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Is *DOG1* Immunoreactivity Specific to Gastrointestinal Stromal Tumor?

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Background: DOG1 *is a novel gene on gastrointestinal stromal tumors (GISTs) that encodes the chloride channel protein anoctamin 1, also known as discovered on GIST-1 (DOG1) protein. DOG1 antibodies are a sensitive and specific marker against GIST positive for CD117 and CD34 and negative for CD117 and CD34.* DOG1 *is also independent of* KIT *or* PDGFRA *mutation status and considered specific for GIST when it was first discovered in 2004.*

Methods: *The previous 10 years of literature was searched for articles relating to* DOG1. *We critically reviewed 12 studies that showed* DOG1 *was positive in 250 cases of 2,360 tested non-GIST neoplasms (10.6%) at different anatomical sites using monoclonal, polyclonal, or nonspecified antibodies. Criteria for positivity varied between the studies.*

Results: *Monoclonal and polyclonal DOG1 antibodies were reactive in various different non-GIST tumor types spanning 9 organ systems in addition to normal salivary and pancreatic tissues. The tumors included were renal oncocytoma (100%), renal cell carcinoma chromophobe type (86%), solid pseudopapillary neoplasm of the pancreas (51%), neoplastic salivary tissue (17%), synovial sarcoma (15%), leiomyoma (10%), pancreatic adenocarcinoma (7%), and leiomyosarcoma (4%).*

Conclusions: *By contrast to the original concept that DOG1 antibodies are specific to GIST neoplasms, the studies reviewed showed that the data suggest* DOG1 *positivity in select non-GIST tumors. Only in the appropriate clinical and pathological context is* DOG1 *positivity specific and helpful in the diagnosis of GIST.*

Introduction

A 58-year-old woman who did not smoke was found to have a 1-cm left upper lobe lingula lung nodule discovered on a screening radiograph as part of a routine physical examination. The patient denied fever, chills, sweats, anorexia, or weight loss. She had a past medical history of thyroid nodule. Her family history was positive for hypertension, heart disease, and lung cancer. She was taking chlorthalidone for hypertension and ibandronate for osteoporosis as well as multivitamin supplements. Subsequent positron emission tomography showed the hypermetabolic uptake of fluorodeoxyglucose in the nodule with a standard uptake value of 7, which is suspicious for a neoplasm. She underwent wedge resection of this lung nodule. Gross examination showed a 1-cm, grey-tan, firm nodule 0.5 cm from the resection margin. Microscopic examination revealed sheets of oncocytic tumor cells with abundant pink, granular cytoplasm, ovoid nuclei, small nucleoli (Fig 1A), and negative surgical margins. Immunohistochemistry was strongly and diffusely positive for *DOG1* (Fig 1B) but negative for CD10, CD34, CD117 (C-kit), CD163, cytokeratin (CK) 7, CK20, CK AE1/AE3, thyroid transcription factor 1, epithelial membrane antigen, renal cell carcinoma, thyroglobulin, and *ERG*.

Based on these findings, a preliminary diagnosis of epithelioid gastrointestinal stromal tumor (GIST) was made. Magnetic resonance imaging of the abdomen was obtained and was within normal limits. The patient came to the H. Lee Moffitt Cancer Center & Research Institute (Tampa, FL) for treatment.

After performing a thorough history and physical examination and reviewing her imaging and pathology findings, her physician indicated that the findings were inconsistent with a diagnosis of GIST. Criteria for diagnosing GIST are reviewed in Table 1.[1] After our pathological review of the case, the *DOG1* immunostain (SP31 clone, prediluted via a dispenser) was repeated and it was diffusely but weakly positive (Fig 1C).

Is This Gastrointestinal Stromal Tumor?

To practice evidence-based medicine, it is necessary to work-up the case in a systemic approach. The steps include:

1. Clinical and radiological correlation with pathology findings: In this case, the overall his-

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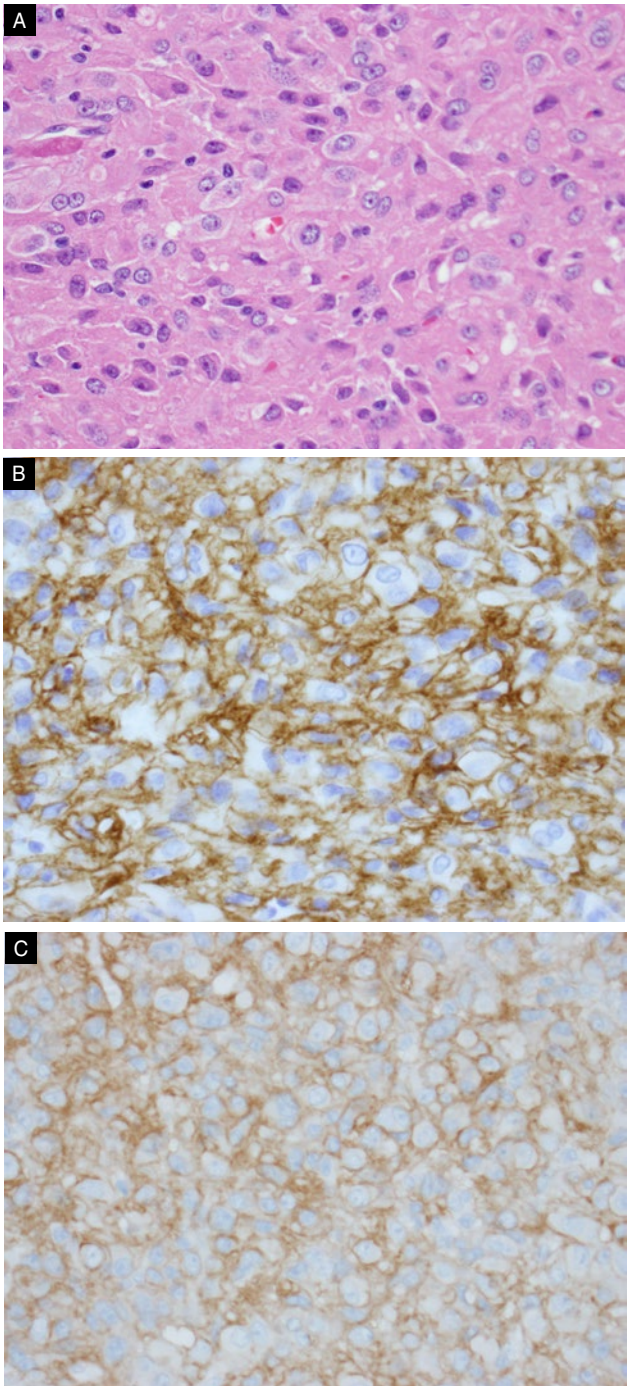

Fig 1A–C. — (A) Hematoxylin and eosin stain showing an epithelioid neoplasm (× 400). (B) *DOG1* (K9 clone) IHC stain showing strong membranous positivity (× 400). (C) *DOG1* (SP31 clone) IHC stain showing weak membranous positivity (× 400). IHC = immunohistochemistry.

Table 1. — Diagnostic Criteria of GIST

Site of Involvement	Stomach: 54%
	Small intestine and duodenum: 32%
	Colon and rectum: 1%
	Others: 9%
Histology	Spindle cell tumor, in most
	Epitheloid: 20%–25%
	Mixed spindle and epithelial cells
Differential Diagnosis	Smooth muscle tumor
	Nerve sheath tumor
	Other spindle or epitheloid tumor
Immunophenotype	CD117 (C-kit) strongly positive, except in 5% of GIST cases with mutant *PDGFRA*
	CD34 is positive in most cases of spindle-cell GIST; less consistent in epitheloid histology
	DOG1 is positive in most cases of GIST ($CD117^+/DOG1^+$); often positive in $CD117^-$ GIST ($CD117^-/DOG1^+$)
When to Diagnose GIST?	CD117 positivity in context with correct clinical and pathological setting
	DOG1 more sensitive than CD117 in detecting GIST of gastric origin, epitheloid morphology, and GIST harboring *PDGFRA* mutation
	CD34 alone not used to diagnose GIST

GIST = gastrointestinal stromal tumor.

From Ríos-Moreno MJ, Jaramillo S, Pereira Gallardo S, et al. Gastrointestinal stromal tumors (GISTs): CD117, DOG-1 and PKCθ expression. Is there any advantage in using several markers? *Pathol Res Pract.* 2012;208(2):74-81.

tological features are not typical for epithelioid GIST; however, the immunoreactivity of *DOG1* was puzzling.[2] Primary GIST of the pleura has been reported in a single case report[2]; however, primary GIST originating in the lung has not been reported.

2. Review the literature and investigate if *DOG1* immunoreactivity is specific for GIST alone and what other tumors are positive for *DOG1* and could be a potential diagnosis: The literature review revealed an interesting result, and our findings are included below so other pathologists may find them useful when dealing with tumors and GISTs positive for *DOG1*.
3. Conduct judicious ancillary testing to rule in or out differential diagnoses (eg, carcinoma, melanoma, granular cell tumor, and epithelial sarcoma).
4. If in doubt, seek an expert opinion.

After pertinent differential diagnoses were ruled out, we felt this was not a case of epithelioid GIST, but rather a primary lung tumor that warranted further subtyping. Christopher D.M. Fletcher, MD, at Brigham & Women's Hospital (Boston, MA) was consulted. Other pertinent stains as well as repeat immunostains were negative for *DOG1*. The tumor was deemed to be an atypical epithelioid neoplasm of the lung; however, this entity is not an established World Health Organization classification but instead is a descriptive diagnosis that reflects the uncertain histogenesis, clinical behavior,

and our current scientific understanding of the lesion. The recommended management is judicious follow-up. At the time of publication, the patient is under surveillance.

DOG1 has shown rates of high sensitivity and specificity in the detection of GISTs, and 74% of GISTs exhibit a positive *DOG1* and CD117 immunoprofile.[3] Discovered on GIST-1 (*DOG1*) protein antibodies are more sensitive than CD117 antibodies in detecting tumors of gastric origin, epithelioid tumors, and tumors harboring *PDGFRA*.[4] Although *DOG1* positivity is generally required for the diagnosis of GIST, the results should be interpreted alongside morphological findings of the tumor and the clinical picture, as shown in the current case. From this, we intend to explore the immunoreactivity of *DOG1* in non-GIST neoplasms to provide useful information for practicing pathologists and other health care professionals.

Methods

A search of the English-speaking medical literature was conducted to identify original articles published from 2004 to 2015 regarding the use of the DOG1 antibody for the pathological diagnosis on formalin-fixed and paraffin-embedded human tissue. We reviewed the relevant studies to examine the results of the clones of DOG1 antibodies as well as immunoreactive and tissue types. The clone refers to DOG1 antibodies made by identical immune cells copies of a unique parent cell (K9, SP31, DOG1.1). These clones were developed from mice and rabbits. Documentation of the findings, a critical review of the results, and the application of this information in the work-up of the index case are discussed.

Results

Antibodies

Twelve relevant studies were identified. The DOG1 antibody used in these studies included monoclonal (SP31, K9, DOG1.1), polyclonal, or nonspecified antibodies. Criteria for positivity varied among the studies.

DOG1 *Expression*

DOG1 is expressed in normal salivary acini gland and pancreatic endocrine tissue (Table 2).[5,6] Table 3 summarizes *DOG1* expression in 250 cases out of 2,360 non-GIST neoplasms tested at different anatomical sites.[5,7-17] Of the 2,360 tumors tested, the average rate of *DOG1* positivity was 10.6%.

As illustrated in Fig 2,[12,14] *DOG1* is 100% positive in cases of renal oncocytoma and chondroblastoma[7,9]; 60% to 97% positive in acinic cell carcinoma, chromophobe renal cell carcinoma, fibroadenoma, adenoid cystic carcinoma, and squamous cell carcinoma[7,11,12,14]; 22% to 53% positive in epithelial-myoepithelial carcinoma, pseudopapillary neoplasm, neoplastic salivary tissue, endometrial adenocarcinoma, gastric adenocarcinoma, and glomus tumor[5,8,10-14]; 7% to 17% positive in cholangiocarcinoma, synovial sarcoma, colonic adenocarcinoma, leiomyoma, and pancreatic adenocarcinoma[8,10-12,14,15]; and 1% to 5% positive in leiomyosarcoma, angiosarcoma, Ewing sarcoma, malignant peripheral nerve sheath tumor, neuroendocrine tumor, melanoma, and schwannoma.[5,8,10-12,14-16] Reports regarding solid pseudopapillary neoplasm of the pancreas are conflicting.[6,8]

Except in cases of renal oncocytoma and chondroblastoma, the tumors mentioned above have various levels of *DOG1* expression. However, of the 2,360 cases, a total of 959 tumors, other than those listed in Table 3, are nonresponsive to *DOG1* immunohistochemistry. The list of these tumors is summarized in Table 4.[7-12,14]

Discussion

This first clue that this lung tumor may not be GIST is that primary GIST has never been reported.[2] If this case was the first discovered primary lung GIST, then molecular confirmation would have been warranted. GIST does not appear to metastasize to the lung, other than in the setting of many years of treatment with tyrosine kinase inhibitors. To the best of our knowledge, primary GISTs in the lung have not been documented in the English literature. The second clue lies in the specificity of *DOG1* immunoreactivity in GIST.

DOG1 was shown to be independent of the *KIT/PDGFRA* mutation status and was considered specific for GIST when it was first discovered in 2004.[18] In other words, *DOG1* should be tested when the tumor is of gastric origin, has epitheloid morphology, and in cases harboring the *PDGFRA* mutation. Data are emerging regarding the expression of *DOG1* in non-GIST tissue. Studies that have reported higher rates of *DOG1* specificity

Table 2. — *DOG1* IHC in Non-Neoplastic Non-GISTs

Study	Anti-*DOG1* Antibody Clone Type	Site	Tumor Type	*DOG1* Expression, n/N (%)
Chenevert[5]	*DOG1.1*	Salivary gland	Salivary tissue	109/109 (100)
Ardeleanu[6]	Polyclonal	Pancreas	Endocrine tissue	
			Adult	11/11 (100)
			Fetal	15/15 (100)

Representative of 135 cases.
GIST = gastroinestinal stromal tumor, IHC = immunohistochemistry.

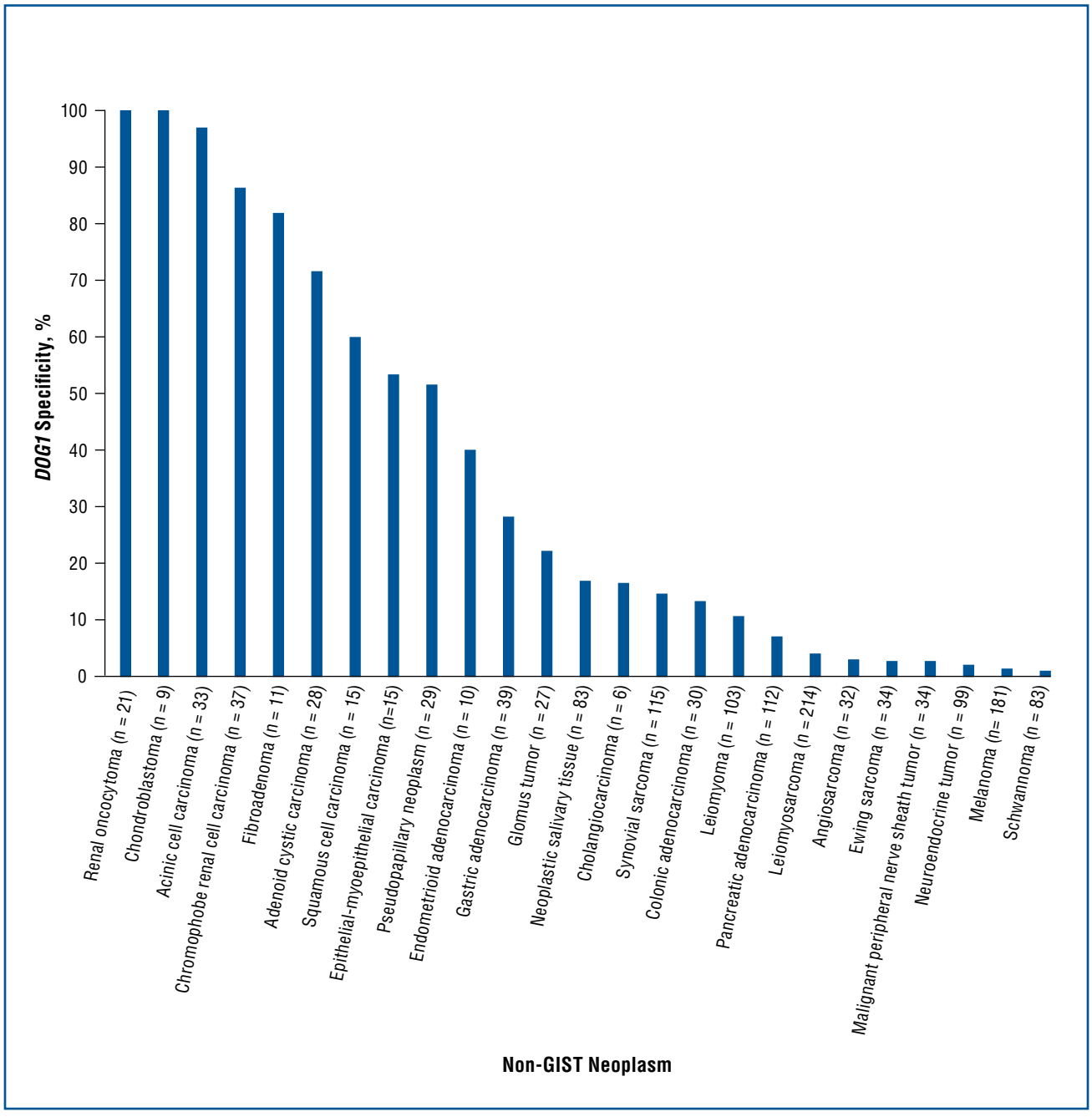

Fig 2. — *DOG1* tumor specificity rates. GIST = gastrointestinal stromal tumor.

have had small sample sizes, whereas larger studies (> 83 cases) have reported that a small percentage of non-GIST tumors were positive for *DOG1* (see Fig 2).[8,10-12,14,16]

As shown in Fig 2, neoplastic salivary gland may show *DOG1* expression. As shown in Table 3, the average *DOG1* specificity rate for neoplastic salivary tissue was 17%.[5,11,14] *DOG1* expression in synovial sarcoma was reported by 4 different studies to have various specificity rates.[10-12,14] The average rates of *DOG1* specificity for synovial sarcoma was 14.8%.

Gastric adenocarcinoma has 28% positivity for *DOG1* compared with 13% for colonic adenocarcinoma (see Fig 2).[12,14] Even though the location of these tumors may overlap with GIST, they rarely overplay histologically with GIST and do not pose diagnostic challenges.

Smooth muscle tumors were reported in 5 studies.[10-12,14,15] The average rates of *DOG1* specificity for leiomyoma and leiomyosarcoma were 10.7% and 4.2%, respectively (see Fig 2). When the smooth muscle tumor occurs in the gastrointestinal tract, it may be diagnostically challenging to be differentiated from GIST. As shown in Fig 2, nerve sheath

Table 3. — *DOG1* IHC in Neoplastic Non-GISTs

Study	Anti-*DOG1* Antibody Clone Type	Site	Tumor Type	*DOG1* Expression, n/N (%)[a]
Zhao[7]	Not specified	Kidney	Renal oncocytoma	21/21 (100)
Hemminger[14]	K9		Chromophobe renal cell carcinoma	32/37 (86)
Akpalo[9]	SP31	Bone	Chondroblastoma	9/9 (100)
Wong[10]	K9		Glomus tumor	6/27 (22)
Lopes[11]			Ewing sarcoma	1/34 (3)
Miettinen[12]				
Bergman[13]	Not specified	Pancreas	Pseudopapillary neoplasm	15/29 (51)
Hemminger[8]	K9		Adenocarcinoma	8/112 (7)
			Neuroendocrine tumor	2/99 (2)
Chenevert[5]	*DOG1.1*	Head and neck Salivary gland	Acinic cell carcinoma	32/33 (97)
Lopes[11]	K9		Adenoid cystic carcinoma	20/28 (71)
Hemminger[14]			Epithelial-myoepithelial carcinoma	8/15 (53)
			Neoplastic salivary tissue	14/83 (17)
Lopes[11]	K9	Breast	Fibroadenoma	9/11 (82)
Wong[10]	*DOG1.1*/K9	Soft tissue	Synovial sarcoma	17/115 (15)
Sah[15]			Leiomyosarcoma	9/214 (4)
Miettinen[12]			Angiosarcoma	1/32 (3)
Hemminger[14]				
Miettinen[12]	K9	Esophagus	Squamous cell carcinoma	9/15 (60)
Sah[15]		Stomach	Adenocarcinoma	11/39 (28)
Wong[10]		Liver	Cholangiocarcinoma	1/6 (17)
Lopes[11]		Abdomen	Leiomyoma	11/103 (11)
Miettinen[12]	K9	Colon	Adenocarcinoma	4/30 (13)
Hemminger[14]				
Hemminger[14]	K9	Uterus (endometrium)	Endometrioid adenocarcinoma	4/10 (40)
Wong[10]		Central nervous system	Malignant peripheral nerve sheath tumor	1/34 (3)
Lopes[11]			Schwannoma	1/83 (1)
Miettinen[12]				
Hemminger[14]	K9/SP31	Skin	Melanoma	3/181 (2)
Gonzalez[16]				
Wong[10]				
Lopes[11]				
Miettinen[12]				
Tang[17]	K9	Prostate	Stromal sarcoma	1/1 (100)

Representative of 1,401 cases.
[a]Combined data from multiple studies.
GIST = gastrointestinal stromal tumor, IHC = immunohistochemistry.

tumors also have a small rate of *DOG1* positivity (< 5%). In these situations, other markers for GIST, such as CD117, CD34, or mutational analysis for CD117 and *PDGFR*, can be used to help definitively diagnose GIST.

Limitations

The various studies we reviewed used different antibodies and various scoring criteria for *DOG1* immunoreactivity. Although no specific practice guideline exists for the diagnosis of GIST, strong, diffuse, and membranous *DOG1* immunoreactivity was generally accepted for the diagnosis.[4] The heterogeneous result of *DOG1* immunoreactivity by various laboratories may present a challenge. For example, in our index case, *DOG1* was tested in 3 different laboratories with 2 different antibodies and yielded different results. If the *DOG1* immunostain was negative, then this lung mass would never have been diagnosed as GIST.

Conclusion

In addition to *DOG1* being detected in cases of gastrointestinal stromal tumor, *DOG1* positivity can be detected in other non-neoplastic and neoplastic tissue; however, only in the appropriate clinical and pathological

Table 4. — IHC Negative for *DOG1* in Neoplastic Non-GISTs

Study	Anti-*DOG1* Antibody Clone Type	Tumor Type	Case, n (N = 959)[a]
Zhao[7] Hemminger[14]	Not specified K9	Clear-cell renal cell carcinoma	35
Akpalo[9]	SP31	Chondromyxoid fibroma	12
Lopes[11]	K9/DOG1.1	Giant cell tumor	40
Wong[10]	K9	Dedifferentiated liposarcoma	27
Lopes[11]	K9/DOG1.1	Desmoid tumor	59
Miettinen[12]	K9	Desmoplastic small round cell tumor	16
Hemminger[14]	K9	Endometrial stromal sarcoma	28
		Extraskeletal myxoid chondrosarcoma	16
		Gastrointestinal ganglioneuroma	3
		Inflammatory fibroid polyp	67
		Inflammatory myofibroblastic tumor	23
		Kaposi sarcoma	24
		Neurofibroma	27
		Perivascular epithelioid cell tumor/angiomyolipoma	8
		Sarcomatoid carcinoma	6
		Small cell carcinoma	12
		Solitary fibrous tumor	49
Hemminger[8]	K9	Serous cystadenoma	28
Lopes[11]	K9/DOG1.1	Mesenchymal tumors other than GIST	261
Miettinen[12]	K9	Extramedullary myeloid tumor	5
Lopes[11]		Mastocytoma, skin	3
		Neuroblastoma	10
		Seminoma testicular	16
		Undifferentiated sarcoma	26
		Small intestine carcinoid tumor	6
		Undifferentiated sarcomatoid carcinoma	21
Hemminger[8]	K9	Alveolar soft-part sarcoma	3
Lopes[11]	K9/DOG1.1	Chordoma	5
		Clear cell sarcoma	5
		Epithelioid sarcoma	8
		Granular cell tumor	6
		Low-grade fibromyxoid sarcoma	8
		Lung adenocarcinoma	5
		Perineurioma	4
		Pleomorphic undifferentiated sarcoma	77
		Prostate adenocarcinoma	10

[a]Combined data from multiple studies.
GIST = gastrointestinal stromal tumor, IHC = immunohistochemistry.

context is *DOG1* positivity specific and helpful for diagnosing true cases of gastrointestinal stromal tumor.

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