycogen and Gomori for reticulin. No fresh tissue was available in any of the 14 cases, and only Bouin or formalin-fixed, paraffin-embedded material could be used for immunohistochemistry. We have used the indirect immunohistochemical method as well as the unlabelled antibody-peroxidase-antiperoxidase method (Taylor and Burns 1974; Burns 1982). Endogenous peroxidase activity was blocked with 0.5% hydrogen peroxid in methanol for ten minutes and the non-specific reactivity was prevented by covering the slides with normal rabbit serum. For each antibody tested, negative (first antibody omitted) and positive controls were simultaneously employed. Following the specific incubations, the tissue was washed in PBS for 10 min, and then incubated with rabbit antimouse immunoglobulin antiserum conjugated to peroxidase (DAKO Labs Santa Barbara Cal.). Finally the reaction was revealed with 0.05% of 3.3′ diaminobenzidine-tetrahydrochloride with 0.03% hydrogen peroxide in PBS. The following antibodies to antigens were used: NSE (polyclonal) and gamma NSE (monoclonal), protein S-100, glial fibrillary acidic protein (GFAP), anti 68-kd neurofilament protein (NFP). The monoclonal antibody HNK-1 (leu-7) was kindly supplied by Dr. Lipinski (Gustave Roussy Institute, Villejuif, France) and was employed with the avidine-biotin-peroxidase technique (Burns 1982). Other immunosera used were the following: vimentin, alpha-1-antichimotrypsin, myoglobine, beta-2-microglobuline (Funa et al. 1986) and the E-36 monoclonal antibody (Monaghan and Roberts 1985) which was kindly supplied by Dr. Munro Neville (Surrey, UK). The Ulex Europeus-1-lectin was also employed for the detection of endothelial cells. Table 1 summarizes the antibodies tested, their source, method and dilutions.

Only six cases could be studied by EM. One cubic millimeter fragments were fixed in phosphate-buffered glutaraldehyde (pH 6.9 at 4 degrees C), washed in Millonig solution and postfixed in 1% osmium tetroxide. The tissue blocks were then progressively dehydrated in graded solutions of ethanol acetone, immersed in propylene oxide and embedded in EPON. Sections were cut in an LKB ultramicrotome and double-stained with uranyl acetate and lead citrate (Venable and Goggenshau 1965) and examined with both a JEOL 100B and a ZEISS 10A electron microscope.

Results

The clinical features of the 14 patients are summarized in Table 2; there were 3 females and 11 males, ages ranging from 3 to 23 years, the mean being 14.4 years. Three patients were North African, the rest Europeans.

Anatomical location in all cases was radiologically and clinically confirmed as primary malignancy of bone, with Ewing-like features; thus all cases were suspected of being of this tumour type. The primary location was mainly the limbs (fibular 3 cases, tibia 2 cases, femur 1 case, metatarsal bone 1 case, humeral bone 2 cases, radius 1 case), while the trunk was involved in 4 cases (2 pelvic infiltrations and two cases within the scapula).

Table 2. Clinical data and follow-up of 14 patients with malignant peripheral neuroectodermal sarcoma of bone

Patient	Age	Sex	Localization at presentation	Metastasis at diagnosis	Treatment	Current status	Survival (months)
1	15	M	Fibula left	Absent	rad	dead	29
2	15	M	Humerus rt	Absent	rad + chem	dead	25
3	17	M	Fibula left	Rachidian	rad + chem	dead	6
4	3	F	Tibia right	Several Bones	rad + chem	dead	7
5	23	M	Pubis	Absent	rad + chem	dead	49
6	22	M	Metatarsic bone rt foot	Lymphnodes	surg + chem	F.O.D.	108
7	8	M	Fibula left	Absent	rad + chem	dead	47
8	13	M	Humerus left	Absent	rad + chem	dead	13
9	12	M	Scapula rt	Absent	rad + chem	dead	41
10	22	M	Radius left	Absent	rad + chem	dead	51
11	14	M	Tibia rt	Absent	rad + chem	dead	26
12	16	M	Ischiopubic branch	Rachidian	rad + chem	dead	9
13	6	F	Tibia left	Cranial base	rad + chem	dead	12
14	16	F	Femur left	Cranial bone tibial bone	rad + chem	dead	14

rad = radiotherapy; chem = multimodal chemotherapy; surg = surgery; m = male; f = female; f.o.d. = free of disease

Most of these neoplasms possessed soft-tissue infiltration but they also extended deep into the bone shaft. Only one case could be surgically resected (No. 6), the right foot being amputated, with the neoplasm located in the second metatarsal bone; there was scanty soft-tissue invasion. No macroscopic tumour tissue was available in other cases, and no relation with nerves could be found. All the cases were reviewed for the presence of implants, multiple bony metastasis being found in 5 cases at diagnosis, with rachidian and cranial bone involvement. In 1 case there was also infiltration of the regional lymph nodes.

Histologically all cases were primary tumours, consistent with small round blue cell sarcomas (Ewing's type), and were submitted to a protocol of radio- and multimodal chemotherapy (Vincristine, Actinomycine D, Cytoxan and Adriamycine). These patients have been included in the results published from the clinical point of view by Zucker et al. 1977, 1983).

Response to therapy was poor in all cases but one; the mean survival time was 25 months. Only Case No. 6, with the metatarsal primary submitted to surgery and chemotherapy is alive at present, nine years later, and free of disease. Causes of death were local tumour relapse (7 cases) distant metastasis (9 cases) or both, with generalized disease. Patient No. 1 died as a direct result of a traffic accident, having no connection with the disease.

Optical microscopy (Fig. 1 a–c) of this tumour revealed non-cohesive, densely aggregated small round blue cells, distributed in a diffuse form; also, and in almost all cases, a lobular pattern was found, composed of reticular fibres surrounding large cell groups in a basket-like distribution. Reticular fibres were sparse in number and of perivascular arrangement. A faintly fibrillary background was also frequently noted in the diffuse, solid fields of the neoplasm.

Cells were always small with scanty cytoplasm. Nuclei possessed irregular infoldings, variable size, and a coarse, dense chromatine with one or two nucleoli. Isolated large cells with binucleation could be found but no ganglion cells existed. Glycogen was present in 9 out of 14 cases with similar distribution to Es.

A common and distinctive feature of all the cases studied was the presence of rosettes of Homer-Wright type. The number varied from case to case, or even within different fields of the same neoplasm. In the same tumours, areas with more immature and poorly developed pseudorosettes appeared, lacking a central fibrillary core, or surrounding vascular channels of capillary type. All these features were present in standard HE sections, and therefore a differential diagnosis with the conventional Es was suggested.

Several immunohistochemical markers have been tested, including NSE and HNK-1, which proved positive in five cases previously published, of similar tumour type (Llombart-Bosch et al. 1987).

NSE was positive in 11 out of the 14 cases tested, while HNK-1 was positive in 7 cases. The positivity of NSE appeared not only within the cytoplasm of numerous cells, but also in the rosettes and pseudorosette centers, the intensity being pale and the distribution uniform (Fig. 2).

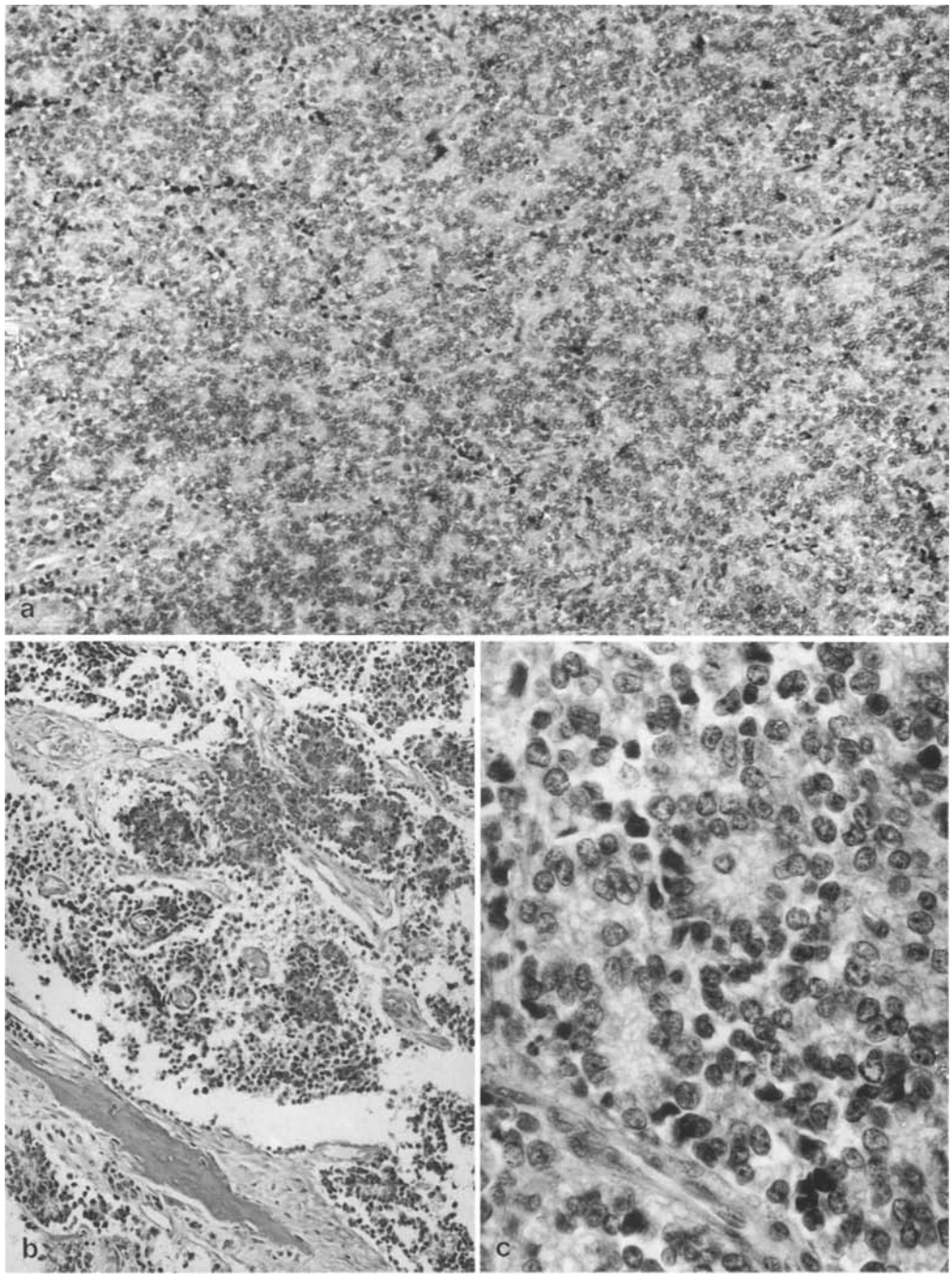

Fig. 1 a–c. Histological view of a peripheral neuroectodermal tumour of bone. **a** Shows a soft-tissue extension of the neoplasm, being composed of a diffuse aggregate of small round blue cells, with a rosette pattern (HE ×5.04). **b** Similar intraosseous extension of the neoplasm with Homer-Wright rosettes (HE ×5.04). **c** Higher magnification of pseudorosettes lacking a central fibrilar core (HE ×32)

However, HNK-1 activity was observed as fine cytoplasmic dots in isolated cell groups, only occasionally were rosettes stained.

A diffuse and intense activity was seen with Beta-2-microglobulin in 12 cases, displaying similar decoration to NSE within the cells and rosettes, while the monoclonal antibody E-36 presented analogous cell distribution such as that found with HNK-1. S-100 antigen was observed only in one tumour, its intensity limited to isolated cells. Neurofilaments and GFAP were not demonstrable in any of the 14 cases tested.

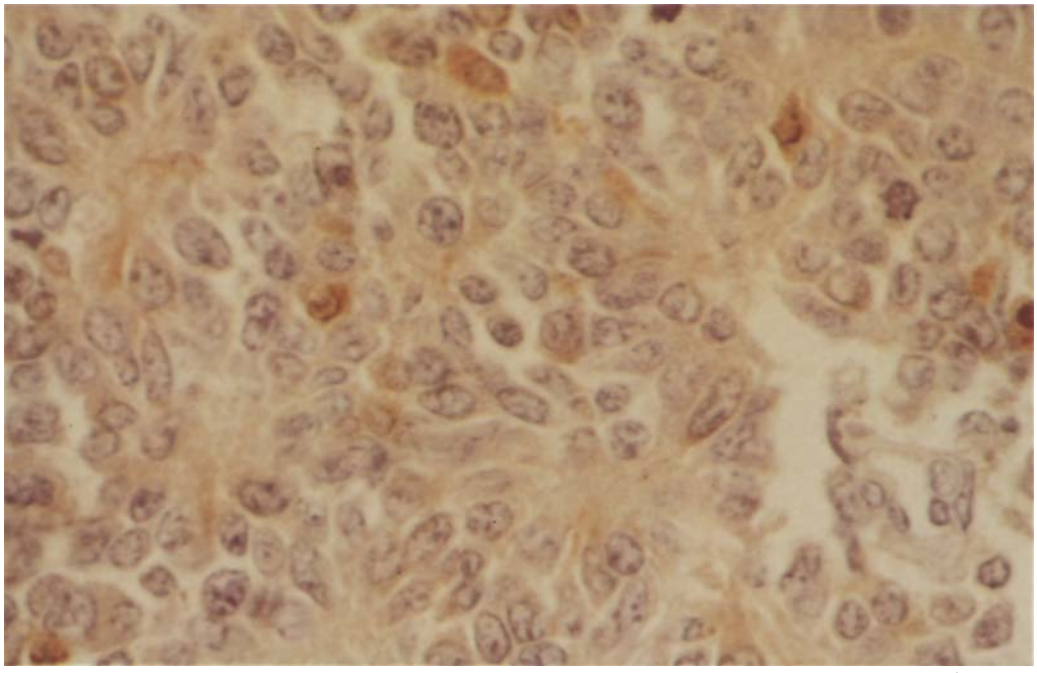

Fig. 2. Immunohistochemistry of the neoplasm, showing two Homer-Wright rosettes displaying diffuse and more condensed NSE activity in the tumour cells (PAP technique; ×32; Kodakchrome)

Vimentin was strictly positive in five tumours, its activity spread on the cytoplasm. Ulex-Europaeus lectin reacted only within the vessels in the stroma, while the tumour cells showed no reactivity. Isolated macrophages possessed Alpha-1-antichymotrypsin activity, and myoglobin was positive within skeletal muscle cells infiltrating the neoplasms.

All six tumours displayed areas of poor differentiation, composed of closely packed small cells in groups with a round-to-polygonal contours. Poorly developed junctional cell-to-cell attachments were seen. Within the cytoplasm the number of organelles was scarce. Clumps of glycogen were present, mainly at a perinuclear level. The nuclei, round or elongated, showed a faintly clumpy chromatin associated with one or two prominent nucleoli. The almost complete absence of specific ultrastructural features conferred the texture attributed to conventional Ewing's sarcoma to these fields and only the presence of isolated cell processes established some ultrastructural differentiation (Fig. 3).

The fields which suggested a neuroectodermal maturation were made up of groups of cells with rosette-like figures, their nuclei elongated or polygonal but oriented toward a center. This central core was filled with cytoplasmic processes, composed of dendritic-like structures, loosely intermingled in a complex interdigitated fashion. Short cytoplasmic tangles also extended between the cells, contacting each other by blunt ends. Neurophil-like structures formed by such tangled masses of neurite-like processes contained neurotubule-like figures and bands of intermediate filaments, 7–10 nm in diameter (Fig. 4a).

Groups of neurosecretory granules (dense core granules enveloped by a membrane of 50–200 nm in size) were found not only within the cell processes but also in the cytoplasm and close to the Golgi fields. An elaboration of these granules has been observed in one case; the newly synthesized granules were located within the Golgi fields and showed a progressive central core condensation, being not only round in contour but also elongated. Clear morphological differences with primary lysosomes were found (Fig. 4b, c).

At interstitial level aggregates of basal lamina-like material and long-spaced cross-banded fibrils (Luse-like bodies), were occasionally seen, which has not been the case in Ewing's sarcoma (Fig. 4d).

Discussion

The neuroectodermal tumour of bone constitutes a recently isolated neoplasm which morphologically resembles the peripheral neuroepithelioma of soft tissue.

Clinically this tumour has mainly been considered as a restricted regional anatomical entity, lim-

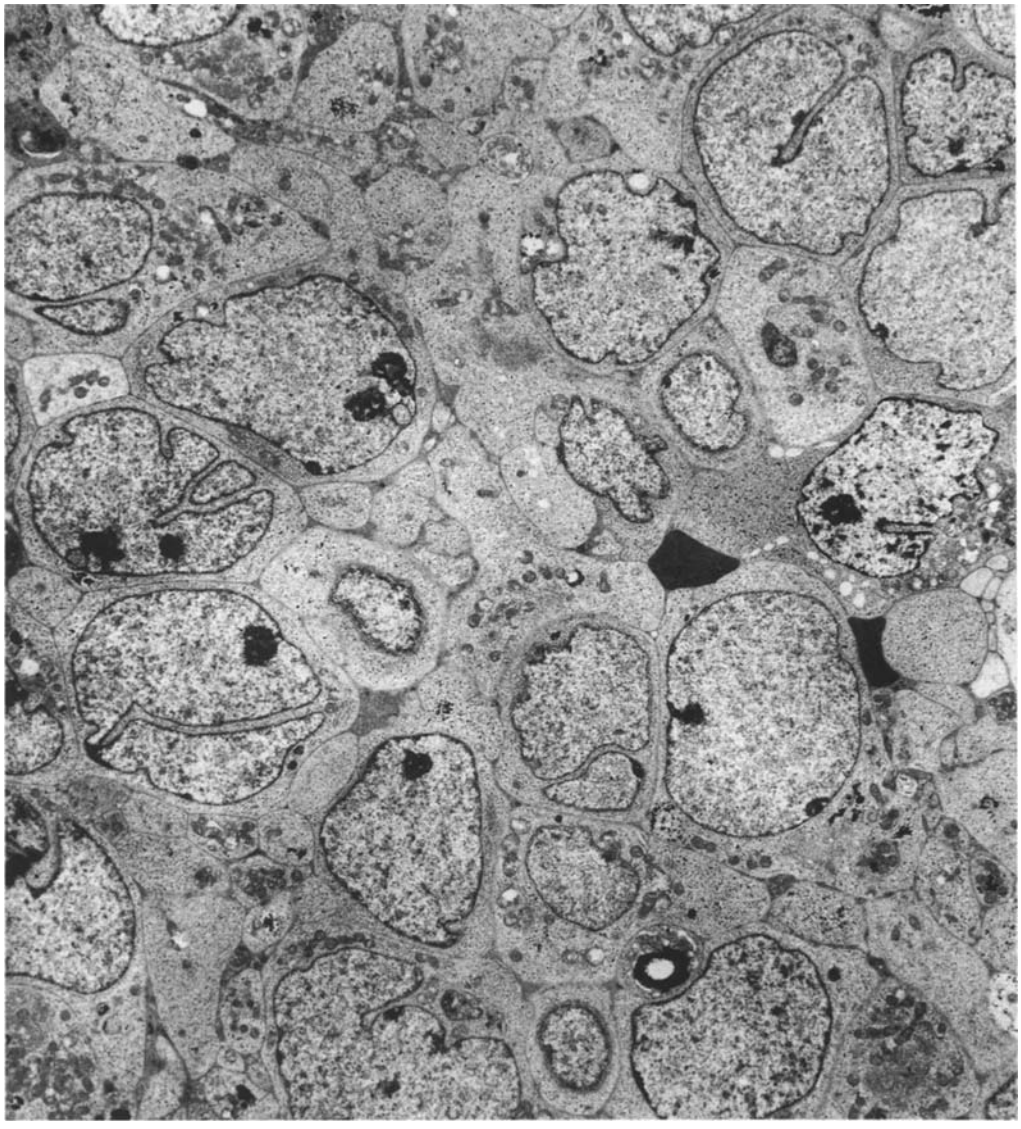

Fig. 3. Electron microscopy of an undifferentiated tumour field, with some cell heterogeneity and presence of cell processes not usually seen in conventional Es of bone (× 3400)

ited to the thoraco-pulmonary region and present in children and adolescents (Askin et al. 1979; Das et al. 1982; Gonzalez-Crussi et al. 1984; Schmidt et al. 1985; Linnoila et al. 1986). At histological level the neoplasm displays rosettes or pseudorosette-like figures, being represented by small round blue cells, while showing NSE positivity at immunohistochemical level (Triche and Askin 1983; Linnoila et al. 1986). Neurosecretory granulae have also been found with EM (Triche et al. 1985; Linnoila et al. 1986). Moreover, the presence of an 11:22 chromosome translocation has been reported (Seemayer et al. 1985) which has also been found in Es of bone (Aurias et al. 1983; Turc-Carel et al. 1983, 1984), in peripheral neuroepithelioma (Wha-Peng et al. 1984) of soft tissues, and in a neuroendocrine neoplasm (Vigfusson et al. 1986).

Recently other cases, similar to Askin's tumours, have also been reported outside the thoracopulmonary region but within the bone tissue by Jaffe et al. (1984). In a previous publication our group (Llombart-Bosch et al. 1987) has reported five cases of bone neoplasms which mimicked atypical Es but displayed neuroectodermal features; only one of those cases was located within the thorax.

In this presentation we report a further 14 new cases of this uncommon tumour entity, all of which were located anatomically outside the thoraco-pulmonary fields (only two cases appeared within the

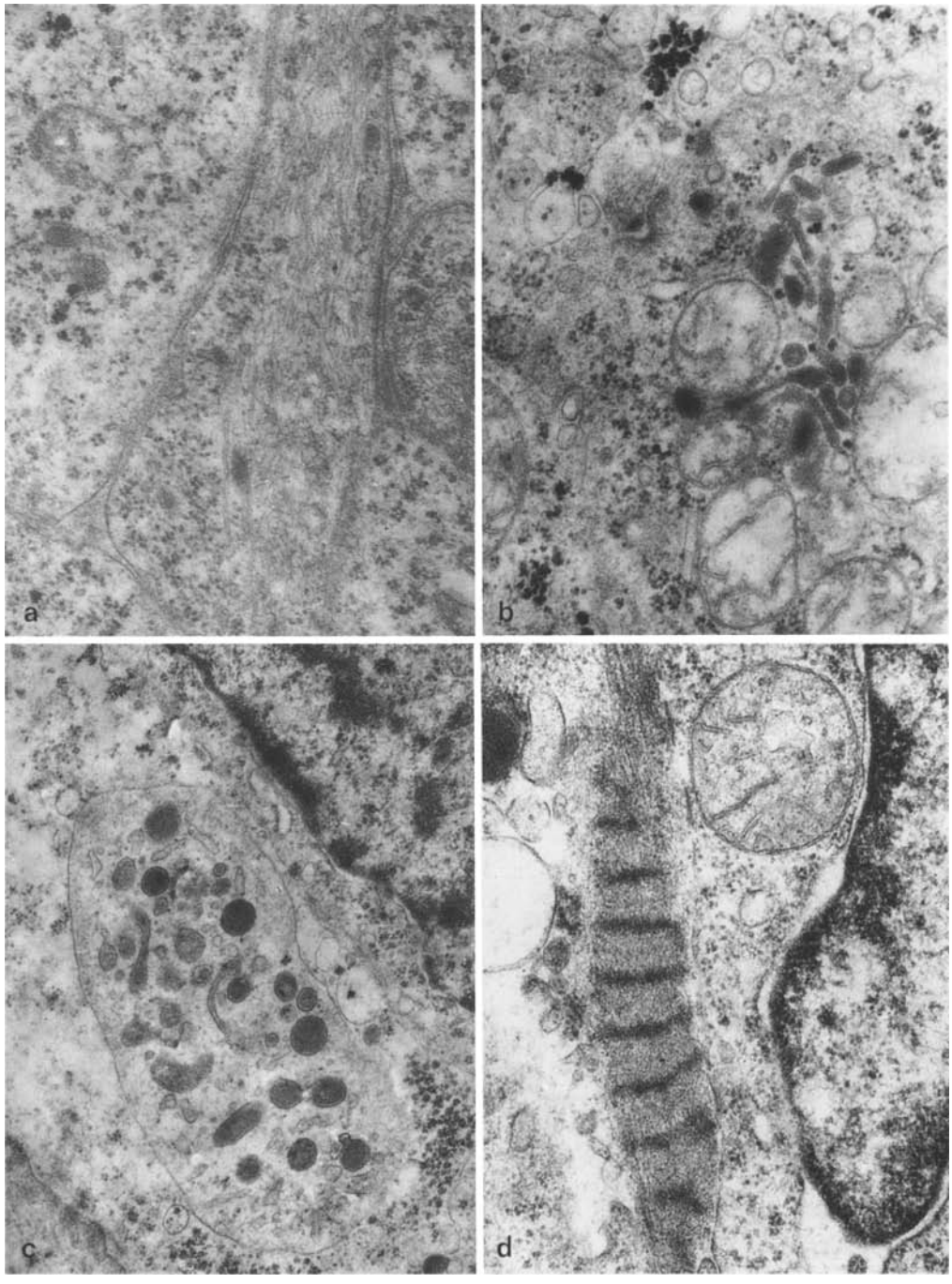

Fig. 4a–d. EM findings of tumoural differentiation within the neoplasm: **a** presents intermediate filaments and neurotubuli grouped in bands within a cell process (×72000). **b** Several types of neurosecretory granules within the neoplastic cells are illustrated: groups of dense-core vesicles (round-to-elongated, but membrane-bound) appear associated to glycogen granules and to mitochondria (×38400). **c** Neurosecretion is present within a dendritic-like cell process (×38400). **d** Illustrates a Luse-like body with long-spaced fibrils located interstitially between two cells (×44000)

scapula) and therefore cannot be included within the consideration of an Askin's neoplasm. As occurred with the cases presented by Jaffe et al. (1984) our patients presented with tumours located in the limbs (upper and lower) as well as in the pelvic girdle (pubis and ischio-pubic branch). Clinically they offered an aggressive behavior with a mean survival rate of 25 months; almost half of them presented with metastasis (5 cases with multiple bone deposits and one case with lymph node

infiltration). Death was caused through tumour relapse, metastasis, or both. Of all these cases, only one is alive and free of disease, being clinically cured after 9 years of follow-up; this is a 22 year-old male who suffered a primary malignancy located in the 2nd metatarsal bone of the right foot, which was amputated.

We have found a number of similarities when comparing this group of neoplasms clinically with the features attributed to conventional Es of bone. The age and sex in these patients are quite similar to those observed in the Es group, with a median age of 14.4 years and a clear male predominance (11:3); there are no differences regarding anatomical location (Zucker and Henry-Amar 1977; Rosen et al. 1981; Zucker et al. 1983; Miser et al. 1985). The only outstanding difference is the high malignancy displayed which resembles, in this tumour type, the behavior observed in the peripheral neuroepithelioma group of soft tissue tumours (Miser et al. 1985). The tendency to present multiple bone and lymph node metastasis is not a common finding in Es.

There are several histological, immunohistochemical and EM features which help in the differential diagnosis of this neoplasm from other small round blue cell sarcomas of undifferentiated character. Histologically three main features are evident in this tumour which are not present in Es: firstly, the presence of numerous Homer-Wright rosettes or pseudorosettes in clusters distributed in an haphazard fashion; secondly the cell and nuclear variation, for although all are small round blue cells with a high nucleo-cytoplasmic ratio they show more heterogeneity than that present in conventional Es (but with similar glycogen deposits). The third typical feature is the existence of a lobular pattern with faintly fibrillary interstitial stroma. These structures have also been described in Askin's tumour (Askin et al. 1979; Triche et al. 1985; Linnoila et al. 1986) and in the neuroectodermal sarcoma of bone (Jaffe et al. 1984). It should nevertheless be remembered that some conventional Es display pseudorosette-like figures and some cell-size irregularity. Thus, as we have previously indicated (Llombart-Bosch et al. 1987), it is hazardous to establish a diagnosis of this tumour based exclusively upon routine histological criteria.

EM has been of help in the characterization of this neoplasm; in all 6 cases studied we were able to find membrane-bound neurosecretory granules, intermediate filaments and neurotubule-like figures within the cytoplasm and in the cell processes. Furthermore, fields composed undifferentiated Ewing-like cells only, and areas in which a progressive maturation toward neural structures resulted were evident. Fibrin-like interstitial deposits and, in one case, long-spaced collagen bands (Luse-like bodies) were seen which have also been found in other sarcomas of neural origin (Luse 1960; Weller and Cervos-Navarro 1977). In fact all these EM features resemble those previously published for Askin's tumour, and for neuroectodermal tumour of bone (Askin et al. 1979; Das et al. 1982; Schmidt et al. 1982; Jaffe et al. 1984; Linnoila et al. 1986; Llombart-Bosch et al. 1987), being superimposeable on those seen in peripheral neuroepithelioma of soft tissue (MacKay et al. 1985; Mierau 1985; Nesland et al. 1985).

Up to now all cases of small round blue cell sarcomas in bone (other than lymphomas) have been termed Ewing's sarcoma, either with typical (conventional) or atypical features (Llombart-Bosch et al. 1978; 1982; Nascimento et al. 1980). The finding of neural markers in some of these neoplasms, similar to those seen in peripheral neuroepithelioma of soft tissue, have raised the question whether all Es are neural neoplasms? Within this situation several authors have claimed, based mainly upon immunohistochemical results with NSE and HNK-1 immunosera positivity in several cases of Es, that we are dealing with a unique type of neural tumour (Caillaud et al. 1984; Jaffe et al. 1984; Lipinski et al. 1987). Further support for this hypothesis has been obtained by in vitro culture of several Es lines, wherein a neural expression could be induced after butil AMP stimulation, but not with other nerve-growth factors (Cavazzana et al. 1987). Primary bone sarcomas, considered to be conventional Es, were negative when tested for NSE and HNK-1 in paraffin-embedded material (Triche and Askin 1983). These same cases became positive not only for those immunosera but also for cytokeratin and neurofilaments, after treatment of the tissue sections with collagenase Type IV (Cavazzana et al. 1987).

In this material, specificity for NSE and for HNK-1 was seen in a large number of cases; the presence of Beta-2-microglobulin was also regularly found (Funa et al. 1986). The monoclonal antibody E-36 stains with specifically neural epitopes the neuroendocrine cells (Monaghan and Roberts 1985), also being positive in several of our cases. Therefore, even if some doubts have arisen for the specificity of NSE and HNK-1 (Carlei et al. 1984; Tsokos et al. 1984; Mierau et al. 1985; Sasaki et al. 1985; Carazzana et al. 1987), the presence of other positive neural markers, as well as the histological and EM features herein discussed gives support for a neural character for these 14 tumours.

As a tentative conclusion based upon the present analysis, which supports previously published cases (Llombart-Bosch et al. 1987), Es of bone is an undifferentiated sarcoma with a restricted maturation capacity which may produce morphologically atypical features (Llombart-Bosch et al. 1978; 1982; 1986). Those atypical Ewing's sarcomas expressing a rosette-like appearance, neurosecretory granules and some neural immunomarkers should therefore be included within the new categorization of "neuroectodermal sarcomas of bone".

References

Askin FB, Rosai J, Sibley PK, Dehner LP, McAlister WH (1979) Malignant small cell tumor of the thoracopulmonary region in childhood: A distinctive clinicopathological entity of uncertain histogenesis. Cancer 43:2438–2452

Aurias A, Rimbaut C, Buffe D, Dubousset J, Mazabraud A (1983) Chromosomal translocation in Ewing's sarcoma. N Engl J Med 309:496

Bolen JW, Thorning D (1980) Peripheral neuroepithelioma: A light- and electron microscopical study. Cancer 46:2456–2462

Burns J (1982) The unlabelled antibody peroxidase-antiperoxidase method. In: Bullock GR, Petrusz P (eds) Techniques in immunohistochemistry. Academic Press, London, pp 91–105

Caillaud TM, Benjeloun S, Bosq J, Braham K, Lipinski M (1984) HNK-1 defined antigen defected in paraffin-embedded neuroectodermal tumors and those derived from cells of Amine Precursor uptake and decarboxylation system. Cancer Res 44:4432–4439

Carlei F, Polak JM, Caccamea A, Marangos PJ, Dahl D, Cocchia D, Michetti F, Lezoche E, Speranza V (1984) Neural and glial markers in tumors of neuroblastic origin. Virchows Arch A (Pathol Anat) 404:313–324

Cavazzana AO, Navarro S, Noguera R, Triche TJ (1987a) Short-term tissue culture in the differential diagnosis of small round cell tumors. Path Res Pract

Cavazzana A, Miser JS, Jefferson HTL, Triche TJ (1987b) Experimental evidence for a neural origin of Ewing's sarcoma. Am J Pathol 127:507–518

Cavazzana A, Navarro S, Triche TJ, Llombart-Bosch A (1987) Immunohistochemical analysis of Ewing's tumor. Lab Investig (Abstract) 12A

Das L, Chang CM, Cushing B, Sewel P (1982) Congenital primitive neuroectodermal tumor (neuroepithelioma) of the chest wall. Med Pediatr Oncol 10:349–358

Donner L, Triche TJ, Israel MA, Seeger RC, Reynolds CP (1985) A panel of monoclonal antibodies which discriminate neuroblastoma from Ewing's sarcoma, rhabdomyosarcoma, neuroepithelioma and hematopoietic malignancies. Evans AE, D'Angio GJ, Seeger DC (eds) Adv in Neuroblastoma Res. Alan R Liss, New York, pp 347–366

Funa K, Gazdar AF, Minna JD, Linnoila RI (1986) Paucity of Beta-2 Microglobulin expression on small cell lung cancer, bronchial carcinoids and certain other neuroendocrine tumors. Lab Investig 2:186–192

Gonzalez-Crussi F, Wolfson SL, Migusi K, Nakajima T (1984) Peripheral neuroectodermal tumors of the chest wall in childhood. Cancer 54:2519–2527

Harper PG, Pringle S, Souhami RL (1981) Neuroepithelioma: A rare malignant peripheral nerve tumor of primitive origin. Report of two new cases and review of the literature. Cancer 48:2282–2287

Hashimoto M, Enjoji M, Nakajima T, Kiryu H, Daimaru Y (1983) Malignant neuroepithelioma (peripheral neuroblastoma). A clinicopathological study of 15 cases. Am J Surg Pathol 7:309–318

Jaffe R, Santamaria M, Yunis EJ, Hrinya Tanejery N, Agostini RM, Medina J, Goodman M (1984) The neuroectodermal tumor of bone. Am J Surg Pathol 8:885–898

Linnoila RI, Tsokos M, Triche TJ, Marangos PJ, Chandra RS (1986) Evidence of neural origin and PAS-positive variants of the malignant small cell tumor of the thoracopulmonary region (Askin's tumor). Am J Surg Pathol 2:124–133

Lipinski M, Braham K, Philip I, Wiels J, Philip T, Ghoridisch M, Lenoir G, Tursz T (1987) Neuroectodermal associated antigens on Ewing's sarcoma cell lines. Cancer Res 47:183–187

Llombart-Bosch A, Blache R, Peydro-Olaya A (1978) Ultrastructural study of 28 cases of Ewing's sarcoma; typical and atypical forms. Cancer 41:1362–1373

Llombart-Bosch A, Blache R, Peydro-Olaya A (1982) Round cell sarcomas of bone and their differential diagnosis (with particular emphasis on Ewing's sarcoma and reticulosarcoma). A study of 233 tumors with optical and electron microscopical techniques. Pathol Ann 17:113–145

Llombart-Bosch A, Contesso G, Henry-Amar M, Lacombe MJ, Oberlin O, Dubousset J, Rouesse J, Sarrazin D (1986) Histopathological predictive factors in Ewing's sarcoma of bone and clinicopathological correlations. Virchows Arch A (Pathol Anat) 409:627–640

Llombart-Bosch A, Lacombe MJ, Contesso G, Peydro-Olaya A (1987) Small round blue cell sarcoma of bone mimicking atypical Ewing's sarcoma, with neuroectodermal features. An analysis of five cases with immunohistochemical and electron microscopical support. Cancer

Llombart-Bosch A, Perez-Bacete A, Triche TJ (1986) Immunohistochemische Untersuchungen der Ewingsarkome und ihre Differentialdiagnose gegenüber anderen "small blue cell" Sarkomen des Kinderalters und der Jugend. Verh Dtsch Ges Pathol 70:321–324

Luse S (1960) Electron microscopic studies of brain tumors. Neurology 10:881–920

Mackay B, Bruner JM, Ordoñez NG (1985) Soft-tissue sarcoma with neural differentiation. Ultrastr Pathol 9:181–188

Mierau GW (1985) Extraskeletal Ewing's sarcoma (peripheral neuroepithelioma). Ultrastruct Pathol 9:91–98

Mierau GW, Berry PJ, Orsini EN (1985) Small round cell neoplasms: Can electron microscopy and immunohistochemical studies accurately classify them? Ultrastr Pathol 9:99–111

Miser J, Steis R, Longo DL, Belasco JB, Triche TJ, Kinsella TJ (1985) Treatment of newly diagnosed high-risk sarcomas and primitive neuroectodermal tumors (PNET) in children and young adults. Proc Am Soc Clin Oncol 4:240 (C-935)

Moll R, Lee E, Gould V, Berndt R, Roessner A, Franke WW (1987) Immunohistochemical analysis of Ewing's tumors. Patterns of expression of intermediate filaments and desmosomal proteins indicate cell type heterogeneity and pluripotential differentiation. Am J Pathol 127:288–304

Monaghan P, Roberts DB (1985) Immunohistochemical evidence for neuroendocrine differentiation in human breast carcinomas. J Pathol 147:281–289

Nascimento AG, Unni K, Pritchard DJ, Cooper KL, Dahlin DC (1980) A clinicopathological study of 20 cases of large cell (atypical) Ewing's sarcoma of bone. Am J Surg Pathol 4:29–36

Nesbitt KA, Vidone RA (1976) Primitive neuroectodermal tumor (neuroblastoma) arising in sciatic nerve of a child. Cancer 37:1562–1570

Nesland TM, Sobrinho-Simoes MA, Holm R, Johannessen JV (1985) Primitive neuroectodermal tumor (peripheral neuroblastoma). Ultrastr Pathol 9:59–64

Rosen G, Caparros B, Niremberg A, Marcove RC, Huvos AG, Kosloff C, Lane JM, Murphy M (1981) Ewing's sarcoma; ten years experience with adjuvant chemotherapy. Cancer 47:2204–2213

Sasaki A, Ogawa A, Nakazato Y, Ishida Y (1985) Distribution of neurofilament protein and neuron specific enolase in peripheral neuronal tumors. Virchows Archiv (Pathol Anat) 407:33–41

Schmidt D, Harms D, Buroach S (1985) Malignant peripheral neuroectodermal tumors of childhood and adolescence. Virchows Arch A (Pathol Anat) 406:351–365

Schmidt D, Mackay B, Ayala AG (1982) Ewing's sarcoma with neuroectodermal-like features. Ultrastruct Pathol 3:143–151

Seemayer TA, Vekemans M, de Chadarevian JP (1985) Histological and cytogenetic findings in a malignant tumor of the chest wall and lung. Virchows Arch A (Pathol Anat) 408:289–296

Taylor CE, Burns J (1974) The demonstration of plasma cells and other immunoglobulin-containing cells in formalin-fixed paraffin-embedded tissues using peroxidase-labelled antibody. J Clin Pathol 27:14–20

Triche TJ, Askin FB (1983) Neuroblastoma and the differential diagnosis of small round blue cell tumors. Hum Pathol 14:569–595

Triche TJ, Tsokos M, Linnoila I, Marangos PJ, Chandra R (1985) NSE in neuroblastoma and other round cell tumors of childhood. In: Evans AE, D'Angio GJ, Seeger DC (eds). Adv in Neuroblastoma Res. Alan R Liss, New York, pp 295–317

Tsokos M, Linnoila RI, Chandra RS, Triche TJ (1984) Neuron-specific enolase in the diagnosis of neuroblastoma and other small round-cell tumors in children. Hum Pathol 15:575–584

Turc-Carel C, Philip L, Berger MP, Philip T, Lenoir GM (1983) Chromosomal translocation in Ewing's sarcoma. N Engl J Med 309:497

Turc-Carel C, Philip L, Berger M, Philip T, Lenoir GM (1984) Chromosomal study of Ewing's sarcoma (Es) cell lines. Consistency of a reciprocal translocation t(11:22) (q24; q12). Cancer Genet Cytogenet 12:1–19

Venable JH, Goggenshau R (1965) A simplified lead citrate stain for use in electron microscopy. J Cell Biol 25:407–408

Vigfusson NV, Allen IJ, Phillips JH, Alschibaja T, Riches WG (1986) A neuroendocrine tumor of the small intestine with a karyotype of 46,XY t(11:22). Cancer Genet Cytogenet 22:211–218

Voss BC, Pysher TJ, Humphrey GB (1984) Peripheral neuroepithelioma in childhood. Cancer 54:3059–3064

Weller RO, Cervos-Navarro J (1977) Pathology of peripheral nerves. Butterworths, London, Boston, pp 154–203

Wha-Peng J, Triche TJ, Knutsen T, Miser J, Douglass EC, Israel MA (1984) Chromosomal translocation in neuroepithelioma. N Engl J Med 311:584–585

Zucker JM, Henry-Amar M (1977) Therapeutic controlled trial in Ewing's sarcoma. Report on the results of a trial by the Clinical Cooperative Group on radio- and chemotherapy of the EORTC. Eur J Cancer 13:1019–1023

Zucker JM, Henry-Amar M, Sarrazin D, Blache R, Patte C, Schweisguth O (1983) Intensive systemic chemotherapy in localized Ewing's sarcoma in childhood. A historical trial. Cancer 52:415–423

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