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KP-1: not a specific marker. Staining of 137 sarcomas, 48 lymphomas, 28 carcinomas, 7 malignant melanomas and 8 cystosarcoma phyllodes

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Abstract This study documents the reactions of the monoclonal antibody KP-1, which detects histiocytes in paraffin sections, with 137 sarcomas, 48 lymphomas, 28 carcinomas, 7 malignant melanomas and 8 cystosarcoma phyllodes. The soft tissue sarcomas had been previously immunophenotyped. Positive staining was obtained in all categories of sarcoma except clear-cell sarcomas. Most categories of sarcoma showed staining in less than 10% of tumour cells although a minority of leiomyosarcomas showed more extensive staining. Five of 7 malignant melanomas were also positive while all lymphomas and carcinomas were negative. We conclude that KP-1 positivity is not helpful in supporting the histiocytic origin of a tumour and is of limited value in the differential diagnosis of soft tissue sarcomas or their separation from other categories of malignancy.

Key words KP-1 · CD 68 · Soft tissue sarcoma
Histiocytic marker

Introduction

A wide and ever-expanding range of immunocytochemical markers is now available to the histopathologist. Many are of potential but unproven value in tumour diagnosis. A number of immunocytochemical markers have been proposed as specific markers of histiocytic differentiation and, despite evidence to the contrary (Leader et al. 1987a; Loftus et al. 1991), continue to be quoted on this basis.

KP-1 (CD 68) is a new histiocytic marker (Pulford et al. 1989) that is currently available commercially. The objective of this study was to assess the usefulness of KP-1 in the differential diagnosis of malignant tumours, and in particular soft tissue sarcomas, while at the same time assessing its specificity as a marker of histiocytic neoplasms.

In this report we document the reactions of KP-1 with 137 soft tissue sarcomas, 48 lymphomas, 28 carcinomas, 7 malignant melanomas, 8 cytosarcoma phyllodes and 5 leiomyomas. We also review the literature referring to the staining reactions of KP-1.

Materials and methods

A total of 137 sarcomas were examined for KP-1 positivity. These comprised 10 different categories and included 5 angiosarcomas, 7 clear-cell sarcomas, 13 fibrosarcomas, 6 haemangiopericytomas, 26 leiomyosarcomas, 18 liposarcomas, 26 malignant fibrous histiocytomas, 9 neurofibrosarcomas, 13 rhabdomyosarcomas and 14 synovial sarcomas. In view of the results obtained with leiomyosarcomas, 5 leiomyomas were subsequently stained. Also, 48 lymphomas were examined. These comprised 23 cases of Hodgkin's disease and 25 non-Hodgkin's lymphomas. In addition, 9 squamous carcinomas, 10 adenocarcinomas, 9 basal cell carcinomas, 7 malignant melanomas and 8 cytosarcoma phyllodes were examined, the last including benign and malignant lesions. All material was fixed in neutral buffered formalin and paraffin embedded.

The sarcomas were obtained from the files of the Westminster Hospital and had been examined by Professor D.H. MacKenzie, an acknowledged authority on soft tissue sarcomas (MacKenzie 1970). Electron microscopy was performed where appropriate, and all tumours had been studied with a wide range of immunohistochemical stains (Leader et al. 1986a–c; 1987a–c; 1989). The blocks ranged in age from 2 to 33 years.

The lymphomas were obtained from the files of Beaumont Hospital, Dublin and had been previously immunophenotyped. The sarcomas and melanomas were obtained from the files of the Westminster Hospital and the Royal College of Surgeons in Ireland and the cystosarcoma phyllodes from St. Vincent's Hospital, Dublin.

All of the tumours were examined for reactivity either with a monoclonal antibody to KP-1 obtained from the John Radcliffe

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Hospital, Oxford, UK or a commercially available antibody (Dako Glostrup, Denmark). The antibody was raised against the lysosomal fraction of human lung macrophages.

Each of the antibodies was tested for reliability on a series of positive controls (reactive lymph nodes). Optimal results were obtained with a dilution of 1 in 50 in the case of the commercially available antibody and with neat application in the case of the antibody obtained from Oxford. The antibodies were applied for 1 h at room temperature to 5-μ m paraffin sections that had been trypsinised for 20 min. A secondary antibody, biotinylated rabbit anti-mouse antiserum (Dako E354), diluted 1 in 300 was added for 30 min at room temperature. Avidin-biotin-peroxidase complex was then added for 30 min and diaminobenzidine was applied for 7 min.

The tumours were stained for KP-1 in batches of 20, which each batch including 1 positive and 2 negative controls. Each section was examined by one of two observers (M.C. and B.L.) using × 4, × 10 and × 40 objectives. Suspected positive cases were reviewed. A decision on positivity was made in the absence of the HE diagnosis. A positive result was regarded as discrete, granular cytoplasmic staining against an unstained background in clearly identifiable tumour cells. In view of the possibility of passive uptake of antigen, apparently positive reactions in degenerate cells or cells adjacent to areas of necrosis were excluded. The result was considered equivocally negative when 5 positively staining tumour cells or fewer were identified in a section. Staining intensity of tumour cells was graded from 1 to 3 (Table 1: 1, weak; 2, moderate; 3, strong). The proportion of positively staining tumour cells was also estimated on a 4-point scale (Table 1: 1, under 10%; 2, 10–25%; 3, 25–50%; 4, over 50%).

In view of the KP-1 positivity identified in leiomyosarcomas, a subset of 19 of these tumours was further stained with an alternative antibody to the CD68 antigen, PG-M1 (Dako M876). This was applied for 1 h at room temperature to 5-μm sections that had been previously trypsinised for 15 min. Optimal dilution was determined as for KP-1. Biotynilated rabbit anti-mouse serum at 1 in 300 dilution was applied for 30 min, followed by avidin-biotin complex for 30 min and diaminobenzidine for 3 min.

Results

The results are summarised in Table 1.

Of the 137 sarcomas examined, 59 (43%) showed positivity with KP-1. All categories of sarcoma examined, except for the clear-cell sarcomas, included KP-1-positive tumours (Fig. 1). In the majority of cases, under 10% of tumour cells were positive, although in the case of the leiomyosarcomas 7 of the 26 tumours examined showed positive staining in 25% or more of the tumour cells (Table 1). All of the 28 carcinomas examined were negative, whereas 5 of 7 malignant melanomas showed positive staining. In view of the results obtained with the leiomyosarcomas, 5 leiomyomas (4 uterine, 1 gastric) were also examined, and all 4 uterine lesions showed small numbers of positively staining cells. Positively staining lesional cells were also identified in the 19 leiomyosarcomas stained with PG-M1 (Fig. 2). The intensity and extent of staining broadly paralleled that obtained with KP-1, although 3 cases adjudged positive with KP-1 were regarded as negative with PG-M1. In each of these

Table 1 KP-1 staining of 137 sarcomas, 48 lymphomas, 28 carcinomas, 7 malignant melanomas and 8 cystosarcoma phyllodes

	Total no.	No. positive	No. negative	Equiv	Staining intensity			Extent of positivity			
					+3	+2	+1	+4	+3	+2	+1
Sarcomas (137)											
Malignant fibrous histiocytoma	26	8	18	0	4	2	2			5	3
Neurofibrosarcoma	9	6	3		1	5				1	5
Leiomyosarcoma	26	23	2	1	9	11	3	2	5	4	12
Synovial sarcoma	14	2	10	2		2					2
Fibrosarcoma	13	3	10			3					3
Clear-cell sarcoma	7		7								
Haemangio pericytoma	6	3	3		1	2					3
Angiosarcoma	5	3	2		1	2					3
Liposarcoma	18	6	11	1	1	5				1	5
Rhabdomyosarcoma	13	5	8		1	1	3		1	2	2
Leiomyomas (5)	5	4	1				4				4
Lymphomas (48)											
Hodgkin's disease	23		20	3							
B-cell lymphomas	20		20								
T-cell lymphomas	5		5								
Carcinomas (28)											
Squamous-cell carcinoma	9		9								
Adenocarcinoma	10		10								
Basal-cell carcinoma	9		9								
Malignant melanomas (7)	7	5	2			4	1	3	1	1	
Cystosarcoma phyllodes (8)											
Epithelial component			8								
Stromal component		6	2		4	2				1	5

Equiv = Equivocal

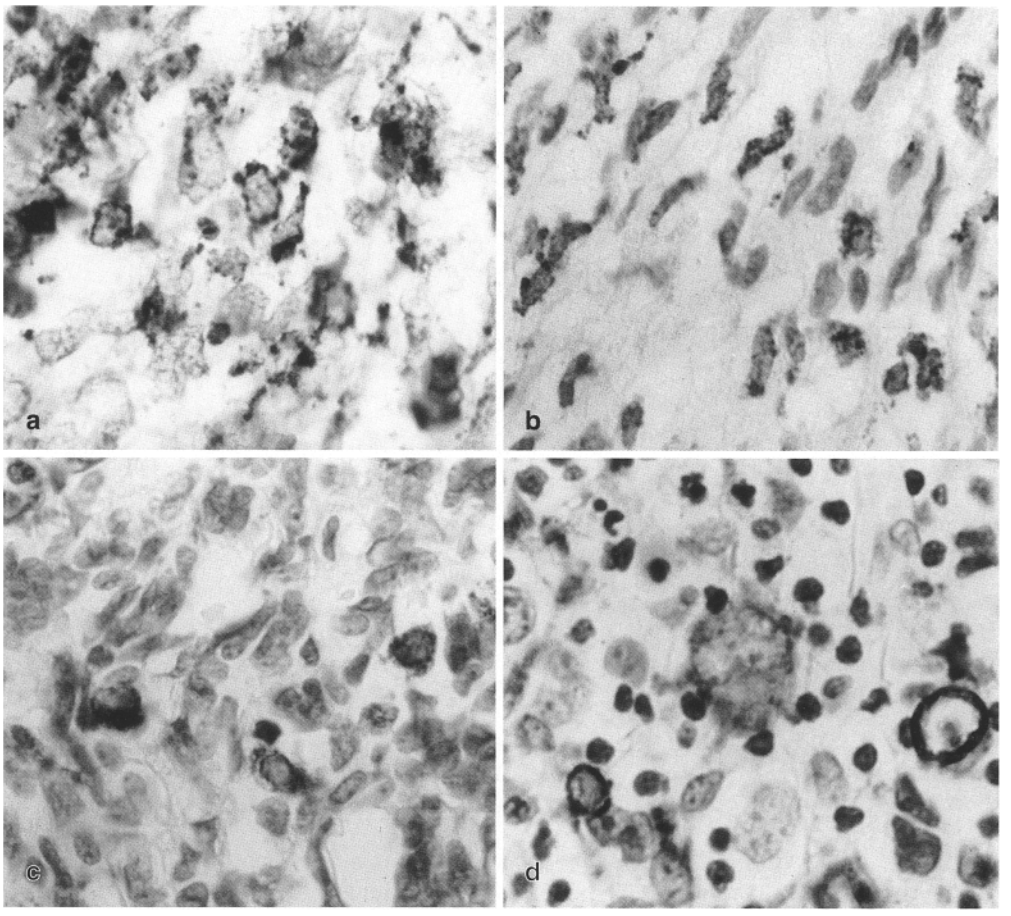

Fig. 1a–d KP-1 positive cells in **a** epithelioid leiomyosarcoma, **b** leiomyosarcoma, **c** angiosarcoma, and **d** Hodgkin's disease. (× 400)

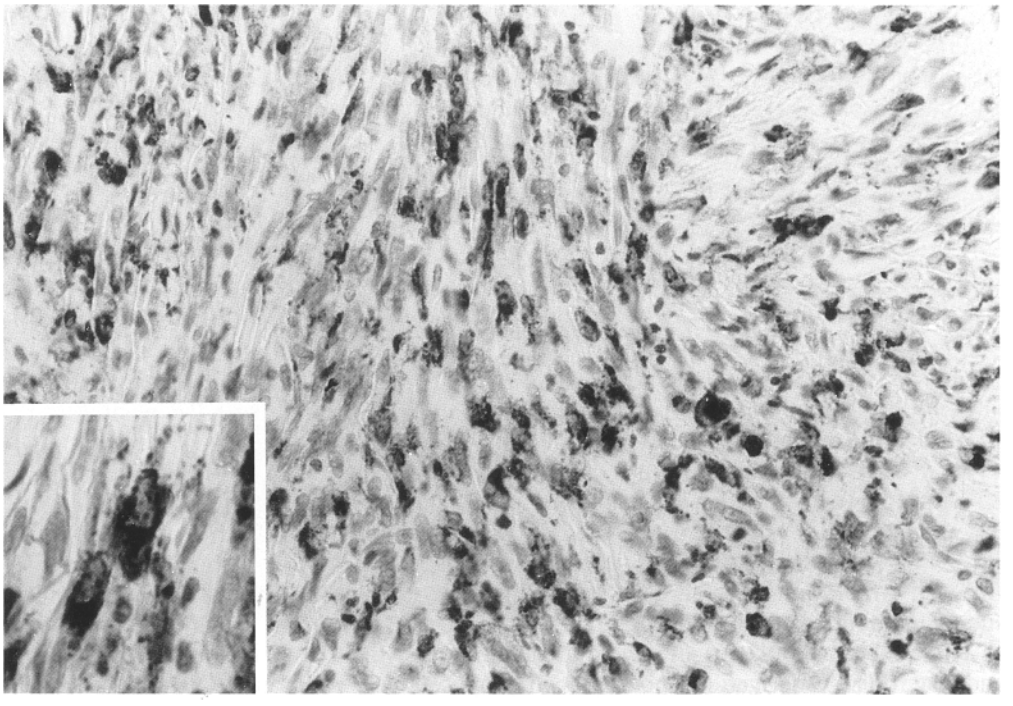

Fig. 2 PGM-1-positive leiomyosarcoma. (× 200) *Inset,* KP-1-positive cells from the same case (× 400)

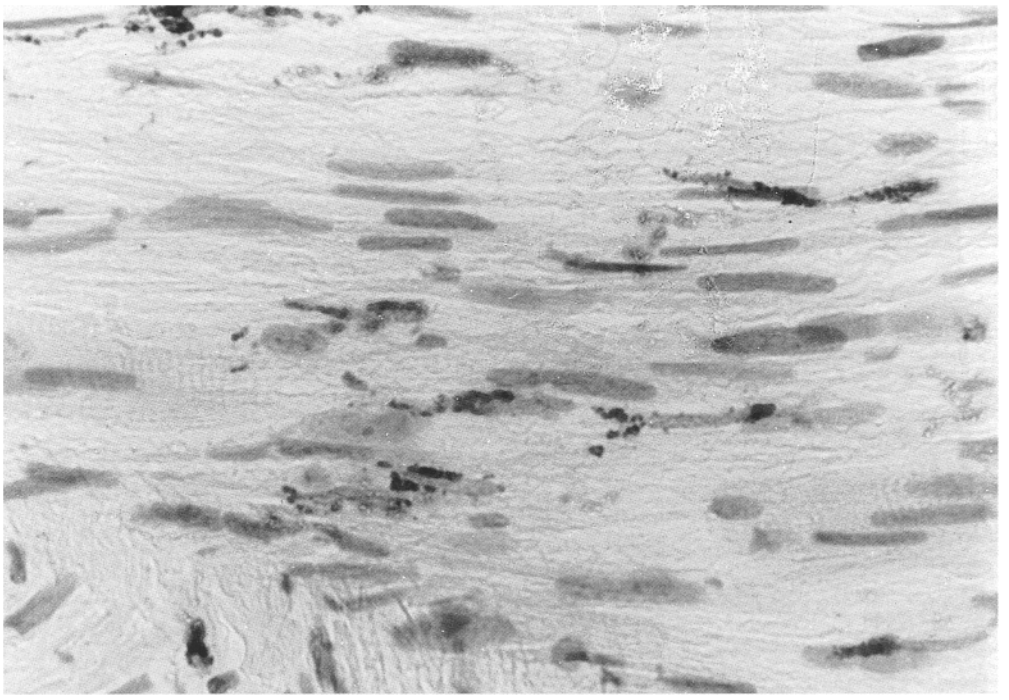

Fig. 3 KP-1-positive leiomyosarcoma. Note positively staining spindle-cells (× 400)

cases the proportion of positive KP-1 cells had been less than 10%. Varying degrees of positivity were also obtained in the stromal component of 6 of 8 cystosarcoma phyllodes. None of the lymphomas examined stained positively with KP-1, although three cases of Hodgkin's disease were judged equivocal in that a very occasional lesional cell showed positive staining.

Histiocytes, mast cells and neutrophils stained positively and generally showed more intense staining than tumour cells. In instances where a clear distinction could not be made between a tumour cell and reactive histiocyte or mast cell the result was recorded as negative. In addition it was noted that a proportion of granulocytes failed to stain with KP-1, and that these cells generally had the morphological appearance of eosinophils. In order to investigate this impression further, two sections of nasal polyps showing heavy eosinophil infiltration of the stroma were also stained for KP-1 and absence of reactivity in eosinophils was confirmed.

Discussion

KP-1 (CD68) is a newly developed monoclonal antibody raised against the lysosomal fraction of human lung macrophages (Pulford et al. 1989). There is evidence that KP-1 recognises a fixation-resistant epitope on the same glycoprotein molecule (MW approx. 110,000) as other macrophage-directed antibodies, such as Y2/131 and EBM11, which however can only be used on frozen tissue (Micklem et al. 1989; Pulford et al. 1989).

KP-1 labels neoplasms of myeloid, myelomonocytic and macrophage derivation and has been proposed as a useful adjunct to the phenotyping of lymphomas (Warnke et al. 1989). Reactivity has also been described in normal and neoplastic mast cells (Horny et al. 1990). Our results support the usage of KP-1 within the group of lymphoreticular neoplasms. In none of the 48 cases we examined would an incorrect diagnosis of histiocytic lymphoma have been made using this antibody.

It has been proposed that KP-1 may have a place in the differential diagnosis of histiocytic versus non-haemopoietic neoplasms. Warnke et al. (1989) describe its use on an unspecified number and type of non-haematopoietic neoplasms without evidence of reactivity. They do, however, note weak KP-1 staining of some epithelial neoplasms in fine-needle aspirate cytospin preparations. All the carcinomas examined in our study were negative.

Positivity for KP-1 has been used as supportive evidence for histiocytic differentiation in a number of recent reports, as in the recently described littoral cell angioma of the spleen, where it was co-expressed with factor VIII-related antigen (Falk et al. 1991); in undifferentiated sarcoma of the liver (Aoyama et al. 1991); and in nodular fasciitis, where expression was interpreted as indicating a limited histiocytic phenotype (Montgomery and Meis 1991). Smith et al. (1991) reported varying degrees of KP-1 positivity in 9 of 19 cases of angiomatoid malignant fibrous histiocytoma (MFH), while 12 other cases of MFH were negative. Twelve other mesenchymal tumours of various types were also stained and all showed negative staining. In our study 8 of the 26 cases of MFH examined showed KP-1 positivity in small numbers of lesional cells, although this may be an underestimate, as particular difficulty in distinguishing histiocytes from neoplastic cells was experienced in this group of tumours. Our results are, however, broadly in line with

those of Soini and Miettinen (1990), who reported KP-1 positivity in one of five MFHs studied.

Iwasaki et al. (1992) reported complete absence of CD68 in MFH cells in a study of 33 tumours of this type, although they report many positively staining reactive histiocytes in the tumour stroma. We were unable to make an absolutely clear-cut distinction between tumour cells and reactive histiocytes in every case, although many of the positively staining cells did appear to be genuine lesional cells.

Fachetti et al. (1991), in a study of benign and malignant melanocytic tumours, recorded positivity in 16 of 20 primary melanomas and 6 of 8 metastatic melanomas and suggested that difficulties may occur with the use of KP-1 in the differential between melanomas and histiocytic neoplasms. These results are supported by our study, in which 5 of 7 malignant melanomas examined were positive. In view of this, and bearing in mind the close relationship between malignant melanoma and clear-cell sarcoma (Swanson and Wick 1989), our failure to identify KP-1 positivity in the 7 clear-cell sarcomas examined may be regarded as surprising. Possibly staining of a larger number of these neoplasms would identify KP-1 positive examples of this tumour.

At first sight, our finding of KP-1 positivity in such a wide range of sarcomas is difficult to explain. However, Pulford et al. (1990) have demonstrated that the CD68 antigen is not confined to cells of the monocyte/macrophage lineage, and in their study of a range of anti-CD68 antibodies applied to a variety of normal tissues they observed that the KP-1 antibody in particular reacted with the largest number of non-macrophage cells. Based on a study of granular cell neoplasms Tsang and Chan (1992) postulate that KP-1 may be more properly viewed as a marker of lysosomes rather than cells of histiocytic derivation.

The prominent expression of KP-1 by malignant smooth muscle tumours is noteworthy. It is of interest that Fletcher (1992) describes co-expression of CD68 (KP-1) and smooth muscle actin in the epithelioid nodular element of plexiform fibrohistiocytic tumour. Pulford et al. (1990) describe weak to moderate staining of connective tissue by KP-1 antibody although they do not specifically describe staining of muscle. Normal smooth muscle and skeletal muscle in our study did not show KP-1 positivity.

The positivity obtained in angiosarcoma is of interest in view of the co-expression of KP-1 and FVIIIRag observed by Falk et al. (1991). KP-1 positivity has also been reported in normal endothelium (Pulford et al. 1990).

The finding of KP-1 positivity in a variety of spindle-cell neoplasms in this study is in accord with findings in the literature (Soini and Miettinen 1990; Aoyama et al. 1991; Montgomery and Meis 1991). However, we suggest in view of the range of sarcomas in which KP-1 was detected in this study, that great caution be exercised in interpreting such positivity as indicating a histiocytic phenotype.

Our observation of absent reactivity in eosinophils (Fig. 2) is in keeping with the findings of Pulford et al. (1990), who noted that eosinophils failed to react with a range of antibodies to the CD68 antigen.

KP-1 has been proposed as a useful marker of histiocytic infiltration as in the study of this phenomenon by Rossi et al. (1991) in oligodendroglioma. Our finding that even in KP-1-positive neoplasms histiocytes generally show more intense staining than lesional cells supports this usage. In addition, and despite the observations of Warnke et al. (1989), our results also suggest that a KP-1-positive tumour of unknown histogenesis is very unlikely to be a carcinoma. The results of our study do not negate the usefulness of KP-1, as we recognise that in practice the antibody would be used as one of a panel of markers; nevertheless we must agree with the view expressed by Brandtzaeg et al. (1992), that CD68 has to be used with great caution as a macrophage marker. The conclusion of this study is that, unless widespread, intense staining is observed, KP-1 positivity is not helpful in either supporting or refuting the histiocytic origin of a tumour and is of limited value in the differential diagnosis of soft tissue sarcomas or in their separation from other categories of malignancy.

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References

Aoyama C, Hachitanda Y, Sato JK, Said JW, Shimada H (1991) Undifferentitated (embryonal) sarcoma of the liver. Am J Surg Pathol 15:615–624

Brandtzaeg P, Dole I, Gabrielsen T (1992) The leucocyte protein L1 (calprotectin): usefulness as an immunohistochemical marker antigen and putative biological function. Histopathology 21:191–196

Facchetti F, Bertalot G, Grigolato PG (1991) KP-1 (CD68) staining of malignant melanomas. Histopathology 19:141–145

Falk S, Stutte HJ, Frizzera G (1991) Littoral cell angioma: a novel splenic vascular lesion demonstrating histiocytic differentiation. Am J Surg Pathol 15:1023–1033

Fletcher CDM (1992) Soft tissue tumours: an update. In: Anthony PP, MacSween RNM (eds) Recent advances in histopathology. Churchill Livingstone, London, pp 113–139

Horny H-P, Schaumburgheuer G, Bolz S, Geerts ML, Kaiserling E (1990) Use of monoclonal antibody KP1 for identifying normal and neoplastic human mast cells. J Clin Pathol 43:719–722

Iwasaki H, Yoshitake K, Ohijimi Y et al. (1992) Malignant fibrous histiocytoma: proliferative compartment and heterogeneity of "histiocytic" cells. Am J Surg Pathol 16:735–745

Leader M, Collins M, Patel J, Henry K (1986a) Staining for factor VIII related antigen and Ulex europeus agglutin I (UEA-I) in 230 tumours. An assessment of their specificity for angiosarcoma and Kaposi's sarcoma. Histopathology 10:1153–1162

Leader M, Collins M, Patel J, Henry K (1986b) Anti-neuron specific enolase staining reactions in sarcomas and carcinomas: its lack of neuroendocrine specificity. J Clin Pathol 39:1186–1192

Leader M, Patel J, Makin CAM, Henry K (1986c) An analysis of the sensitivity and specificity of the cytokeratin marker CAM 5.2 for epithelial tumours. Histopathology 10:1315–1324

Leader M, Patel J, Collins M, Henry K (1987a) Anti-alpha-1-antichymotrypsin staining of 194 sarcomas, 38 carcinomas and 17 malignant melanomas. Its lack of specificity as a tumour marker. Am J Surg Pathol 4:133–139

Leader M, Collins M, Patel J, Henry K (1987b) Desmin: its value as a marker of muscle derived tumours using a commercial antibody. Virchows Arch [A] 411:345–349

Leader M, Collins M, Patel J, Henry K (1987c) Vimentin: an evaluation of its role as a tumour marker. Histopathology 11:63–72

Leader M, Patel J, Henry K (1989) Myoglobin: an evaluation of its role as a marker of rhabdomyosarcomas. Br J Cancer 59:106–109

Loftus B, Loh LC, Curran B, Henry K, Leader M (1991) Mac 387: its non-specificity as a tumour marker or marker of histiocytes. Histopathology 19:251–255

Mackenzie DH (1970) The differential diagnosis of fibroblastic tumours. Blackwell Scientific, Oxford

Micklem K, Rigney E, Cordell J, et al. (1989) A human macrophage associated antigen (CD68) detected by 6 different monoclonal antibodies. Br J Haematol 73:6–11

Montgomery EA, Meis JM (1991) Nodular fasciitis: its morphologic spectrum and immunohistochemical profile. Am J Surg Pathol 15:942–948

Pulford KAF, Rigney EM, Micklem KJ, Jones M, Stross WP, Gatter KC, Mason DY (1989) KP1: a new monoclonal antibody that detects a monocyte/macrophage associated antigen in routinely processed tissue sections. J Clin Pathol 42:414–421

Pulford KAF, Sipos A, Condell JL, Stross WP, Mason DY (1990) Distribution of the CD68 macrophage/myeloid associated antigen. Int Immunol 2:973–980

Rossi ML, Butler J, Heath SA (1991) Monocyte/macrophage infiltration in 41 oligoendrogliomas: paraffin was study. Path Res Pract 187:166–169

Smith MEF, Costa MJ, Weiss SW (1991) Evaluation of CD68 and other histiocytic antigens in angiomatoid malignant fibrous histiocytoma. Am J Surg Pathol 15:757–763

Soini Y, Miettinen M (1990) Immunohistochemistry of markers of histiomonocytic cells in malignant fibrous histiocytomas: a monoclonal antibody study. Path Res Pract 186:759–767

Swanson PE, Wick MR (1989) Clear cell sarcoma. An immunohistochemical analysis of six cases and comparison with other epithelioid neoplasms of soft tissue. Arch Pathol Lab Med 113:55–60

Tsang WYW, Chan JKC (1992) KP-1 (CD68) staining of granular cell neoplasms: is KP-1 a marker for lysosomes rather than the histiocytic lineage? Histopathology 21:84–86

Warnke RA, Pulford KAF, Pallesen G, Ralfkiaer E, Brown DC, Gatter KC, Mason DY (1989) Diagnosis of myelomonocytic and macrophage neoplasms in routinely processed tissue biopsies with monoclonal antibody KP1. Am J Pathol 135:1089–1095