

# A Novel Monoclonal Antibody Against DOG1 is a Sensitive and Specific Marker for Gastrointestinal Stromal Tumors

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**Abstract:** Gastrointestinal stromal tumors (GIST) occur primarily in the wall of the intestine and are characterized by activating mutations in the receptor tyrosine kinases genes *KIT* or *PDGFRA*. The diagnosis of GIST relies heavily on the demonstration of KIT/CD117 protein expression by immunohistochemistry. However, KIT expression is absent in ~4% to 15% of GIST and this can complicate the diagnosis of GIST in patients who may benefit from treatment with receptor tyrosine kinase inhibitors. We previously identified DOG1/TMEM16A as a novel marker for GIST using a conventional rabbit antipeptide antiserum and an in situ hybridization probe. Here, we describe 2 new monoclonal antibodies against DOG1 (DOG1.1 and DOG1.3) and compare their staining profiles with KIT and CD34 antibodies on 447 cases of GIST. These included 306 cases with known mutational status for *KIT* and *PDGFRA* from a molecular consultation service. In addition, 935 other mesenchymal tumors and 432 nonsarcomatous tumors were studied. Both DOG1 antibodies showed high sensitivity and specificity for GIST, with DOG1.1 showing some advantages. This antibody yielded positive staining in 370 of 425 (87%) scorable GIST, whereas CD117 was positive in 317 of 428 (74%) GIST and CD34 in 254 of 430 (59%) GIST. In GIST with mutations in *PDGFRA*, 79% (23/29) showed DOG1.1 immunoreactivity while only 9% (3/32) and 27% (9/33) stained for CD117 and CD34, respectively. Only 1 of 326 (0.3%) leiomyosarcomas and 1 of 39 (2.5%) synovial sarcomas among the 935 soft tissue tumors examined showed positive immunostaining for DOG1.1. In addition, DOG1.1 immunoreactivity

was seen in fewer cases of carcinoma, melanoma, and seminoma as compared with KIT.

**Key Words:** GIST, DOG1, monoclonal antibody, immunohistochemistry

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**G**astrointestinal stromal tumor (GIST) is the most common mesenchymal tumor arising in the gastrointestinal tract. Most contain an activating mutation in the juxtamembrane domains of either *KIT* or *PDGFRA* that result in constitutive, ligand-independent activation of these receptor tyrosine kinases (RTK).<sup>10–12,22</sup> Imatinib mesylate (Gleevec), a small molecule inhibitor with activity against KIT and PDGFRA is the primary therapy for metastatic or unresectable GIST.<sup>5</sup> Furthermore, the use of imatinib as a neoadjuvant or adjuvant therapy is being examined in ongoing clinical trials. The next generation of RTK inhibitors, including drugs such as sunitinib (Sutent), is providing options for GIST patients who fail imatinib therapy.<sup>6</sup> With the growing effectiveness and availability of RTK directed therapies, the accurate diagnosis of GIST has become imperative.

Histologically, GIST demonstrates considerable morphologic overlap with other tumors. Mutation screening of *KIT* or *PDGFRA* can serve in confirming the diagnosis of GIST, but only a few centers in United States perform this analysis clinically. Moreover, up to 15% of GIST lack a mutation in both of these kinase genes (so-called wild-type GIST). In routine practice, the diagnosis of GIST is based on the anatomic location of the tumor and immunohistochemical evidence of KIT (CD117) and/or CD34 expression. CD34 is not a specific marker for GIST and is positive in many other soft tissue tumors that may enter into the differential diagnosis of GIST.<sup>21</sup> Consequently, its utility in the diagnosis of GIST is limited. In contrast, within the sarcomas, KIT is a relatively specific marker for GIST. However, about ~4% to 15% of GIST in reported series show weak or negative staining for KIT/CD117.<sup>19,23</sup> Many of these

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“KIT-negative” GIST possess *PDGFRA* mutations and a subset of these cases is sensitive to treatment with imatinib.<sup>4,9,19</sup> Diagnosis of these tumors remains a significant challenge.

Using gene expression profiling, we previously identified *DOG1* (*TMEM16A*) as a gene with high levels of expression in GIST and developed a rabbit polyclonal antibody and an in situ hybridization probe that target *DOG1*.<sup>31</sup> This study involving a series of 149 GIST and 438 other mesenchymal tumors showed that the polyclonal *DOG1* antibody was superior in sensitivity and specificity compared with commercially available anti-CD117 polyclonal antiserum. However, we were unable to generate large amounts of polyclonal anti-*DOG1* antiserum. Here, we describe 2 novel mouse monoclonal antibodies against *DOG1* (*DOG1.1* and *DOG1.3*) that have superior sensitivity and specificity compared with anti-CD117 and anti-CD34 reagents in a large series of GIST and other tumors. Our results indicate that monoclonal *DOG1* antibodies are useful diagnostic markers for GIST and will help to identify additional patients with GIST who may benefit from targeted therapy.

## MATERIALS AND METHODS

### Case Material

The cases analyzed for this study consisted of 447 GIST, 935 other mesenchymal tumors (Table 1), and 432 nonsarcomatous tumors distributed over 10 tissue microarrays (TMAs). A diagnosis of GIST was made based on tumor location, morphology, and immunostaining for KIT. For 306 GIST cases, mutational analysis of the *KIT* and *PDGFRA* genes was obtained. Of the 39 mutation-negative (WT) tumors, 27 were located in the wall of the intestine and showed no histologic or immunophenotypic support for smooth muscle differentiation, 24 of these cases showed staining for KIT or CD34. The 12 remaining WT tumors were metastatic lesions or were located in the abdomen without a definite site. Eight of these 12 tumors were positive by immunostaining for KIT and/or CD34. The remaining 4 were accepted as GIST based on histologic features alone. The TMAs were constructed using a manual tissue arrayer from Beecher Instrument, Silver Spring, MD. Cores of 0.6 mm were taken from paraffin-embedded soft tissue tumors from the Stanford University Medical Center, the Oregon Health and Science University, Portland, Oregon, and the University of British Columbia, Vancouver. A significant proportion of GIST (306 cases) had been submitted for molecular consultation (C.L.C., M.C.H.) and had known mutational status for the *KIT* and *PDGFRA* genes. The GIST were analyzed for mutations in exons 9, 11, 13, and 17 of the *KIT* gene using a combination of denaturing high pressure liquid chromatography and direct sequencing, as previously described.<sup>3,9</sup> *KIT* wild-type tumors were subsequently screened for mutations in exons 12, 14, and 18 of the *PDGFRA* gene.<sup>9</sup>

**TABLE 1.** Mesenchymal Tumors Other Than GIST Included in the Study

Tumors	No. Cases
Alveolar soft part sarcoma	2
Phyllodes tumor	2
Pleomorphic liposarcoma	3
Epithelioid sarcoma	3
Fibrosarcoma	3
Chondrosarcoma	3
Inflammatory pseudotumor	5
Clear cell sarcoma	7
DSRCT	7
Dedifferentiated liposarcoma	10
Myxoid liposarcoma	10
Ewing/PNET	10
Desmoplastic melanoma	10
Well-differentiated liposarcoma	11
Extraskeletal myxoid chondrosarcoma	11
Low-grade fibromyxoid sarcoma	11
Fibroadenoma	11
Epithelioid hemangioendothelioma	12
Endometrial stroma sarcoma	13
Rhabdomyosarcoma	13
Angiosarcoma	14
Osteosarcoma	14
Dermatofibrosarcoma protuberans	20
Solitary fibrous tumor	21
Leiomyoma	22
Desmoid fibromatosis	35
Synovial sarcoma	44
Schwannoma	46
Neurofibroma	55
Malignant peripheral nerve sheath tumor	86
Undifferentiated sarcoma	87
Leiomyosarcoma	334

DSRCT indicates desmoplastic small round cell tumor; PNET, primitive neuroectodermal tumor.

### Immunohistochemistry

Slides were cut at 4  $\mu$ m, deparaffinized in xylene, and hydrated in a graded series of alcohol. The primary antibodies used were *DOG 1.1* (mouse monoclonal, 1/50; Stanford University), *DOG1.3* (mouse monoclonal, 1/1 supernatant; Applied Genomics Inc), CD117 (rabbit polyclonal, 1/400; DAKO, Carpinteria, CA), and CD34 (mouse monoclonal, 1/80; clone 581/CD34, BD Biosciences, San Jose, CA). The antigen retrieval solution for *DOG1.1*, *DOG1.3*, and CD34 was citrate, pH: 6 and for CD117 was EDTA, pH:8. Slides were boiled by microwaving in antigen retrieval solution for 12 minutes. The immunohistochemical reactions were visualized using rabbit or mouse versions of the biotin-free EnVision+ system (DAKO, Carpinteria, CA) using diaminobenzidine.

### In Situ Hybridization

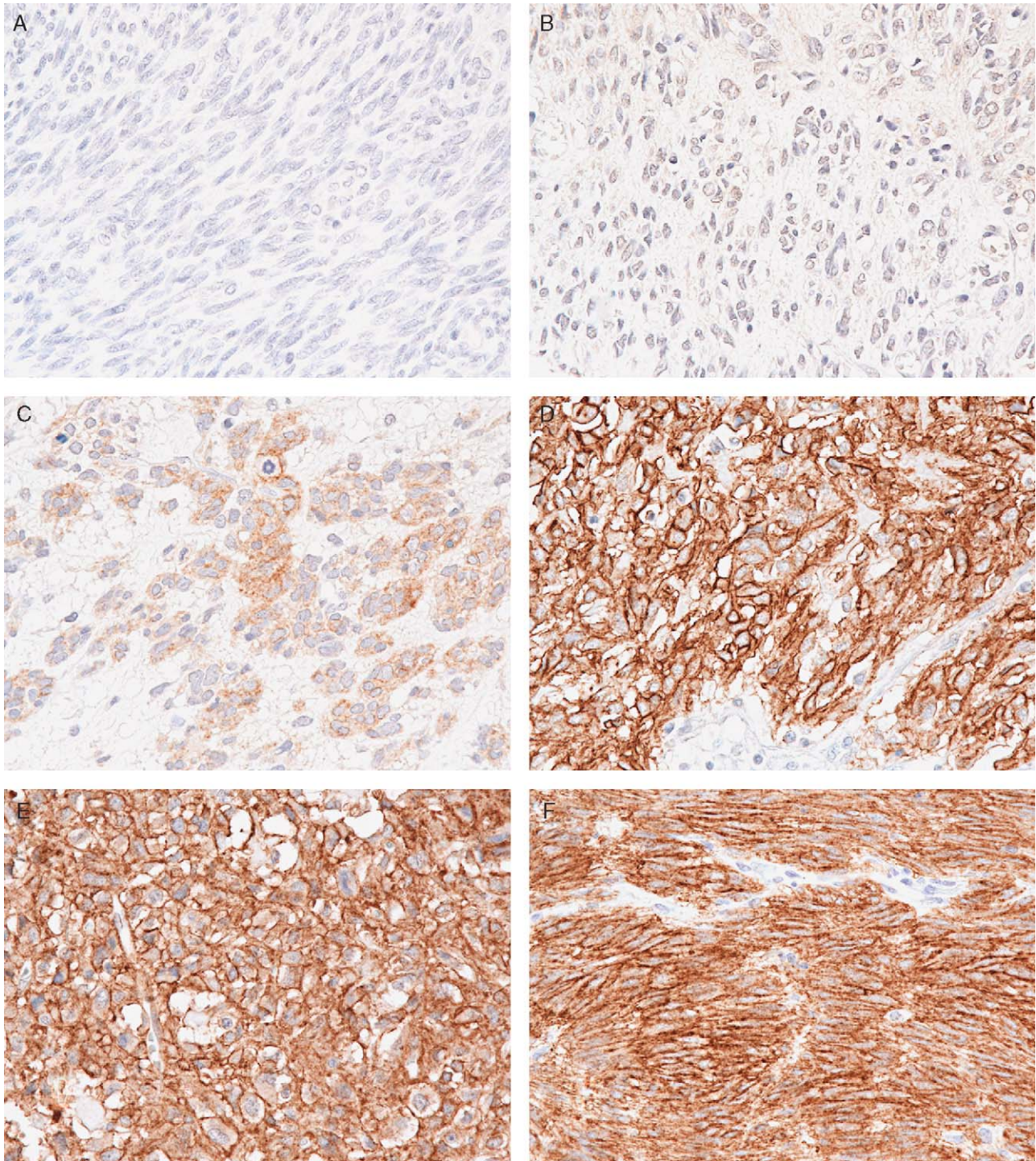
In situ hybridization of TMA sections was performed based on a protocol published previously.<sup>13,26,31</sup>

### Scoring of Immunohistochemistry and In Situ Hybridization

Cores were scored as follows: 0 indicates the absence of any staining; 1 indicates equivocal staining;

2 indicates any moderate membranous staining whether diffusely or focally present in the tumor; 3 indicates strong complete membranous staining whether diffusely or focally present in the tumor (Figs. 1A–D). Score 2 and 3 were considered positive. The cores were independently

reviewed for 2 pathologists (I.E. and C.H.L.) and any disagreements were reviewed together with a third pathologist (M.v.d.R.) to achieve a consensus score. Digital images of stained cores can be viewed at [http://tma.stanford.edu/tma\\_portal/DOG1\\_mcab](http://tma.stanford.edu/tma_portal/DOG1_mcab).



**FIGURE 1.** DOG1.1 immunohistochemistry in GIST ( $\times 40$ ). A, Score 0. B, Score 1. C, Score 2. D, Score 3. E, Strong membranous staining in an epithelioid GIST. F, Strong cytoplasmic staining in a spindle cell GIST.

## Antibody Generation

The peptides sequences to generate the DOG1.1 and DOG1.3 monoclonal antibodies were MSDFVDWVIP DIPKDISQQIHKEKVLVVELFMREEQDKQQLLETCE MEKER QKDEPPCNHHNTKACPDSLGSPPASHAYH GGVL and KVDYILVYHH KRPSGNRTLVRVQHS DTPSGARSVKQDHLPLPGKGASLDAGSGEPPMDYHE DDKRFRREEYEGNLLLEAGLELERDEDTKIHGVGF VKIHAP, respectively. These peptides were injected intraperitoneally with Freund complete adjuvant into a mouse and boosted 4 times weekly. Splens were taken for hybridoma preparation as described by Köhler and Milstein.<sup>7,16</sup>

The fused cells were distributed in 96-well plates and the antibody producing wells were detected by enzyme-linked immunosorbent assay using DOG1 peptide-coated plates. Positive wells were next screened by immunohistochemistry on a mini-TMA containing 5 cases of GIST and 2 cases of leiomyosarcoma.

## RESULTS

### Clinicopathologic Features of the GIST Cases

All but 2 of the 447 cases of GIST cases were primary tumors. The 2 exceptions were a liver metastasis and an abdominal metastasis of GIST (Table 2). In the cases with available clinicopathologic information, the features were typical of GIST. The median age of the patients was 59 years (range, 3 to 95 y) and the majority of the patients were over 60 years old. There was equal sex distribution and the tumor size ranged from 0.4 to 32 cm (median, 6.5 cm). The most common location for the

**TABLE 2.** Clinicopathologic Features of GIST Cases

Total No. Patients	447
Age (n = 341; 3-95 y; median: 59)	
< 18 y	9 (3%)
≥ 18 to < 40 y	31 (9%)
≥ 40 to < 60 y	139 (41%)
≥ 60 y	162 (48%)
Sex (n = 357)	
Female	179 (50%)
Male	178 (50%)
Size (n = 303)	
≤ 2 cm	21 (7%)
> 2 to ≤ 5 cm	94 (31%)
> 5 to ≤ 10 cm	96 (32%)
> 10 cm	92 (30%)
Location (n = 337)	
Stomach	179 (53%)
Small bowel	101 (30%)
Large bowel	27 (8%)
EGIST	24 (7%)
Esophagus	3 (1%)
Gallbladder	3 (1%)
Risk (n = 70)	
Very low	5 (7%)
Low	30 (43%)
Intermediate	10 (14%)
High	25 (36%)

EGIST indicates extragastrointestinal GIST.

tumor in this series was stomach (53%), followed by small bowel (30%), large bowel (8%), nongastrointestinal (7%), esophagus (1%), and gallbladder (1%).

### DOG1 Staining in GIST in Comparison With KIT and CD34

DOG1.1 reactivity was seen in 370 GISTs cases (370/425; 87%), whereas the expression of KIT and CD34 was found in 317 (317/428; 74%) and 254 cases (254/430; 59%), respectively. In the majority of cases, immunohistochemistry with DOG1.1 resulted in strong staining involving the majority of tumor cells [282 GIST with strong staining, score 3(66%) and 88 GIST with moderate staining, score 2 (21%)]. Examples of different staining intensities are shown in Figures 1A to D. The predominant staining pattern of DOG1 was membranous. This staining pattern was most evident in the epithelioid GIST cases, whereas in the spindle cell cases the membranous staining was often accompanied by cytoplasmic staining (Figs. 1E, F). This staining pattern is in keeping with the DNA sequence for DOG1/TMEM16, which predicts 8 transmembrane regions.

The reactivity of DOG1.1, KIT, and CD34 with GISTs containing a mutation in the *KIT* gene was 92% (200/218), 81% (180/221), and 64% (142/224), respectively. In the wild-type GISTs, DOG1.1 was expressed in 33 of 37 cases (89%), KIT in 29 of 35 cases (83%), and CD34 in 20 of 38 cases (51%). DOG1.1 expression was not related to the type of mutation (Table 3A), site, or size of the tumor (Table 3B), the grade of the tumor, or the age of the patient. The DOG1.3 monoclonal antibody showed a very similar reactivity in KIT mutation positive and WT-GIST, of 88% (197/223) and 84% (31/37) of cases, respectively. Overall, only 6 GIST cases that were negative for DOG1.1 were positive for DOG1.3 and 3 of these were positive for KIT. The DOG1.3 therefore gave a minimal increase in the number of cases recognized as GIST and was omitted from further studies.

Importantly, in GIST with *PDGFRA* mutations, DOG1.1 was positive in 23 of 29 scorable GISTs (79%), whereas KIT was positive only in 3 of 32 cases (9%). CD34 was positive in 9 of 33 cases (27%) (Figs 2, 3). In contrast, the other monoclonal antibody against DOG1, DOG1.3, was positive in only 12 of 29 (41%) cases with *PDGFRA* mutations. Among 19 *PDGFRA*-mutant GIST that were positive for DOG1.1 and had failed to react for KIT, 7 harbored mutations predicted to be sensitive to imatinib<sup>4</sup>: 4 exon 12 mutations [V561D (2 cases), InsER561-562 (1 case), and SPDGHE566-571R (1 case)], and 3 exon 18 mutations (all Del DIMH842-845). Eleven of the 19 cases had exon 18 mutations [D842V (10 cases) and Del HDSN844-848P (1 case)] and 1 case had a mutation in exon 12 (Del RV560-561). These confer imatinib resistance; nevertheless, the findings suggest that DOG1.1 can identify KIT-negative GIST that may respond to kinase inhibitor therapy.

Data on *PDGFRA* expression in GIST are scant because of the absence of a reliable antibody for *PDGFRA* in paraffin-embedded tissue. By in situ

**TABLE 3.** Staining Results for KIT, CD34, and DOG1.1 Based on Genotype (A) and Primary Tumor Location (B)

	DOG1.1	KIT	CD34	DOG1.1/KIT/CD34
<b>A</b>				
KIT exon 11 (n = 207)	180/197 (91%)	158/196 (81%)	129/200 (65%)	183/189 (97%)
KIT exon 9 (n = 20)	16/16 (100%)	17/19 (89%)	10/18 (61%)	15/15 (100%)
KIT exon 13 (n = 6)	4/5 (80%)	5/6 (83%)	3/6 (50%)	5/5 (100%)
PDGFRA exon 18 (n = 26)	18/23 (78%)	3/24 (13%)	8/26 (31%)	18/22 (82%)
PDGFRA exon 12 (n = 7)	5/5 (100%)	0/7 (0%)	1/6 (17%)	5/5 (100%)
PDGFRA exon 14 (n = 1)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
WT (n = 39)	33/37 (89%)	29/35 (83%)	20/38 (53%)	31/33 (94%)
Unknown (n = 141)	112/139 (81%)	104/138 (75%)	81/133 (61%)	118/133 (89%)
<b>B</b>				
Stomach (n = 179)	148/169 (88%)	120/169 (71%)	140/174 (80%)	157/164 (96%)
Small bowel (n = 101)	88/97 (91%)	78/95 (82%)	28/95 (29%)	85/88 (97%)
Large bowel (n = 27)	21/27 (78%)	18/27 (67%)	16/27 (59%)	21/27 (78%)
EGIST (n = 24)	16/21 (76%)	12/23 (52%)	14/23 (61%)	17/20 (85%)
Esophagus (n = 3)	1/2 (50%)	2/3 (67%)	3/3 (100%)	2/2 (100%)
Gallbladder (n = 3)	2/3 (67%)	2/3 (67%)	2/3 (67%)	2/3 (67%)
Unknown (108)	93/104 (89%)	84/106 (79%)	50/103 (49%)	91/99 (92%)

EGIST indicates extragastrointestinal GIST.

hybridization on the current set of TMAs, we found results similar to those reported by West et al.<sup>31</sup> The majority of the *PDGFRA*-mutant GISTs were positive for *PDGFRA* expression by in situ hybridization (24/32; 75%). *PDGFRA* expression was also seen in 37 GIST with mutations in the *KIT* gene. In our prior study, we showed that other sarcomas like leiomyosarcomas (LMS), undifferentiated sarcomas, synovial sarcoma (SS), and liposarcomas also express *PDGFRA* by in situ hybridization.<sup>32</sup> *PDGFRA* expression therefore is not a specific marker for GIST.

### DOG1.1 Staining on Other Soft Tissue Tumors

A variety of soft tissue tumors fall within the morphologic differential diagnosis of GIST. These include smooth muscle tumors, nerve sheath tumors, desmoid fibromatosis, undifferentiated sarcomas, inflammatory pseudotumors, solitary fibrous tumor, melanoma, SS, dedifferentiated liposarcoma, and dermatofibrosarcoma protuberans (Table 4A). DOG1.1 was expressed in 1/326 LMS (0.3%), 1/39 SS (2.5%), and in 1 desmoplastic

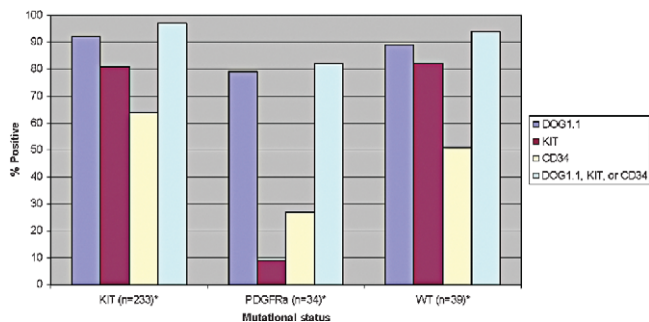
melanoma (1/10; 10%). In contrast, expression of KIT was found in 3/331 LMS (0.9%), 1/87 undifferentiated sarcomas (1.1%), and in 1 desmoplastic melanoma (1/10; 10%). CD34 was more widely expressed. It was found in 5 LMS (1.5%), 9 undifferentiated sarcomas (11%), 11 malignant peripheral nerve sheath tumors (13%), 38 neurofibromas (69%), 1 SS (2.2%), 4 schwannomas (9%), 12 solitary fibrous tumor (60%), 15 dermatofibrosarcoma protuberans (88%), 3 dedifferentiated liposarcomas (37%), and 1 desmoplastic melanoma (10%).

### DOG1 Staining in Nonsarcomatous Tumors

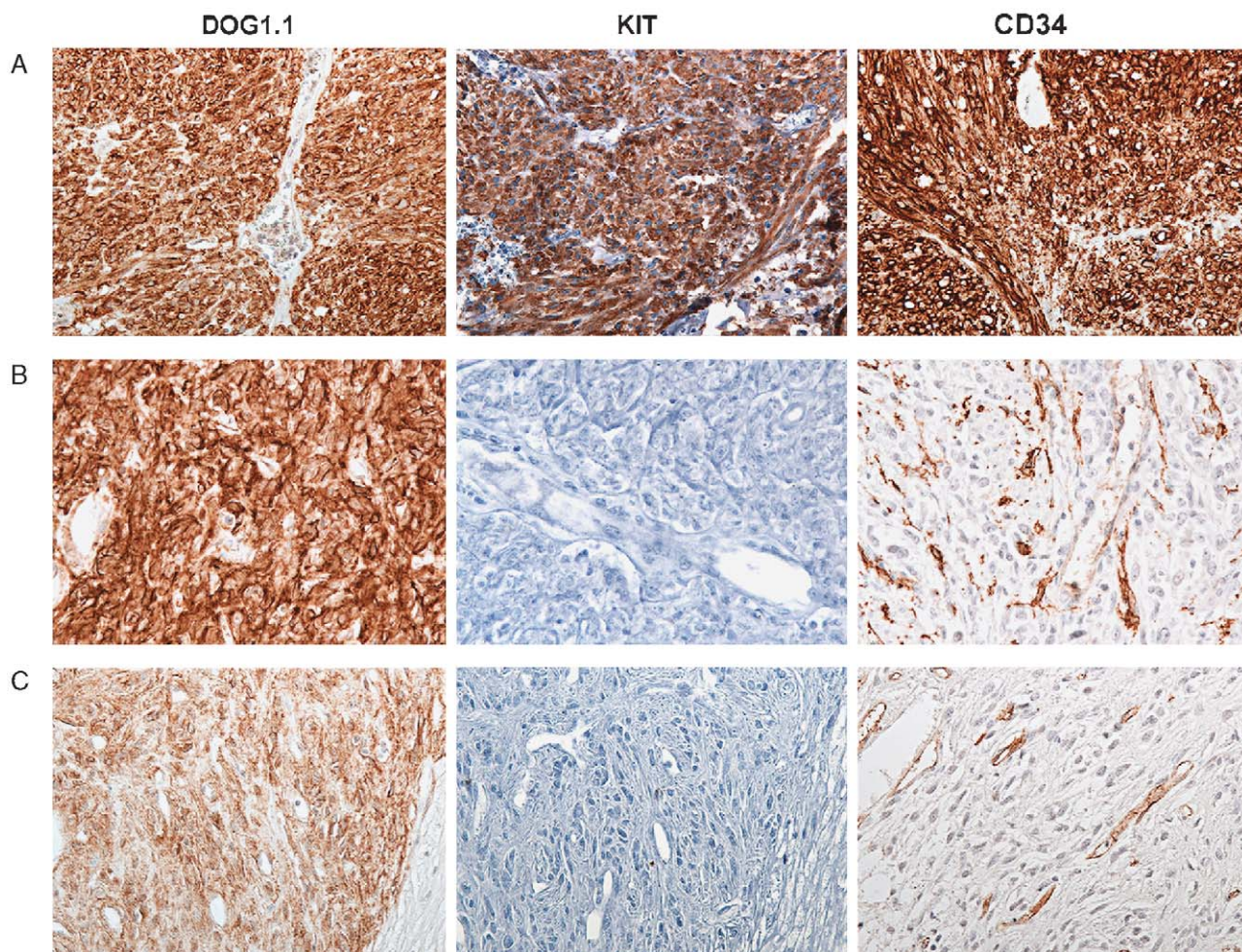
We compared the reactivity of DOG1.1 on 432 cases of nonsarcoma lesions and compared it with staining results obtained with KIT and CD34 antibodies. The TMA contained a wide variety of carcinomas, from 25 primary sites and a limited number of lymphomas, brain tumors, and other types of tumors. Within all tumor types, only rare cases with DOG 1.1 reactivity were seen, summarized in Table 5 (a complete table of the staining results is available as Web supplement Table 1). Most of the cases were negative for DOG1.1. In contrast, occasional cases of liver, pancreas, kidney, bladder, endometrial, and other carcinomas stained for KIT. As reported previously by others<sup>18,20</sup> a significant number of seminomas (18/21) and melanomas (8/21) stained for KIT, whereas only 1 desmoplastic melanoma and no seminomas were positive for DOG1.1. This is clinically relevant as melanomas and retroperitoneal seminomas can be part of the differential diagnosis for GIST.

### DOG1 mAb Staining in Interstitial Cells of Cajal in Non-neoplastic Gastrointestinal Tract

In non-neoplastic esophagus, stomach, small bowel, and colon, the staining obtained with DOG1.1 was highly similar to that seen with KIT antiserum. In the small bowel (Fig. 4), the DOG1.1 and KIT positive cells were



**FIGURE 2.** Immunohistochemical results for KIT, CD34, and DOG1.1 in KIT or *PDGFRA* mutation positive and KIT/*PDGFRA* mutation negative (WT) GISTs.



**FIGURE 3.** Patterns of DOG1.1, KIT, and CD34 in GISTs with different genotypes ( $\times 40$ ). A, KIT exon 11 mutant with expression of DOG1.1, KIT, and CD34. B, KIT exon 11 mutant with expression of DOG1.1, and negative for KIT and CD34. C, PDGFRA exon 18 mutant with expression of DOG1.1 and negative for KIT and CD34.

located in the myenteric plexus, between the circular and longitudinal muscle layer. These cells possess numerous thin cytoplasmic processes and form a cellular network around the ganglion cells of the myenteric plexus. However, in contrast to DOG1 mAb, KIT pAb also stained numerous mast cells and the basal portion of the gastric epithelium, as reported previously.<sup>1,20</sup> This is in contrast with our experience with the original DOG1 rabbit antiserum, where mast cells reacted as well.<sup>31</sup> A TMA with 31 different normal human tissues failed to show DOG1 reactivity other than that described in the myenteric plexus.

### DISCUSSION

With the recent development of effective targeted therapies for GIST,<sup>5</sup> the correct diagnosis of these tumors has a considerable clinical impact. Most GIST can be identified based on the combination of tumor location, histologic appearance, and the presence of KIT by

immunohistochemistry.<sup>8</sup> However, in a significant proportion of GISTs (~4% to 15%), KIT expression is equivocal or negative, leaving the diagnosis in question.<sup>23</sup> Screening for *KIT* and *PDGFRA* mutations can be helpful in this setting, but this approach adds to the time and cost of diagnosis and is better reserved for use in decisions concerning kinase inhibitor therapy. What is needed to aid in routine diagnosis is a marker that reliably stains GIST that are KIT-weak/negative.

DOG1 is a protein of unknown function that was found to be selectively expressed in GIST using gene expression profiling.<sup>31</sup> The *DOG1* gene (aka *TMEM16*), is localized on the chromosome 11 (11q13). It contains 26 exons and encodes for a 960 amino acid protein with an expected size of 114Kb. On the basis of DNA sequence analysis, the protein has 8 transmembrane domains. Its function is unknown but the high number of transmembrane regions suggest that it may be an ion channel.<sup>2,14</sup> Human DOG1 protein shows homology with other proteins including TMP16B

**TABLE 4.** Immunohistochemical Results of KIT, DOG1.1, and CD34 With Lesions in the Differential Diagnosis of GIST (A) and in Others Tumors (B)

	DOG1.1	KIT	CD34
<b>A</b>			
<b>Differential diagnostic tumors (n = 775)</b>			
Leiomyosarcoma (n = 334)	1/326 (0.3%)	3/331 (0.9%)	5/334 (1.5%)
Undifferentiated sarcoma (n = 87)	0/79 (0%)	1/87 (1.1%)	9/85 (11%)
Malignant peripheral nerve sheath tumor (n = 86)	0/85 (0%)	0/86 (0%)	11/83 (13%)
Neurofibroma (n = 55)	0/53 (0%)	0/55 (0%)	38/55 (69%)
SS (n = 44)	1/39 (2.5%)	0/44 (0%)	1/44 (2.2%)
Schwannoma (n = 46)	0/44 (0%)	0/45 (0%)	4/44 (9%)
Desmoid fibromatosis (n = 35)	0/35 (0%)	0/32 (0%)	0/31 (0%)
Leiomyoma (n = 22)	0/22 (0%)	0/21 (0%)	0/22 (0%)
Solitary fibrous tumor (n = 21)	0/20 (0%)	0/21 (0%)	12/20 (60%)
Dermatofibrosarcoma protuberans (n = 20)	0/20 (0%)	0/20 (0%)	15/17 (88%)
Dedifferentiated liposarcoma (n = 10)	0/9 (0%)	0/10 (0%)	3/8 (37%)
Desmoplastic melanoma (n = 10)	1/10 (10%)	1/10 (10%)	1/10 (10%)
Inflammatory pseudotumor (n = 5)	0/4 (0%)	0/5 (0%)	0/5 (0%)
<b>B</b>			
<b>Other tumors (n = 160)</b>			
Angiosarcoma (n = 14)	0/14 (0%)	1/11 (9%)	7/11 (64%)
Osteosarcoma (n = 14)	0/14 (0%)	0/14 (0%)	0/14 (0%)
Endometrial stroma sarcoma (n = 13)	0/13 (0%)	0/12 (0%)	0/13 (0%)
Rhabdomyosarcoma (n = 13)	0/12 (0%)	0/13 (90%)	1/13 (8%)
Epithelioid hemangioendothelioma (n = 12)	0/12 (0%)	3/12 (25%)	4/12 (33%)
Well-differentiated liposarcoma (n = 11)	0/11 (0%)	0/9 (0%)	0/8 (0%)
Extraskeletal myxoid chondrosarcoma (n = 11)	0/11 (0%)	6/11 (54%)	0/11 (0%)
Low-grade fibromyxoid sarcoma (n = 11)	0/11 (0%)	0/11 (0%)	0/11 (0%)
Fibroadenomas (n = 11)	0/11 (0%)	0/11 (0%)	9/11 (82%)
Myxoid liposarcoma (n = 10)	0/10 (0%)	0/10 (0%)	0/9 (0%)
Ewing/PNET (n = 10)	0/10 (0%)	1/10 (10%)	0/9 (0%)
Clear cell sarcoma (n = 7)	0/7 (0%)	1/7 (14%)	0/7 (0%)
DSRCT (n = 7)	0/6 (0%)	1/7 (14%)	0/7 (0%)
Pleomorphic liposarcoma (n = 3)	0/3 (0%)	0/3 (0%)	0/3 (0%)
Epithelioid sarcoma (n = 3)	0/3 (0%)	0/3 (0%)	1/3 (33%)
Fibrosarcoma (n = 3)	0/3 (0%)	0/3 (0%)	0/3 (0%)
Chondrosarcoma (n = 3)	0/3 (0%)	0/3 (0%)	0/3 (0%)
Alveolar soft part sarcoma (n = 2)	0/2 (0%)	2/2 (100%)	0/2 (0%)
Phyllodes tumor (n = 2)	0/2 (0%)	0/2 (0%)	0/2 (0%)

DSRCT indicates desmoplastic small round cell tumor; PNET, primitive neuroectodermal tumor.

(79%), TMP16E (57%), TMP16C (57%), TNP16F (56%), the gene encoding for hypothetical protein C691.05C (44%), and TMP16H (41%), but shows no homology at the DNA or amino acid level with KIT. The human DOG1 protein has a high degree of homology with the mouse DOG1 protein (89%). The 11q13 locus is amplified in several cancers: head and neck squamous cell carcinoma, bladder tumors, and breast cancer, but we failed to demonstrate DOG1 expression in these tumors by immunohistochemistry.

The rabbit serum used in our previous studies proved difficult to generate in additional rabbits, despite repeated immunization attempts with the original DOG1 peptide. We therefore generated 2 monoclonal antibodies (DOG1.1 and DOG1.3) against different peptide sequences of DOG1. Both antibodies showed high specificity in the immunostaining of GIST. In particular, the DOG1.1 monoclonal antibody has potential for clinical use in the routine diagnosis of GIST.

Among GIST cases with *KIT* mutations, most of which are imatinib sensitive, the DOG1.1 antibody

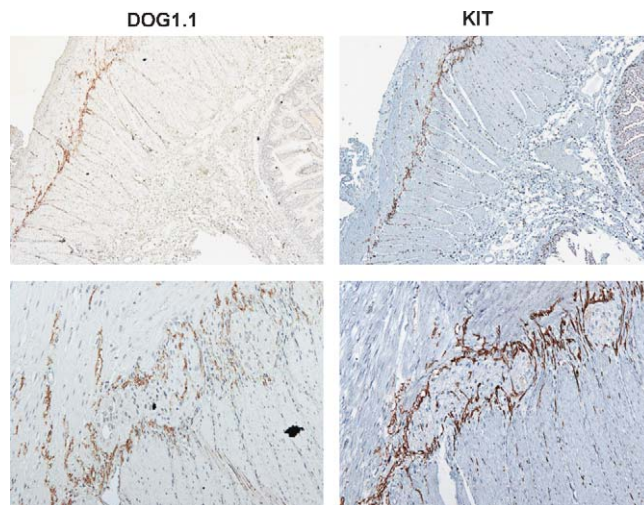
identified 11% more cases than KIT. GIST cases with mutations in *PDGFRA* differ in their gene expression profile from KIT-mutant GIST,<sup>27</sup> are often weak or negative for KIT by immunohistochemistry, and tend to have an epithelioid morphology.<sup>19,24,29–31</sup> As such, they are more likely to be misdiagnosed for epithelial neoplasms. In our study, only 9% of *PDGFRA*-mutant GIST (3/32) were positive for KIT, whereas DOG1.1 was positive in the majority (23/29; 78%). Moreover, a third of the *PDGFRA*-mutant GIST that were DOG1+ and KIT– harbored mutations predicted to be imatinib sensitive.<sup>4</sup> The lack of KIT staining in these cases might otherwise consign them to a non-GIST diagnosis, potentially denying patients the opportunity to be treated with imatinib.

The high number of KIT-negative GIST in our study (26%) almost certainly reflects referral bias. Many of the tumors were received in consultation because of the absence of KIT expression and were subjected to mutational analyses to substantiate the suspected diagnosis of GIST.

**TABLE 5.** Nonsarcoma Neoplasms Positive for DOG1.1 (A) and KIT (B)

A		
Organ	Tumor	DOG1.1+
Liver	Hepatocellular carcinoma	1/4
Salivary gland	Basal cell adenoma	1/1
	Adenoid cystic carcinoma	1/7
Lung	Adenocarcinoma	1/8
Skin	Desmoplastic melanoma	1/10
B		
Organ	Tumor	KIT+
Breast	Ductal carcinoma	1/9
Pancreas	Mucinous cystic tumor	1/8
	Papillary cystic tumor	1/1
Adrenal gland	Neuroblastoma	1/1
Kidney	Clear cell carcinoma	1/4
	Papillary renal cell carcinoma	1/4
	Chromophobe carcinoma	4/4
	Oncocytoma	2/2
	Transitional cell carcinoma from the renal pelvis	1/4
	Neuroblastoma	1/2
Colon	Adenoma	2/3
Ovary	Dysgerminoma	1/1
	Yolk sac tumor	1/1
Urinary bladder	Transitional cell carcinoma	3/14
	Adenocarcinoma	1/3
Uterus	Endometrial carcinoma	2/7
Uterine cervix	Adenocarcinoma	1/5
Testis	Seminoma	18/21
	Yolk sac tumor	1/2
	Teratoma	1/2
	Mixed germ cell tumor	3/6
Skin	Merkel cell carcinoma	3/3
	Melanoma (nondesmoplastic)	8/21
	Desmoplastic melanoma	1/10
	Oligodendroglioma	2/2
Brain	Medulloblastoma	2/3
	Ependymoma	1/2
	Esthesioneuroblastoma	1/1
	Adenocarcinoma	1/4
Duodenum	Oncocytoma	2/2
Salivary gland	Warthin tumor	1/2
	Adenoid cystic carcinoma	6/6
Lung	Adenocarcinoma	1/8
	Squamous cell carcinoma	1/5
	Small cell carcinoma	2/3
	Low-grade mucoepidermoid carcinoma	1/1
	Adenoid cystic carcinoma	1/1
	Carcinoid tumor	1/1
Thyroid gland	Papillary carcinoma	2/3
	Follicular carcinoma	1/1
Lymph node	Follicular adenoma	1/2
	Diffuse large B-cell lymphoma	1/3

One of the principal differential diagnoses in GIST is leiomyosarcoma. In our series of LMS cases used for this study, there were 3 cases that stained for DOG1.1. The first case (no. 832) was initially diagnosed as LMS of the lung; however, additional molecular analysis performed after DOG1.1 staining was positive showed a point mutation in exon 18 of *PDGFRA* (D842V), confirming a diagnosis of GIST. No additional clinical

**FIGURE 4.** DOG1.1 and KIT show similar staining patterns consistent with ICC staining in the small bowel.

history was available and we interpreted this case as either an extragastrointestinal GIST or a metastasis from a clinically silent intestinal GIST. The second case (no. 10057) was initially diagnosed as a LMS metastatic to liver. Sequence analysis failed to show a mutation in either the *KIT* or *PDGFRA* genes. However, the tumor was immunopositive for CD34 and KIT and the patient had a gastric primary. This case was reclassified as a wild-type GIST. The third case (no. 10180) was considered to be a true LMS, as no mutation in *KIT* or *PDGFRA* was found and the tumor was from the thigh. As a result, DOG1.1 was positive in only 1 case of LMS (1/324; 0.3%). DOG1.1 also stained 1 case of desmoplastic melanoma and 1 case of SS. The diagnosis of SS was confirmed by fluorescence in situ hybridization and by staining for TLE1.<sup>28</sup> The desmoplastic melanoma was from the skin and expressed both S100 and HMB45.

GIST are believed to originate from the interstitial cells of Cajal (ICC) or their stem cell precursor.<sup>15,25</sup> ICC express KIT and are localized to the myenteric plexus and in the muscular layers throughout the gastrointestinal tract.<sup>17</sup> In the normal gastrointestinal tract, the pattern of expression of DOG1 was very similar to KIT, supporting the idea that DOG1 is also expressed in the ICC.

In conclusion, we have demonstrated that DOG1 is a very sensitive and specific marker for GIST that works in paraffin-embedded tissue and is highly expressed in *KIT*-mutant and *PDGFRA*-mutant GIST. The use of DOG1.1 in clinical practice as either a backup to KIT or as a part of a panel can allow the identification of more GIST cases. In our study, 63 patients (DOG1.1+ KIT-) would merit from this approach. These results have an important clinical implication because most GIST patients can be benefited from the imatinib treatment. As a result of its localization in the cell membrane, its absence in the majority of normal tissue (with the exception of the myenteric plexus) and the presence in

most of the GIST, DOG1 may be an additional target in the treatment of GIST.

### Supplementary Data

Supplementary data are available at *The PAS Journal* Online ([http://tma.stanford.edu/tma\\_portal/DOG1\\_mcab](http://tma.stanford.edu/tma_portal/DOG1_mcab)).

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