Histopathology 2005, **46**, 522–531. DOI: 10.1111/j.1365-2559.2005.02128.x

PDGFR expression in differential diagnosis between KIT-negative gastrointestinal stromal tumours and other primary soft-tissue tumours of the gastrointestinal tract

G Rossi, R Valli, F Bertolini,[1] A Marchioni,[2] A Cavazza,[3] C Mucciarini,[1] M Migaldi, M Federico,[1] G P Trentini & A Sgambato[4]

Department of Pathologic Anatomy and Legal Medicine, Section of Pathologic Anatomy, [1]Department of Oncology and Haematology, Section of Oncology and [2]Department of Medical Sciences, University of Modena and Reggio Emilia, and [3]Operative Unit of Pathology, Hospital S. Maria Nuova, Reggio Emilia, and [4]Centro di Ricerche Oncologiche 'Giovanni XXIII', Institute of General Pathology, Catholic University, Rome, Italy

Date of submission 2 April 2004
Accepted for publication 22 May 2004

Rossi G, Valli R, Bertolini F, Marchioni A, Cavazza A, Mucciarini C, Migaldi M, Federico M, Trentini G P & Sgambato A (2005) *Histopathology* **46**, 522–531

PDGFR expression in differential diagnosis between KIT-negative gastrointestinal stromal tumours and other primary soft-tissue tumours of the gastrointestinal tract

Aims: To investigate the value of platelet-derived growth factor receptors (PDGFRs) by immunohistochemistry in discriminating KIT-negative gastrointestinal stromal tumours (GISTs) from other soft-tissue neoplasms of the digestive tract.

Methods and results: One-hundred and sixty-seven primary gastrointestinal mesenchymal tumours (125 GISTs, 15 intra-abdominal desmoids, 12 leiomyomas, eight leiomyosarcomas, three schwannomas, two solitary fibrous tumours, and one case each of inflammatory pseudotumour and fibroid polyp) were reclassified based on morphology and on the immunohistochemical panel recommended by the National Institutes of Health consensus on GIST. All cases were then tested with antibodies specific for PDGFR α and β. Of 125 GISTs, 117 were KIT-positive (93.6%) and eight KIT-negative (6.4%). All the KIT-positive GISTs were negative for both PDGFRs, while all the eight KIT-negative GISTs expressed PDGFR-α, with two of them also coexpressing PDGFR-β. Among the 42 non-GIST tumours, only a small percentage (26.6%) of desmoids immunostained for PDGFR-α, two of them coexpressing PDGFR-β.

Conclusions: Immunostaining with PDGFR-α is a helpful marker in discriminating between KIT-negative GISTs and other gastrointestinal mesenchymal lesions: all KIT-negative GISTs were positive for PDFGR-α, while none of the other gastrointestinal mesenchymal tumours analysed, except a small subset of desmoids, was reactive with anti-PDGFRs. These preliminary data demonstrate the suitability of commercially available antibodies to detect immunohistochemically the mutually exclusive expression of KIT and PDGFR-α previously reported in GISTs by molecular biological techniques. Since PDGFR exists in the form of a homodimer (αα, ββ) or heterodimer (αβ) and two of the KIT-negative GISTs coexpressed both PDGFR isoforms, further investigations are required to elucidate the role of PDGFR-β in GISTs.

Keywords: CD117, desmoid, GIST, immunohistochemistry, KIT, PDGFR

Abbreviations: GIST, gastrointestinal stromal tumour; PDGFR, platelet-derived growth factor receptor

Introduction

Gastrointestinal stromal tumour (GIST) is a phenotypically and genotypically distinct entity representing the most common primary mesenchymal neoplasm of the digestive tract.[1–3] Although GIST may be identified by light microscopy, pathologists commonly employ a panel of immunohistochemical

Address for correspondence: Giulio Rossi MD, Department of Pathologic Anatomy and Legal Medicine, Section of Pathologic Anatomy, University of Modena and Reggio Emilia, via del Pozzo, 71-41100 Modena, Italy. e-mail: rossi.giulio@unimo.it

markers to confirm the morphological impression, thus distinguishing GISTs from other potential soft-tissue mimics occurring in the intestine such as smooth muscle and neurogenic tumours, desmoids, solitary fibrous tumours, inflammatory pseudotumours and fibroid polyps.[4–7] The recommended panel commonly comprises antibodies anti-CD34, smooth-muscle actin, desmin, S100 protein, and CD117, a specific marker corresponding to the KIT protein, the product of the *c-kit* proto-oncogene, currently appearing as the most specific marker for GIST.[5–10] Immunohistochemistry should be carried out in all cases since KIT expression in GIST is usually related to gain-of-function mutations in the *c-kit* gene,[2,3,11,12] that would make tumours eligible for treatment with the KIT inhibitor STI571 (formerly imatinib mesylate; Glivec or Gleevec, Novartis Pharmaceuticals Corp.), a small molecule that gives alternative and promising clinical results in advanced GISTs that usually do not respond to conventional chemotherapy or radiotherapy.[13–16] However, it is well known that a small number of GISTs that do not show functional mutations of the *c-kit* gene and are unstained for CD117 may still respond to STI571.[3,16,17] Recently, Heinrich *et al.*[18] and Hirota *et al.*[19] independently found that GISTs display mutually exclusive mutations of *c-kit* and *platelet-derived growth factor receptor-alpha* (*PDGFR-α*) genes, KIT-negative GIST therefore presenting a point mutation, with increased expression, of the *PDGFR-α* gene but a wild-type *c-kit*. It is noteworthy that both genes are located on chromosome 4q and the corresponding proteins share high amino acid identity.[18,19] Similar to the KIT protein, PDGFR is a type III tyrosine kinase and acts as a receptor for the relevant ligand platelet-derived growth factor (PDGF).[20] Both ligand and receptor present two isoforms (α and β) and may exist in the form of homodimer (αα or ββ) or heterodimer (αβ) possessing different affinity for the various ligands.[21] Interestingly, PDGFR is significantly blocked by various tyrosine kinase inhibitors, including STI571.[22–25]

In this study, we retrospectively collected and reclassified 167 primary gastrointestinal mesenchymal tumours by means of careful histological examination as well as by immunohistochemistry applying the panel of markers recently recommended by the National Institutes of Health (NIH) consensus conference on GIST.[5,6] Most important, for the first time we examined by immunohistochemistry the diagnostic role of PDGFR expression in identifying KIT-negative GISTs and in discriminating this subset of GISTs from other gastrointestinal soft-tissue neoplasms.

Materials and methods

The archival files and the clinical charts of the Section of Pathology and the Tumours Registry, respectively, of the University of Modena and Reggio Emilia were searched for primary mesenchymal tumours of the gastrointestinal tract or tumours with a spindle cell morphology, diagnosed from 1988 to 2003.

Among the 186 originally collected cases, nine cases were excluded from this study since they consisted of a tiny endoscopic biopsy precluding a confident histopathological examination by light microscopy and/or immunohistochemical analysis. After a careful review of clinical data and pathological findings, another 10 were reclassified, seven as metastatic lesions (four retroperitoneal spindle cell liposarcomas, two retroperitoneal malignant fibrous histiocytomas and one uterine leiomyosarcoma) and three as sarcomatoid carcinomas. Finally, 167 tumours were selected for subsequent immunohistochemical study. In each case, all the haematoxylin and eosin-stained sections obtained from routinely formalin-fixed paraffin-embedded blocks were reviewed by three pathologists (G.R., R.V., G.P.T.) at a multiheaded microscope. The final diagnosis was reached from the analysis of clinical findings, morphological features at light microscopy as well as from the immunuhistochemical results using the panel of markers recently suggested by the NIH consensus conference on GIST.[5,6] On this basis, the present series consisted of 125 GISTs (113 surgical resections and 12 endoscopic biopsies), 15 intra-abdominal desmoids, 12 leiomyomas, eight leiomyosarcomas, three schwannomas, two solitary fibrous tumours, and one case each of inflammatory pseudotumour and fibroid polyp (all consisting of surgical specimens). Immunohistochemistry was performed on 4 μm thick paraffin-embedded sections obtained from a representative block. Briefly, sections were air-dried overnight at 37°C, deparaffinized in xylene and rehydrated through decreasing concentrations of alcohol to water. Troublesome immunostaining, even on changing antibody dilutions or antigen retrieval methods, was initially observed when immunohistochemistry for PDGFR-α and PDGFR-β was performed in the usual manual manner. The main problem was related to a background blush probably due to non-specific staining in several GISTs (KIT-positive and -negative) and other soft-tissue tumours. This kind of staining precluded a correct interpretation of the immunohistochemical results. Thus, we decided to perform immunostaining using an automated immunostainer (Benchmark; Ventana, Tucson, AZ, USA) with a closed system. Even at a lower dilution of the antibodies (1 : 100), the first results

were much better than before, displaying clear specific staining with only a weak background. To further reduce the non-specific staining, several attempts were performed testing different antibody dilutions with or without antigen retrieval, until a 1 : 200 dilution with microwave antigen treatment was selected since it gave reproducible results with a clean background and appropriate staining of the internal control (submucosal and myenteric ganglion cells). 3′-3-Diaminobenzidine was used as the chromogen and Harris's haematoxylin as the counterstain. The primary antibodies together with the relevant sources and immunostaining conditions are listed in Table 1. Controls for specificity of staining were performed by immunostaining duplicate sections with non-immune mouse IgG, at the same concentration as that of the corresponding primary antibody. Tumour samples with known immunoreactivity served as a positive control for each antibody. Positive and negative control slides were included with each batch of slides. The following internal positive controls served to ensure preservation of the immunoreactivity and antibody specificity: mast cells (CD117), endothelial cells (CD34), smooth muscle (smooth-muscle actin), vessels and/or intestinal smooth muscle wall (desmin), mature ganglion cells and nerve fibres of the submucosal and/or myenteric plexus (S100 protein, PDGFR-α and PDGFR-β).

All the immunostains were reviewed by two pathologists (G.R., R.V.). The intensity of the immunostaining was graded as negative (no staining), weak (1+), moderate (2+) or strong (3+). Tumours with 2+ or 3+ staining and the relevant subcellular localization in more than 10% of the tumour cells were considered to be positive. In case of disagreement, the immunostains were re-evaluated at a multiheaded microscope by four pathologists (G.R., R.V., A.C., G.P.T.) and a final consensus was reached in all cases.

The correlation between clinicopathological parameters and immunohistochemical results was performed using contingency table methods and tested for significance using the Pearson's χ^2 test. A difference in probability (P) values of ≤ 0.05 was considered significant.

Results

PDGFR-α AND PDGFR-β DISTRIBUTION IN NORMAL INTESTINAL TRACT

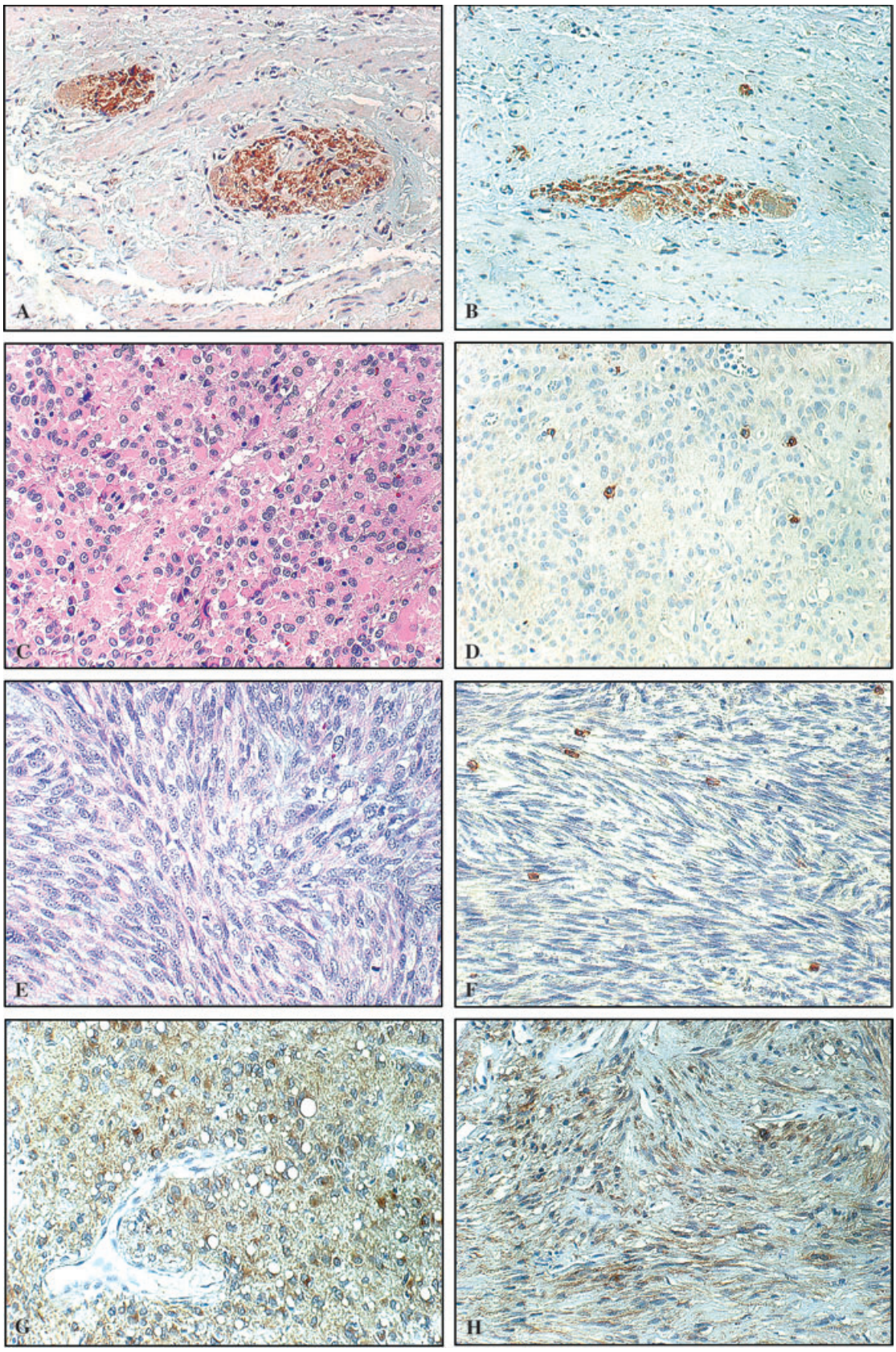
In normal gastrointestinal tract, both PDGFR-α and PDGFR-β were expressed in the cytoplasm of the mature ganglion cells and the nerves of the myenteric and submucosal plexus (Figure 1A,B). This kind of staining clearly served as a positive internal control,

Table 1. Details of antibodies used for immunohistochemical analysis of 167 primary mesenchymal tumours of the gastrointestinal tract

Antibody (clone or catalogue no.)	Source	Dilution	Antigen retrieval
CD117 (pAb, no. A4502)	Dako (Glostrup, Denmark)	1 : 200	None
S100 (pAb, no. RB044-A)	NeoMarkers (Fremont, CA, USA)	1 : 5	None
Smooth-muscle actin (mAb, 1A4)	Biogenex (San Ramon, CA, USA)	1 : 20	None
Desmin (mAb, D33)	Dako	1 : 10	MW
CD34 (mAb, QB-END/10)	Novocastra (Newcastle upon Tyne, UK)	1 : 40	MW
PDGFR-α (pAb, no. sc-338)	Santa Cruz Biotechnology (Santa Cruz, CA, USA)	1 : 200	MW
PDGFR-β (pAb, no. sc-339)	Santa Cruz Biotechnology	1 : 200	MW

pAb, Polyclonal antibody; mAb, monoclonal antibody; PDGFR, platelet-derived growth factor receptor; MW, microwave treatment.

Figure 1. Expression of platelet-derived growth factor receptor (PDGFR)-α (**A**) and PDGFR-β (**B**) in mature ganglion cells and nerves of myenteric plexus served as appropriate internal positive controls. A gastrointestinal stromal tumour (GIST) of epithelioid type (**C**) completely unstained with CD117 (**D**) and a spindle-cell type GIST (**E**) also negative immunostained with CD117 (**F**). Note the scattered CD117-immunoreactive mast cells as positive internal control. The same cases showed positive cytoplasmic immunostaining of tumour cells with the anti-PDGFR-α antibody (**G,H**).



ensuring preservation of tissue immunoreactivity and allowing exclusion of equivocal reactions and false negatives. All the other normal structures, including smooth muscle and endothelial cells, adipose tissue and lymphoid elements were completely unstained. Preabsorption of the primary antibodies with the respective blocking peptide provided by the manufacturer (with an excess of blocking peptide) before use abolished the staining for PDGFR-α and PDGFR-β, thus further confirming the specificity of the reaction.

EXPRESSION OF KIT, PDGFR-α, PDGFR-β AND OTHER IMMUNOHISTOCHEMICAL MARKERS IN GISTs

The most relevant clinicopathological findings of the GISTs included in this study are summarized in Table 2. The 125 patients with GIST included 65 males and 60 females with a median age at diagnosis of 67 years (range 26–87 years). The stomach represented the most commonly affected site (82 cases; 65.6%), followed by the small intestine (32 cases, 25.6%). When tumour size and mitotic index were recorded, the majority of GISTs displayed a high mitotic index (> 5 × 50 high-power fields, 40×) and a large tumour size (> 50 mm), thus falling into the high-risk category according to Fletcher *et al.*[5,6] (64 cases; 51.2%). Despite the high quality of biopsies, assessment of tumour size and mitotic index was not reliable in 12 cases. Eighty-nine GISTs (71.2%) had a spindle cell appearance and the remaining 36 cases presented an epithelioid (22 cases; 17.6%) and mixed (spindle and epithelioid) (14 cases; 11.2%) morphology.

Immunoreactivity for CD117/KIT (Table 3) was observed in the great majority of GISTs (117 cases; 93.6%), whereas eight (6.4%) of them were entirely negative (Figure 1C–F). CD34 immunostaining was found in 78 cases (62.4%) and smooth-muscle actin in 23 cases (18.4%). Four tumours (3.2%) showed focal S100 protein positivity. Moderate staining for desmin was observed in only one case. Of the eight KIT-negative GISTs, all showed cytoplasmic immunoreactivity for PDGFR-α (Figure 1G,H) and two also for PDGFR-β (Figure 2A,B). The neoplastic elements (all of epithelioid type) focally showed a marked dot-like, paranuclear accentuation with anti-PDGFR antibodies, while a membranous pattern of expression was observed in two cases (one spindle and one epithelioid type) for PDGFR-α and in one case for PDGFR-β. Preabsorption of primary antibodies with specific blocking peptide before use abolished the staining, as did omission of the primary antibodies (data not shown), thus confirming the specificity of the staining. Six (75%) KIT-negative GISTs also expressed CD34 (Table 3). Of note, all the KIT-negative GISTs displayed the classical morphology of the more conventional KIT-positive ones and no histopathological features allowed discrimination between KIT-negative and KIT-positive GISTs.

Statistical analysis showed that none of the tested markers, except CD34, was significantly associated with the analysed prognostic parameters (size, mitotic index and risk category). CD34 positivity, in fact, appeared significantly correlated with a lower mitotic index ($P = 0.02$) and a lower risk group ($P = 0.047$). In addition, a statistically significant association was observed between CD34+ GISTs and spindle cell morphology ($P = 0.012$).

EXPRESSION OF IMMUNOHISTOCHEMICAL MARKERS IN OTHER GASTROINTESTINAL MESENCHYMAL TUMOURS

Immunohistochemical results in tumours other than GIST are summarized in Table 3. As expected, smooth-muscle tumours (leiomyomas and leiomyosarcomas) were characterized by strong immunoreactivity for smooth-muscle actin and desmin, but not for the other tested markers except for one case of leiomyoma displaying moderate cytoplasmic immunoreactivity for CD34.

Schwannomas immunoreacted exclusively with anti-S100 antibody and solitary fibrous tumours were only positive for CD34. The inflammatory pseudotumour and the fibroid polyp present in our series stained positively for smooth-muscle actin and CD34, respectively. Most importantly, none of the above tumours showed immunopositivity for CD117, PDGFR-α and/or PDGFR-β. Conversely, all but one of the desmoid tumours were moderately immunoreactive with anti-smooth-muscle actin antibody and four out of 15 (26.6%) showed cytoplasmic positivity for PDGFR-α. Finally, two of these latter cases also coexpressed PDGFR-β (Figure 2C–E).

Discussion

Traditionally, the diagnosis of GIST has been a challenge for the pathologist. Nowadays, GIST is regarded as a distinct entity at both the genotypic and phenotypic level and represents the most common soft-tissue tumour of the digestive tract.[1,5–7,26] Moreover, it is the solid tumour in which the presence of specific molecular alterations correlates more strictly with the immunophenotype and with the possibility of applying novel therapeutic approaches using molecular targeted therapies.[2,3,16]

Table 2. CD117 expression and clinicopathological characteristics in 125 gastrointestinal stromal tumours

	Total	Positive	Negative
Site			
Oesophagus	1	1	0
Stomach	82	77	5
Duodenum	6	6	0
Digiunum	3	3	0
Ileum	23	21	2
Right colon	3	3	0
Left colon	4	3	1
Sigma-rectum	3	3	0
Mitotic index (x 50 HPF)			
<5	50	49	1
6–10	34	31	3
>10	29	27	2
NA	12	10	2
Size (mm)			
<20	17	16	1
20–50	21	21	0
>50–100	39	38	1
>100	36	32	4
NA	12	10	2
Risk groups			
Very low	13	12	1
Low	13	13	0
Intermediate	23	23	0
High	64	59	5
NA	12	10	2
Morphology			
Spindle	89	87	2
Epithelioid	22	18	4
Mixed	14	12	2

HPF, High-power fields; NA, not available.

Following the fundamental work by Hirota *et al.*,[11] who first demonstrated the presence of activating constitutive mutations of the proto-oncogene *c-kit* in GIST, several authors have confirmed that this molecular event is critical in the development of almost all GISTs, independent of the site of origin, tumour size, morphology, and clinical behaviour.[3,12,27–29] In 1998, Hirota *et al.*[11] and Kindblom *et al.*[8] reported that the vast majority of GISTs stained positively for CD117, a molecular marker corresponding to the tyrosine kinase KIT, the *c-kit* proto-oncogene product. Shortly thereafter, other authors showed that CD117 appeared to be an extremely helpful marker in discriminating GISTs from other mesenchymal tumours of the gastrointestinal tract, such as smooth muscle and neural tumours, desmoids, solitary fibrous tumours, inflammatory pseudotumours and fibroid polyps.[9,10] It is therefore generally accepted that none of these lesions shows positivity for CD117/KIT.[1,5,6] Apart from its diagnostic role, CD117 also has potentially important therapeutic value, since recent trials have confirmed that advanced and metastatic GISTs may be successfully treated with the selective KIT inhibitor imatinib mesylate.[13–16] This latter finding strongly supports the contention that a diagnosis of GIST should always be confirmed by the immunohistochemical expression of CD117, although positivity for this marker does not always predict the presence of KIT functional activation.[3–6] However, since CD117 expression in GIST is usually related to molecular alterations in different exons of *c-kit* and mutational analysis in GIST cannot be routinely performed in all surgical pathology laboratories, the detection of CD117 immunostaining is at present the most currently and least expensive method employed to identify GISTs, thus permitting the selection of patients for imatinib mesylate-based therapy.[4–6,16]

Given the clinical value of KIT expression, Hornick and Fletcher[30,31] and Lucas *et al.*[32] have recently underlined that the choice of appropriate immunohistochemical reagents and optimal technical conditions in routine immunohistochemistry represent critical issues in preventing conflicting results and false CD117 positivity in this setting. While it is still debatable whether a diagnosis of GIST can be performed on morphological grounds alone or in the absence of CD117 immunopositivity,[1,4–7] it is well known that a small subset of GISTs are KIT-negative and do not have point mutations of *c-kit*.[1,4–6] Interestingly, the majority of such tumours seem to respond to treatment with imatinib mesylate.[17] Despite the extremely heterogeneous spectrum of morphological patterns reported in GIST,[33–36] we found that careful histological examination coupled with the lack of a clear-cut differentiation at immunohistochemistry adopting the panel of antibodies recommended by the NIH consensus conference on GIST[5,6] gave us sufficient confidence in the

Table 3. Immunohistochemical comparison between gastrointestinal stromal tumours (GISTs) and other soft-tissue tumours of the gastrointestinal tract

Histotype	No. of cases	CD34*	Actin*	Desmin*	S100*	CD117*	PDGFR-α*	PDGFR-β*
GIST	(125)	78 (62.4)	23 (18.4)	1 (0.8)	4 (3.2)	117 (93.6)	8 (6.4)	2 (1.6)
Desmoids	(15)	0	14 (93.3)	0	0	0	4 (26.6)	2 (13.3)
Leiomyomas	(12)	1 (8.3)	12 (100)	12 (100)	0	0	0	0
Leiomyosarcomas	(8)	0	8 (100)	7 (87.5)	0	0	0	0
Schwannomas	(3)	0	0	0	3 (100)	0	0	0
Solitary fibrous tumours	(2)	2 (100)	0	0	0	0	0	0
Inflammatory pseudotumour	(1)	0	1 (100)	0	0	0	0	0
Inflammatory fibroid polyp	(1)	1 (100)	0	0	0	0	0	0

PDGFR-α, platelet-derived growth factor receptor-alpha; PDGFR-β, platelet-derived growth factor-beta.
*The number and the percentage (in parentheses) of positive cases have been reported for each marker.

identification of KIT-negative GISTs collected in the current series. Unfortunately, given the retrospective design of the study, all KIT-negative GISTs herein occurred in the pre-STI571 era and none of these patients underwent molecular therapy. As recently and independently demonstrated by Heinrich *et al.*[18] and Hirota *et al.*,[19] almost all KIT-negative GISTs have point mutations in the *PDGFR-α* gene and express high levels of the corresponding protein. In addition, both authors found that mutations in the *c-kit* and *PDGFR-α* genes were mutually exclusive.[18,19] From a clinical standpoint, Heinrich *et al.*[17] recently presented the results of a clinical trial showing that patients with KIT-positive GISTs were more sensitive to STI571 than those with mutant PDGFR-α. However, little is known about the clinical response to STI571 in PDGFR-positive/KIT-negative GISTs, since these cases represent only a minor subset of all GISTs and a large multicentre clinical trial with STI571 would be advisable in order to evaluate definitively whether targeted therapy might also be effective in this subgroup of tumours.

These observations prompted us to test the suitability of commercially available antibodies against PDGFRs to detect KIT-negative GISTs by immunohistochemistry and for discriminating this subset of GISTs from other mesenchymal tumours of the gastrointestinal tract potentially mimicking GISTs. As previously reported,[37–39] PDGFRs are usually expressed in Schwann cells of the peripheral nervous system, including the enteric nervous system, contributing to normal gangliogenesis through an autocrine growth regulatory loop involving PDGFs/PDGFRs. Accordingly, we used PDGFR expression of the myenteric plexus as a positive internal control to validate our immunohistochemical results. Moreover, the specificity of the staining was further confirmed by demonstrating its loss following preincubation of the specific polyclonal antibodies with an excess of the respective blocking peptide before use or by omitting the primary antibodies (data not shown). The percentage of KIT-negative GISTs in our series (6.4%) is in line with that (4.7%) reported in the recent trial by Heinrich *et al.*,[17] though a little higher than that suggested by the NIH consensus statement[5,6] (less than 2%) and lower than the 15% reported by Sarlomo-Rikala *et al.* in 1998.[9] According to previous studies,[26,40,41] the majority of GISTs occur in the stomach (65.6%) and small intestine (25.6%), present a spindle cell morphology (71.2%) and fall within the high-risk group (51.2%). Our immunohistochemical results have confirmed that CD117 is certainly the most specific marker in distinguishing GISTs from other soft-tissue tumours. In fact, no tumour type other than GIST showed immunoreactivity for CD117, including desmoids, for which conflicting data have been previously reported in the literature.[42–44] Among all the markers tested, only CD34 was significantly correlated with pathological prognostic parameters in our series of GISTs, with CD34 immunostaining being more frequent in tumours with a lower mitotic index ($P = 0.02$) and belonging to a lower risk group ($P = 0.047$). This finding is not entirely surprising and is in agreement with previous results by Sarlomo-Rikala *et al.*,[9] who reported a higher incidence of CD34 negativity in GISTs with malignant clinical behaviour. Moreover, a similar phenomenon was also described

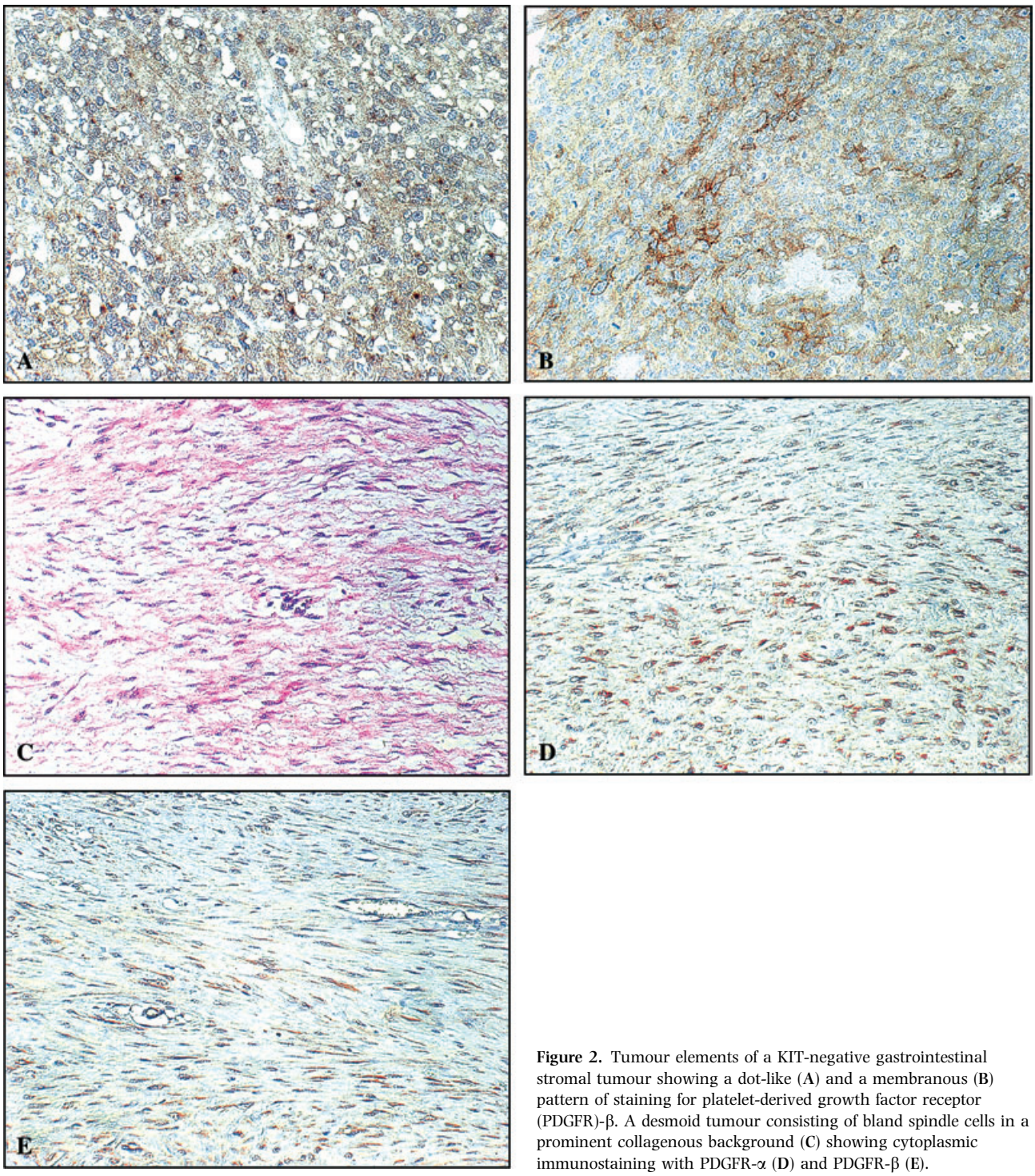


Figure 2. Tumour elements of a KIT-negative gastrointestinal stromal tumour showing a dot-like (**A**) and a membranous (**B**) pattern of staining for platelet-derived growth factor receptor (PDGFR)-β. A desmoid tumour consisting of bland spindle cells in a prominent collagenous background (**C**) showing cytoplasmic immunostaining with PDGFR-α (**D**) and PDGFR-β (**E**).

by Goldblum[45] in dermatofibrosarcoma protuberans, another CD34+ soft-tissue tumour in which CD34 immunoreactivity disappeared when fibrosarcomatous overgrowth occurred within the same lesion. Taken together with the results of our study, these findings support the idea that loss of CD34 might be associated with tumour progression and with acquisition of a more aggressive behaviour, although further studies will be needed to confirm this hypothesis definitively.

To our knowledge, this is the first study demonstrating the suitability of commercially available antibodies to detect variations in PDGFR expression occurring

in KIT-negative GISTs by immunohistochemistry. Our results on the expression of PDGFR in GISTs are in agreement with those of Heinrich *et al.*[18] demonstrating the mutually exclusive expression of these two proteins in GISTs. In fact, all KIT-positive GISTs were unstained with the anti-PDGFR-α antibody, while KIT-negative GISTs showed clear-cut cytoplasmic and/or membranous immunostaining for PDGFR-α. Interestingly, of the eight KIT-negative GISTs, two cases showed coexpression of both PDGFR-α and PDGFR-β isoforms. Since PDGFR may exist as homodimer (αα, ββ) or heterodimer (αβ),[20,21] it could be that a minority of KIT-negative GISTs possesses PDGFR in the form of the heterodimer, but further molecular investigation will be needed to assess definitively whether or not PDGFR-β expression in GISTs is related to additional activating mutation(s) in the relevant gene locus.

As stated by Greenson, GISTs are mainly confused with desmoids in routine practice, especially when they appear as a pure spindle cell proliferation.[7] Desmoids consist of bland lesions with a collagen-rich stroma and spindled to stellate neoplastic cells, and some workers have reported a high frequency of CD117 immunoreactivity in this group of tumours.[42,44] This finding led Mace *et al.*[44] to perform a preliminary study in which they treated two patients affected by recurrent extra-abdominal desmoid fibromatosis with STI571, demonstrating a significant clinical improvement. The same authors also reported that six out of nine (67%) desmoids stained positively with CD117 and all cases were equally immunoreactive with anti-PDGFR-α and anti-PDGFR-β antibodies.[44] We did not experience any positive immunostaining in our 15 desmoids with respect to CD117, but four cases (27%) were positive for PDGFR-α and two of them (13%) also coexpressed PDGFR-β, suggesting that the positive clinical response obtained with STI571 in desmoid patients might be related to inhibition of PDGFR rather than KIT. From a diagnostic point of view, our results suggest that PDGFR expression does not allow a reliable differential diagnosis between KIT-negative GISTs and desmoids.

In summary, in our series KIT-negative GISTs always expressed PDGFR-α, another type III tyrosine kinase inhibited by STI571, that might be helpful as an additional marker in identifying KIT-negative GISTs and in discriminating this small subset of GISTs from the majority of other KIT-negative mesenchymal tumours of the gastrointestinal tract mimicking GIST, apart from desmoids. About one-third of these latter tumours, in fact, showed positive staining for PDGFR-α and in two desmoids coexpression of PDGFR-α and PDGFR-β was noted. These preliminary data await further confirmation on a larger series and using more sophisticated methods. Moreover, since two of eight KIT-negative GISTs showed combined immunoreactivity for both PDGFR-α and PDGFR-β, further investigations are needed on a larger number of KIT-negative GISTs to determine whether this finding is merely related to the presence of PDGFR in a heterodimer form or to the occurrence of activating mutations involving the *PDGFR-β* locus.

Acknowledgements

This work was supported by a grant of the 'Associazione contro il Cancro Angela Serra'. The authors thank Paola Manni and Claudia Malagoli for their skilful technical assistance in performing the immunohistochemical staining and Luca Fabbiani for production of microphotographs.

References

1. Miettinen M, Lasota J. Gastrointestinal stromal tumours—definition, clinical, histological, immunohistochemical and molecular genetic features and differential diagnosis. *Virchows Arch.* 2001; **438;** 1–12.
2. Heinrich MC, Rubin BP, Longley BJ, Fletcher JA. Biology and genetic aspects of gastrointestinal stromal tumors. KIT activation and cytogenetic alterations. *Hum. Pathol.* 2002; **33;** 484–495.
3. Kitamura Y, Hirota S, Nishida T. Gastrointestinal stromal tumors (GIST): a model for molecule-based diagnosis and treatment of solid tumors. *Cancer Sci.* 2003; **94;** 315–320.
4. Berman J, O'Leary TJ. Gastrointestinal stromal tumor workshop. *Hum. Pathol.* 2001; **32;** 578–582.
5. Fletcher CDM, Berman JJ, Corless C *et al.* Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Int. J. Surg. Pathol.* 2002; **10;** 81–89.
6. Fletcher CDM, Berman JJ, Corless C *et al.* Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Hum. Pathol.* 2002; **33;** 459–465.
7. Greenson JK. Gastrointestinal stromal tumors and other mesenchymal lesions of the gut. *Mod. Pathol.* 2003; **16;** 366–375.
8. Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. *Am. J. Pathol.* 1998; **152;** 1259–1269.
9. Sarlomo-Rikala M, Kovatich AJ, Barusevicius A, Miettinen M. CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. *Mod. Pathol.* 1998; **11;** 728–734.
10. Miettinen M, Sobin LH, Sarlomo-Rikala M. Immunohistochemical spectrum of GISTs at different sites and their differential diagnosis with reference to CD117 (KIT). *Mod. Pathol.* 2000; **13;** 1134–1142.
11. Hirota S, Isozaki K, Moriyama Y *et al.* Gain-of-function mutations of c-KIT in human gastrointestinal tumors. *Science* 1998; **279;** 577–580.
12. Rubin BP, Singer S, Tsao C *et al.* KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res.* 2001; **61;** 8118–8121.

13. Joensuu H, Roberts PJ, Sarlomo-Rikala M *et al.* Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N. Engl. J. Med.* 2001; **344;** 1052–1056.
14. Van Oosterom AT, Judson I, Verweji J *et al.* Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet* 2001; **358;** 1421–1423.
15. Demetri G, von Mehren M, Blanke CD *et al.* Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med.* 2002; **347;** 472–480.
16. Joensuu H, Fletcher C, Dimitrijevic S, Silberman S, Roberts P, Demetri G. Management of malignant gastrointestinal stromal tumours. *Lancet Oncol.* 2002; **3;** 655–664.
17. Heinrich MC, Corless CL, Demetri GD *et al.* Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J. Clin. Oncol.* 2003; **21;** 4342–4349.
18. Heinrich MC, Corless CL, Duensing A *et al.* PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003; **299;** 708–710.
19. Hirota S, Ohashi A, Nishida T *et al.* Gain-of-function mutations of platelet-derived growth factor receptor α gene in gastrointestinal stromal tumors. *Gastroenterology* 2003; **125;** 660–667.
20. Yarden Y, Escobedo JA, Kuang WJ *et al.* Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. *Nature* 1986; **323;** 226–232.
21. Heldin CH, Ostman A, Ronnstrand L. Signal transduction via platelet-derived growth factor receptors. *Biochim. Biophys. Acta* 1998; **1378;** F79–F113.
22. Ostman A, Heldin CH. Involvement of platelet-derived growth factor in disease: development of specific antagonists. *Adv. Cancer Res.* 2001; **80;** 1–38.
23. George D. Platelet-derived growth factor receptors: a therapeutic target in solid tumors. *Semin. Oncol.* 2001; **28;** 27–33 (Suppl. 17).
24. Buchdunger E, Cioffi CL, Law N *et al.* Abl protein-tyrosine kinase inhibitor STI571 inhibits *in vitro* signal trasduction mediated by c-Kit and platelet-derived growth factor receptors. *J. Pharmacol. Exp. Ther.* 2000; **295;** 139–145.
25. Mendel DB, Laird DA, Xin X *et al. In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin. Cancer Res.* 2003; **9;** 327–337.
26. Emory TS, Sobin LH, Lukes L, Lee DH, O'Leary TJ. Prognosis of gastrointestinal smooth-muscle (stromal) tumors. *Am. J. Surg. Pathol.* 1999; **23;** 82–87.
27. Lux ML, Rubin BP, Biase TL *et al.* KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. *Am. J. Pathol.* 2000; **156;** 791–795.
28. Corless CL, McGreevey L, Haley A, Town A, Heinrich MC. KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. *Am. J. Pathol.* 2002; **160;** 1567–1572.
29. Singer S, Rubin BP, Lux ML *et al.* Prognostic value of KIT mutations type, mitotic activity, and histologic subtype in gastrointestinal stromal tumors. *J. Clin. Oncol.* 2002; **20;** 3898–3905.
30. Hornick JL, Fletcher CDM. Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution. *Am. J. Clin. Pathol.* 2002; **117;** 188–193.
31. Hornick JL, Fletcher CDM. Validating immunohistochemical staining for KIT (CD117). *Am. J. Clin. Pathol.* 2003; **119;** 325–327.
32. Lucas DR, Al-Abbadi M, Tabaczka P, Hamre MR, Weaver DW, Mott MJ. C-Kit expression in desmoid fibromatosis. Comparative immunohistochemical evaluation of two commercial antibodies. *Am. J. Clin. Pathol.* 2003; **119;** 339–345.
33. Suster S. Gastrointestinal stromal tumors. *Semin. Diagn. Pathol.* 1996; **13;** 297–313.
34. Yoneda E, Teramura K, Hiruma S, Satou T, Hashimoto S. Malignant gastrointestinal stromal tumor showing remarkable whorl formations. *Arch. Pathol. Lab. Med.* 2001; **125;** 665–668.
35. Leung KM, Wong S, Chow TC, Cheong Lee K. A malignant gastrointestinal stromal tumor with osteoclast-like giant cells. *Arch. Pathol. Lab. Med.* 2002; **126;** 972–974.
36. Suarez-Vilela D, Izquierdo-Garcia FM. Cytotoxic T-lymphocyte-rich gastrointestinal stromal tumor. *Histopathology* 2003; **32;** 397–410.
37. Eccleston PA, Funa K, Heldin CH. Expression of platelet-derived growth factor (PDGF) and PDGF alpha- and beta-receptors in the peripheral nervous system: an analysis of sciatic nerve and dorsal root ganglia. *Dev. Biol.* 1993; **155;** 459–470.
38. Soriano P. The PDGF alpha receptor is required for neural crest cell development and for normal patterning of the somites. *Development* 1997; **124;** 2691–2700.
39. Hoch RV, Soriano P. Roles of PDGF in animal development. *Development* 2003; **130;** 4769–4784.
40. Chandu de Silva MV, Reid R. Gastrointestinal stromal tumors (GIST): c-kit mutations, CD117 expression, differential diagnosis and targeted cancer therapy with imatinib. *Pathol. Oncol. Res.* 2003; **9;** 13–19.
41. Lin SC, Huang MJ, Zeng CY, Wang TI, Liu ZL, Shiay RK. Clinical manifestations and prognostic factors in patients with gastrointestinal stromal tumors. *World J. Gastroenterol.* 2003; **9;** 2809–2812.
42. Yantiss RK, Spiro IJ, Compton CC, Rosenberg AE. Gastrointestinal stromal tumor versus intra-abdominal fibromatosis of the bowel wall. A clinically important differential diagnosis. *Am. J. Surg. Pathol.* 2000; **24;** 947–957.
43. Miettinen M. Are desmoid tumors KIT positive? *Am. J. Surg. Pathol.* 2001; **25;** 549–550 (letter).
44. Mace J, Biermann JS, Sondak V *et al.* Response of extraabdominal desmoid tumors to therapy with imatinib mesylate. *Cancer* 2002; **95;** 2373–2379.
45. Goldblum JR. CD34 positivity in fibrosarcomas which arise in dematofibrosarcoma protuberans. *Arch. Pathol. Lab. Med.* 1995; **119;** 238–241.