

# KIT-negative gastrointestinal stromal tumor of the abdominal soft tissue : A clinicopathological and genetic study of 10 cases

Yamamoto, Hidetaka

Department of Anatomic Pathology, Pathological Sciences, Graduate School of Medical Sciences, Kyushu University

Kojima, Aya

Department of Anatomic Pathology, Pathological Sciences, Graduate School of Medical Sciences, Kyushu University

Nagata, Shigenori

Department of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases

Tomita, Yasuhiko

Department of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases

他

<https://hdl.handle.net/2324/25597>

---

出版情報 : American Journal of Surgical Pathology. 35 (9), pp.1287-1295, 2011-09. Lippincott Williams & Wilkins

バージョン :

権利関係 : (C) 2011 Lippincott Williams & Wilkins, Inc.

## **KIT-negative gastrointestinal stromal tumor of the abdominal soft tissue:**

### **A clinicopathological and genetic study of 10 cases**

*Hidetaka Yamamoto, M.D., Ph.D.,<sup>1</sup> Aya Kojima, M.D.,<sup>1</sup> Shigenori Nagata, M.D., Ph.D.,<sup>2</sup>*

*Yasuhiko Tomita, M.D.,<sup>2</sup> Ph.D., Satsuki Takahashi, M.D.,<sup>3</sup> Yoshinao Oda, M.D., Ph.D.<sup>1</sup>*

1. Department of Anatomic Pathology, Pathological Sciences, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

2. Department of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan

3. Department of Pathology, Hiraka General Hospital, Yokote, Japan

**Address correspondence and reprint requests to:** Hidetaka Yamamoto, M.D., Ph.D.

Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University,  
3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan

TEL: 81-92-642-6061      FAX: 81- 92-642-5968

e-mail: hidetaka @surgpath.med.kyushu-u.ac.jp

## **Acknowledgement**

This study was presented in part at the 100th annual meeting of the United States and Canadian Academy of Pathology (USCAP) in San Antonio, TX, February 26-March 4, 2011. This study was supported in part by Grants-in-Aid for Scientific Research from the Japan Society of the Promotion of Science, Tokyo, Japan (Grant Number 22790346; H. Yamamoto). The English in this article was edited by KN international (<http://www.kninter.com/>).

**Abstract**

Gastrointestinal stromal tumor (GIST) typically occurs in the gastrointestinal (GI) tract and expresses KIT protein associated with *KIT* or *platelet-derived growth factor receptor-alpha* (*PDGFRA*) gene mutation. Extragastrointestinal stromal tumors (EGISTs) are a minor subset of GIST that occurs in the soft tissue outside the GI tract, and in very rare cases, these tumors can be KIT-negative. We examined the clinicopathological and molecular characteristics of 10 cases of KIT-negative EGIST by using immunohistochemical staining and gene mutation analysis. The tumors occurred in the omentum (n=5), mesentery (n=2), retroperitoneum (n=1), pelvic cavity (n=1) and not-otherwise-specified regions of the abdominal cavity (n=1). They ranged from 4 to 33 cm (median 15 cm) in maximum diameter with relatively low mitotic counts (median 3.5 per 50 high-power-fields). Morphologically, most cases were of epithelioid cell (n=9) or mixed epithelioid and spindle cell (n=1) type, accompanied by variable amounts of myxoid stroma. By immunohistochemical staining, the tumors were positive for CD34 (80%), PKC theta (90%) and DOG1 (90%), but negative for KIT (0%). The majority of the examined cases (7/9 cases; 78%) had *PDGFRA* mutations in exon 12 (n=1) or exon 18 (n=6). One case (11%) had a mutation in *KIT* exon 11, and the remaining one had no mutation in either *KIT* or *PDGFRA*. Distant metastasis and local recurrence occurred in 1 (10%) and 2 (20%) patients, respectively, and adverse outcome was correlated with larger (>10 cm) tumor size and high mitotic counts (>5/50 high-power-fields).

Therefore, KIT-negative EGISTs can be characterized by preferential omental origin, epithelioid cell type, low mitotic activity and mutation of the *PDGFRA* gene, and these features are similar to those of KIT-negative gastric GIST. As KIT-negative EGIST should be considered a potential abdominal soft tissue neoplasm, immunohistochemical staining panel and molecular analysis are necessary not only to confirm the diagnosis but also to determine the therapeutic strategy.

**Key Words:** gastrointestinal stromal tumor, extragastrointestinal, KIT, PDGFRA, DOG1

## Introduction

Gastrointestinal stromal tumor (GIST) is a mesenchymal tumor occurring in the stomach and small intestine and more rarely in the large intestine and esophagus. Histologically, GISTs consist of spindle cells, epithelioid cells, or a mixture of both, and they typically express the KIT (c-kit) protein [19]. These tumors are thought to originate from, or to show differentiation toward the interstitial cells of Cajal (ICC), which are the pacemaker cells present in the gastrointestinal (GI) tract. GISTs frequently harbor a gain-of-function mutation of the *KIT* or *platelet-derived growth factor receptor-alpha (PDGFRA)* gene [8,9,14,21]. *KIT* exon 11 mutation is the most common type (accounting for about 70% of all GISTs), and a small subset of GISTs (5-10%) have a *PDGFRA* mutation at exon 12 or 18. A majority of GISTs with *PDGFRA* mutations are reported to be of gastric origin, epithelioid cell type, and to have the KIT-positive phenotype [13].

A minor subset (3-5%) of GISTs are negative KIT by routine immunohistochemical staining [18]. Such “KIT-negative” GISTs tend to be gastric primary, with epithelioid cell morphology and mutations of the *PDGFRA* gene [18, 27]. Furthermore, this type of GIST generally has low mitotic activity and relatively favorable prognosis.

Recent gene expression profiling studies have shown that Discovered on GIST-1 (DOG1) and Protein Kinase C (PKC) theta are constantly expressed in GIST [1,29]. In addition, these markers are expressed in both KIT-positive and KIT-negative GISTs, suggesting that they are highly

sensitive markers for the identification of GISTs, irrespective of the KIT expression level [5, 6, 12, 15, 23-25].

Although GISTs typically occur in the stomach and small intestine, similar KIT-positive tumors can occur in soft tissue outside the gastrointestinal tract, such as in the mesentery, omentum and retroperitoneum; these cases are classified as extragastrointestinal stromal tumors (EGISTs) [20, 26, 28, 31]. Our previous study revealed the presence of frequent *KIT* or *PDGFRA* gene mutations in EGISTs [31]. Since that study, we have encountered several cases of intra-abdominal soft tissue tumors lacking immunohistochemical expression of KIT but having an otherwise consistent morphology of EGIST. These tumors were considered to be the extragastrointestinal counterpart of KIT-negative GIST; however, the clinicopathological, phenotypic and genotypic features of such tumors have not been detailed to date. In this study, we aimed to characterize KIT-negative EGIST.

## **Materials and Methods**

### **Case materials**

We obtained 10 cases of KIT-negative EGIST from the files of the Department of Anatomic Pathology of Kyushu University between 1986 and 2009. In contrast, there were 42 cases of

KIT-positive EGISTs including weakly to strongly KIT-positive tumors in the same files. All cases of EGISTs occurred in the abdominal soft tissue, such as the omentum, mesentery and retroperitoneum, and had no definite connection with the gastrointestinal tract wall by intraoperative or pathological (both gross and microscopic) observations.

### **Histopathological evaluation**

Each tumor was evaluated for clinicopathological and histological features, including tumor size and mitotic count. Tumors were classified into subgroups (groups 1-6b) depending on tumor size (cut off: 2, 5 or 10 cm) and mitotic count (cut off: 5 per 50 high-power fields [HPFs]); this stratification is used for risk assessment of conventional GIST (**Table 1**) [19, 21]. The histological patterns were evaluated in accordance with previous reports with slight modification [19, 21, 27].

### **Immunohistochemical staining**

Histological sections (4  $\mu$  m-thick) of 10% formalin-fixed, paraffin-embedded samples were used for the immunohistochemical examination. The primary antibodies were as follows: KIT (rabbit polyclonal, A4502; dilution: 1/100; Dako, Carpinteria, CA), CD34 (mouse monoclonal, QB-end-10; dilution: 1/50; Leica Biosystems, Newcastle Upon Tyne, UK), alpha-smooth muscle actin (mouse monoclonal, 1A4; dilution: 1/5000; Sigma BioSciences, St. Louis, MO), desmin



(mouse monoclonal, D33; dilution: 1/100; Dako), S-100 protein (rabbit polyclonal; dilution: 1/400; Dako), DOG1 (mouse monoclonal, K9; dilution: 1/100; Leica Biosystems), PKC theta (goat polyclonal, c-19; dilution: 1/100; Santa Cruz Biochemistry, Santa Cruz, CA) and Ki-67 (mouse monoclonal, MIB-1; dilution: 1/100; Dako). Heat-induced epitope retrieval (HIER) was performed in EDTA (DOG1) or in citrate buffer (PKC theta and Ki-67) for 20 minutes at 95°C by using a microwave. As for CD34 and S-100 protein, pretreatment with trypsin for 30 minutes at 37°C was performed. The sections were incubated with primary antibodies for one hour at room temperature, and reacted with the streptavidin-biotin-peroxidase method (Histofine SAB-PO Kit; Nichirei, Tokyo, Japan) for PKC theta or by the biotin-free, horseradish peroxidase enzyme-labeled polymer method (Envision+ system; Dako) for the remaining primary antibodies. The sections were then reacted in a 3,3'-diaminobenzidine peroxytrichloride substrate solution and counterstained with hematoxylin.

We used the polyclonal primary antibody for KIT (A4502, Dako) without HIER in the current study as well as in routine diagnosis for GIST. It is reported that this polyclonal antibody at low dilution with HIER may lead nonspecific staining in endothelial cells and non-GIST tumor cells such as desmoid tumor [17]. We defined “KIT-negative” EGIST as a tumor completely lacking immunoreactivity for KIT by using the above mentioned method (without HIER). The similar criteria for “KIT-negative” GIST and immunohistochemical staining procedure were applied in a

previous study [18].

In addition, 2 specimens of normal omental tissue obtained from the patients with ovarian carcinomas were immunohistochemically examined to check the presence or absence of KIT-positive cells.

### **Analysis of KIT and PDGFRA mutations**

Mutations of exons 9, 11, 13, and 17 of the *KIT* gene and those of exons 12 and 18 of the *PDGFRA* gene were examined by polymerase chain reaction (PCR) using the direct sequencing method, as previously reported [31].

## **Results**

### **Clinicopathological findings**

The clinicopathological findings are summarized in **Table 2**. The 10 patients comprised 5 men and 5 women, ranging in age from 41 to 84 years (median, 60.5 years). The tumors were located in the omentum (n=5), mesentery (n=2), retroperitoneum (n=1), pelvic cavity (n=1) and not-otherwise-specified regions of the abdominal cavity (n=1). They ranged from 4 to 33 cm (median 15 cm) in maximum diameter.

## **Gross findings**

The cut surface was solid with variable cystic and myxoid areas in most tumors. One case (Case 7) was a huge thin-walled cystic tumor containing serous-like fluid, accompanied by a focally thickened cyst wall or solid component (**Figure 1**).

## **Histological and immunohistochemical findings**

Morphologically, all but one of the cases were of epithelioid cell type (n=9), and the remaining case was of mixed epithelioid and spindle cell (n=1) type. The epithelioid tumor cells were arranged in a diffuse or sheet-like pattern, accompanied by a variable amount of collagenous and myxoid stroma. Among the epithelioid cell-type tumors, there were the following representative histologic patterns: sclerosing epithelioid, cohesive epithelioid, discohesive myxoid epithelioid, hypercellular epithelioid and sarcomatous epithelioid (**Figure 1**). These patterns were similar to that of gastric epithelioid GISTs, as previously described [21, 27]. Most cases were made up of a mixture of more than 2 patterns, and the histologic appearance varied from one tumor to another, or even from one microscopic field to another in an individual tumor (**Table 2**). In addition to the basic histologic patterns described above, several morphologic variations were focally present (**Figure 2**); microcystic degeneration was occasionally observed, and was often present in a

myxoid area. Myxoid appearance with a delicate capillary network as seen in myxoid/round cell liposarcoma was also observed in a focal area with a discohesive myxoid pattern. In addition, tumor cells with cytoplasmic vacuolation and eccentric nuclei (signet ring cell-like cells) were present in some cases. Hemangiopericytoma-like vasculatures with hyalinized vessel walls were focally observed in some cases. In part, tumor cells were arranged in a cord-like pattern with densely sclerotic, hyalinized collagenous stroma. Skenoid fibers were not observed in any of the cases. Mitotic counts ranged from 0 to 18 per 50 HPFs (median: 3.5/50HPFs).

By immunohistochemical staining, the tumors were positive for CD34 (8/10 cases; 80%), PKC theta (9/10; 90%) and DOG1 (9/10; 90%), but negative for KIT (0/10; 0%) (**Figure 3, Table 3**). Alpha-smooth muscle actin, desmin and S-100 protein were positive in 2 (20%), 0 (0%) and 0 (0%) cases, respectively. We failed to find the apparently KIT-positive mesenchymal cells in 2 specimens of normal omental tissue, although mast cells were scattered.

### **Mutations of the *KIT* and *PDGFRA* genes**

Of the 9 cases available for molecular study, 7 (78%) had the *PDGFRA* gene mutations at exon 12 (n=1) or exon 18 (n=6) (**Table 3**). The most common type (n=4) was the missense mutation affecting codon 842 on exon 18 (D842V or D842Y). Inframe deletions between codons 842 and 846 (del DIMH842-845 or del IMHD843-846) were also detected in two cases. The mutation at

exon 12 (n=1) was a missense mutation with a substitution of aspartic acid for valine at codon 561 (V561D).

One case (11%) showed a missense mutation of *KIT* exon 11 (P577S). The remaining one case (11%) was wild for both the *KIT* and *PDGFRA* genes.

### **Prognostic analysis**

Follow-up information was available for 10 patients, and the follow-up period ranged from 2 to 192 months (median, 24.5 months) (**Tables 1 and 2**). None of the patients were treated with imatinib or sunitinib before or after the surgery.

Local recurrence was observed in 2 (20%) patients (Cases 1 and 9) at 9 and 1 months after the initial operation, respectively. Distant metastasis to the lung was seen in one (10%) patient (Case 1), 7 months after the operation. Both patients with recurrence (Cases 1 and 9) died of tumor, at 22 or 14 months after the initial operation. These two cases were group 6b GISTs (size >10 cm, mitoses >5/50HPFs).

One patient (Case 10) died of cachexia related with the tumor; in this patient, biopsy and surgical excision were not performed because of the poor performance status on admission, and the autopsy revealed a huge abdominal tumor without foci of distant metastases. Another patient (Case 2) died of unrelated disease, 5 months after the surgical operation. These two tumors (Cases

2 and 10) belonged to group 3b (size >10 cm, mitoses <5/50HPFs).

The remaining 6 patients (all 3 cases of groups 2 and 3a, and 3/5 cases of group 3b) were still alive without any evidence of recurrence after the initial operation.

## Discussion

Although GIST rarely occurs outside the GI tract, such tumor (EGIST) is usually positive for KIT [20, 26, 28, 31]. Miettinen et al. reported 95 cases of omental GIST that included 51 cases of solitary tumor, and 3 of the 41 (7.3%) cases of solitary omental GIST examined were immunohistochemically negative for KIT (although half of the solitary omental GISTs involved the gastric wall in their series) [22]. Thus, KIT-negative EGIST is so rare that its clinicopathological and genetic features have not been well documented. In the earlier reports, small number of cases of KIT-negative EGIST were included in a lump of KIT-negative GISTs, irrespective of the primary site [15, 18, 27]. According to these studies, a total of 15 cases of KIT-negative EGIST were located in the omentum (n=10), mesentery (n=3) and retroperitoneum (n=2). Most tumors were histologically epithelioid cell type (n=11) or mixed cell type (n=2). In addition, among the 10 cases examined, 9 cases had the *PDGFRA* gene mutation in exon 18 or exon 12, while only one case had the *KIT* exon 11 mutation. However, the details of histologic

variations, mitotic counts and prognostic information had not been described. In the current study, we confirmed that KIT-negative EGISTs were characterized by preferential omental origin, epithelioid cell type and mutation of the *PDGFRA* gene, and found that these tumors had low mitotic activity and relatively favorable prognosis.

The basic histologic patterns (sclerosing, discohesive myxoid, cohesive, hypercellular and sarcomatous) of epithelioid tumor cells of KIT-negative EGISTs were essentially the same as those of gastric epithelioid GISTs previously described by Miettinen et al. [21] and Sakurai et al. [27]. Interestingly, in the present study, both omental and mesenteric KIT-negative EGISTs showed similar gastric-type morphology. In contrast, among KIT-positive EGISTs, the spindle cell-type and epithelioid cell-type cases were of equal frequency, and *KIT* mutation (particularly in exon 11) rather than *PDGFRA* mutation was predominant [26, 28, 31]. These findings indicate a distinctive phenotypic-genotypic correlation in KIT-negative EGIST, which resembles the phenomenon found in KIT-negative gastric GIST (**Figure 4**).

The most common type of mutation found in our series of KIT-negative EGISTs was the missense mutation at codon 842 on *PDGFRA* exon 18 (D842V, n=3; D842Y, n=1), followed by inframe deletions between codons 842 and 846 on *PDGFRA* exon 18 (del DIMH842-845, n=1; del IMHD843-846, n=1) and missense mutation at codon 561 on *PDGFRA* exon 12 (n=1). The *KIT* exon 11 mutation was present in only one case. Among 10 cases of KIT-negative EGISTs

previously reported [15, 18, 27], 8 cases had the gene mutation in *PDGFRA* exon 18 (D842V, n=5; del DIMH 842-845, n=3) and each one case had the mutation in *PDGFRA* exon 12 and *KIT* exon 11. These results including ours strongly suggest that the *PDGFRA* gene mutation, particularly that affecting the codon 842 on exon 18, is the predominant genotype of KIT-negative EGISTs. The prevalence of the *PDGFRA* mutation subtype in KIT-negative EGISTs is similar to that previously reported in gastric GISTs [13].

Imatinib is a selective tyrosine kinase inhibitor against the KIT and PDGFRA proteins, and its efficacy is closely related with the *KIT* and *PDGFRA* genotypes [4, 10]. The preliminary clinical data and *in vitro* studies have demonstrated that GISTs with a *PDGFRA* D842V substitution (the most frequent mutation in KIT-negative EGISTs) were resistant to imatinib, but some populations of other *PDGFRA* mutants were sensitive [2, 7]. Sunitinib is an alternative tyrosine kinase inhibitor for blocking KIT and PDGFRA proteins, and some imatinib-resistant GISTs are sensitive for sunitinib [3]. Further studies will be needed to develop a therapeutic strategy based on the *KIT* or *PDGFRA* genotype for patients with KIT-negative EGIST.

In our series of KIT-negative EGIST, the majority (80%) of cases showed low mitotic activity (<5/50 HPFs), and local recurrence and distant metastasis were relatively rare (20% and 10%, respectively). Even large (>10 cm) tumors with low mitotic activity (the Group 3b cases) showed neither local recurrence nor distant metastasis. In contrast, all of the cases of Group 6b (tumors



with large size [ $>10$  cm] and high mitotic count [ $>5/50$  HPFs]) had local recurrence and/or distant metastasis. Likewise, mitotic count rather than tumor size was correlated with worse prognosis in KIT-positive EGISTs in our previous study [31]. These data may provide the evidence that risk stratification mainly based on the mitotic count is useful to predict the biological behavior of EGIST. However, further examinations with larger numbers of EGIST cases will be needed to establish the criteria of risk for aggressive behavior, because the number of cases and follow-up period were limited in the present study.

DOG1, also known as anoctamin-1 (ANO1), which encodes a membrane protein associated with calcium-dependent chloride channel activity, was recently shown to be up-regulated in GIST by using gene expression profiling [29]. Subsequently, several kinds of monoclonal antibodies against DOG1 (DOG1.1, DOG1.3 and K9) were developed, and these antibodies were reported to be highly specific and sensitive for GIST irrespective of the underlying genotype or KIT expression level [6, 15, 23]. Furthermore, DOG1 expression has been reported to be positive in approximately 90% of EGISTs [23].

Protein kinase C (PKC) theta, an isotype of PKC, is also reported to be a novel marker for GIST [5, 12]. According to studies in the literature, both KIT-positive and KIT-negative GISTs are positive for PKC theta, and some populations of non-GIST tumors can also express PKC theta [5, 24, 25], suggesting that PKC theta may be less specific to confirm the diagnosis of GIST. In the

current study, the majority of KIT-negative EGISTs were positive for both markers. Therefore, immunohistochemical stainings for DOG1 and PKC theta are suggested to be useful as ancillary tools for the identification of KIT-negative EGIST.

The histogenesis of EGIST has been a subject of controversy. Some investigators have reported the presence of ICC-like cells in abdominal soft tissue and viscera, and have speculated that these cells are closely related to EGIST [11, 28]. We failed to find the apparently KIT-positive mesenchymal cells in 2 specimens of normal omental tissue, although mast cells were scattered; the result was consistent with the previous study [22]. Miettinen et al. revealed that most of solitary omental EGIST were characterized by gastric GIST-like morphology and predominant PDGFRA mutation genotype [22]. In addition, because about half of those EGISTs involved or had attachment to the gastric wall, the authors speculated that at least some of omental GISTs initially occurred in the gastric wall, but then extended to the soft tissue and eventually lost their connection to the wall. The current series of KIT-negative EGISTs had no connection with the GI wall by intraoperative, macroscopic and microscopic examinations. Recently, Long et al. reported the occurrence of EGIST in the thoracic cavity, far from the esophagus wall [16]. We also have an experience of a case of primary hepatic GIST which was completely separate from the GI wall. Interestingly, this hepatic EGIST was KIT-negative and of epithelioid cell type with *PDGFRA* gene mutation [30]. We think that it is more clinically important to recognize that KIT-positive or

negative GIST-type tumors may occur as a primary tumor in the abdominal soft tissue, rather than to prioritize determination of the origin of EGIST. This idea may be of great benefit for the clinical management of patients with this kind of tumor, particularly those receiving molecular-targeted therapy.

The diagnosis of KIT-negative EGIST can be very challenging: the unusual location, epithelioid-to-round cell morphology, and KIT-negative/CD34-positive immunoprofile might lead to a broad differential diagnosis and a misdiagnosis as other types of tumors. The distinction from solitary fibrous tumor (SFT) can be difficult, because SFT is CD34-positive, and sometimes shows round cell morphology. Furthermore, KIT-negative EGISTs focally have hemangiopericytoma-like vasculatures with a hyalinized vessel wall, which are characteristic features of SFT. Myxoid/round cell liposarcoma is another possible morphologic mimicker. In this study, some cases of KIT-negative EGIST focally showed a myxoid appearance with a delicate capillary network as seen in myxoid/round cell liposarcoma. In addition, tumor cells with cytoplasmic vacuolation and eccentric nuclei (signet ring cell-like cells) of KIT-negative EGIST superficially resemble carcinoma cells or immature adipocytes of liposarcoma. Focally, EGIST cells are arranged in a cord-like pattern with sclerotic, hyalinized collagenous stroma. This pattern may be similar to that of sclerosing epithelioid fibrosarcoma. However, awareness of the distinctive histologic features of KIT-negative EGIST and application of an appropriate

immunohistochemical panel and molecular genetic study confirming the *PDGFRA* gene mutation may allow for proper diagnosis. Most non-GIST mesenchymal tumors including SFT and myxoid liposarcoma are reported to be negative for DOG1 [6, 23, 25].

In conclusion, we described the characteristics of KIT-negative EGISTs that had predominantly epithelioid cell morphology and *PDGFRA* gene mutation. This type of GIST lacked demonstrable KIT expression and occurred in unusual locations, but otherwise had consistent morphologic, phenotypic and genetic features. Therefore, the soft tissue counterpart of KIT-negative GIST should be considered as a potential primary abdominal neoplasm. A panel of immunohistochemical markers including DOG1 and PKC theta and gene mutation analysis of the *KIT* and *PDGFRA* genes are necessary not only to confirm the diagnosis but also to help to determine the therapeutic strategy for patients with this kind of tumor.

## References

1. Allander SV, Nupponen NN, Ringnér M, et al. Gastrointestinal stromal tumors with KIT mutations exhibit a remarkably homogeneous gene expression profile. *Cancer Res.* 2001;61:8624-8628.
2. Corless CL, Schroeder A, Griffith D, et al. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. *J Clin Oncol.* 2005;23:5357-5364.
3. Demetri GD, van Oosterom AT, Garrett CR, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet.* 2006;368:1329-1338.
4. Demetri GD, 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.
5. Duensing A, Joseph NE, Medeiros F, et al. Protein Kinase C theta (PKCtheta) expression and constitutive activation in gastrointestinal stromal tumors (GISTs). *Cancer Res.* 2004;64:5127-5131.
6. Espinosa I, Lee CH, Kim MK, et al. A novel monoclonal antibody against DOG1 is a sensitive and specific marker for gastrointestinal stromal tumors. *Am J Surg Pathol.* 2008;32:210-218.

7. 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.
8. Heinrich MC, Corless CL, Duensing A, et al. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science.* 2003;299:708-710.
9. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science.* 1998;279:577-580.
10. Hornick JL, Fletcher CD. The role of KIT in the management of patients with gastrointestinal stromal tumors. *Hum Pathol.* 2007;38:679-687.
11. Huizinga JD, Faussone-Pellegrini MS. About the presence of interstitial cells of Cajal outside the musculature of the gastrointestinal tract. *J Cell Mol Med.* 2005;9:468-473.
12. Kim KM, Kang DW, Moon WS, et al. PKC $\theta$  expression in gastrointestinal stromal tumor. *Mod Pathol.* 2006;19:1480-1486.
13. Lasota J, Dansonka-Mieszkowska A, Sobin LH, et al. A great majority of GISTs with PDGFRA mutations represent gastric tumors of low or no malignant potential. *Lab Invest.* 2004;84:874-883.
14. Lasota J, Miettinen M. KIT and PDGFRA mutations in gastrointestinal stromal tumors (GISTs). *Semin Diagn Pathol.* 2006;23:91-102.
15. Liegl B, Hornick JL, Corless CL, et al. Monoclonal antibody DOG1.1 shows higher

- sensitivity than KIT in the diagnosis of gastrointestinal stromal tumors, including unusual subtypes. *Am J Surg Pathol.* 2009;33:437-446.
16. Long KB, Butrynski JE, Blank SD, et al. Primary extragastrointestinal stromal tumor of the pleura: report of a unique case with genetic confirmation. *Am J Surg Pathol.* 2010;34:907-912.
  17. Lucas DR, al-Abbadi M, Tabaczka P, et al. c-kit expression in desmoid fibromatosis. comparative immunohistochemical evaluation of two commercial antibodies. *Am J Clin Pathol.* 2003;119:339-345.
  18. Medeiros F, Corless CL, Duensing A, et al. KIT-negative gastrointestinal stromal tumors: proof of concept and therapeutic implications. *Am J Surg Pathol.* 2004;28:889-894.
  19. Miettinen M, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. *Semin Diagn Pathol.* 2006;23:70-83.
  20. Miettinen M, Monihan JM, Sarlomo-Rikala M, et al. Gastrointestinal stromal tumors/smooth muscle tumors (GISTs) primary in the omentum and mesentery: clinicopathologic and immunohistochemical study of 26 cases. *Am J Surg Pathol.* 1999;23:1109-1118.
  21. Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. *Am J Surg Pathol.* 2005;29:52-68.

22. Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors presenting as omental masses: a clinicopathologic analysis of 95 cases. *Am J Surg Pathol.* 2009;33:1267-1275.
23. Miettinen M, Wang ZF, Lasota J. DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases. *Am J Surg Pathol.* 2009;33:1401-1408.
24. Motegi A, Sakurai S, Nakayama H, et al. PKC theta, a novel immunohistochemical marker for gastrointestinal stromal tumors (GIST), especially useful for identifying KIT-negative tumors. *Pathol Int.* 2005;55:106-112.
25. Novelli M, Rossi S, Rodriguez-Justo M, et al. DOG1 and CD117 are the antibodies of choice in the diagnosis of gastrointestinal stromal tumours. *Histopathology.* 2010;57:259-270.
26. Reith JD, Goldblum JR, Lyles RH, et al. Extragastrointestinal (soft tissue) stromal tumors: an analysis of 48 cases with emphasis on histologic predictors of outcome. *Mod Pathol.* 2000;13:577-585.
27. Sakurai S, Hasegawa T, Sakuma Y, et al. Myxoid epithelioid gastrointestinal stromal tumor (GIST) with mast cell infiltrations: a subtype of GIST with mutations of platelet-derived growth factor receptor alpha gene. *Hum Pathol.* 2004;35:1223-1230.
28. Sakurai S, Hishima T, Takazawa Y, et al. Gastrointestinal stromal tumors and KIT-positive mesenchymal cells in the omentum. *Pathol Int.* 2001;51:524-531.



29. West RB, Corless CL, Chen X, et al. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. *Am J Pathol.* 2004;165:107-113.
30. Yamamoto H, Miyamoto Y, Nishihara Y, et al. Primary gastrointestinal stromal tumor of the liver with PDGFRA gene mutation. *Hum Pathol.* 2010;41:605-609.
31. Yamamoto H, Oda Y, Kawaguchi K, et al. c-kit and PDGFRA mutations in extragastrointestinal stromal tumor (gastrointestinal stromal tumor of the soft tissue). *Am J Surg Pathol.* 2004;28:479-488.

## Figure legends

**Figure 1.** Macroscopic findings (a) and representative histologic patterns (b-f) in KIT-negative gastrointestinal stromal tumors of the soft tissue.

a. The omental tumor is composed of solid and cystic parts (Case 7).

b. Sclerosing epithelioid pattern. Oval-to-polygonal cells without a distinctive cell border are set in a syncytial pattern with a sclerosing stroma.

c. Discohesive myxoid epithelioid pattern. Epithelioid tumor cells show a less cohesive pattern of growth and proliferate haphazardly in a loose collagenous or myxoid matrix. Bi- or multi-nucleated tumor cells with eosinophilic cytoplasm are noted.

d. Cohesive epithelioid pattern. Epithelioid cells with eosinophilic cytoplasm are surrounded by a lacunar space and distinctive cell borders. Interstitial matrix is scant.

e. Hypercellular epithelioid pattern. Oval-to-rounded cells of relatively uniform shape and size proliferate in a diffuse sheet-like pattern.

f. Sarcomatous epithelioid pattern. Round-to-polygonal cells show moderate nuclear pleomorphism. Some tumor cells have small but distinctive nucleoli. Mitotic figures are evident.

**Figure 2.** Morphologic variations focally present in a KIT-negative gastrointestinal stromal tumor

of soft tissue.

a. Microcystic degeneration is occasionally associated with myxoid area.

b. The pseudoglandular structure is lined by epithelioid tumor cells mimicking cuboidal epithelial cells.

c. Myxoid area with capillary-sized vessels and hypocellular proliferation of epithelioid-to-rounded cells. Transition to a more hypercellular area (left upper field) is noted.

This appearance superficially resembles myxoid/round cell liposarcoma.

d. Signet ring cell-like appearance. Tumor cells show cytoplasmic vacuolation and eccentric nuclei.

e. Hemangiopericytoma-like vessels with hyalinization are focally observed.

f. Tumor cells are arranged in a cord-like pattern with sclerotic, heavily hyalinized collagenous stroma.

**Figure 3.** Immunohistochemical findings. Tumor cells are completely negative for KIT, whereas infiltrating mast cells are positive for KIT (a). Tumor cells show diffuse cytoplasmic positivity for CD34 (b), membranous and cytoplasmic positivity for DOG1 (c) and cytoplasmic positivity for PKC theta (d).

**Figure 4.** Variants of gastrointestinal stromal tumors showing the correlation among KIT expression, primary site, cell type and genotype of the *KIT* and *PDGFRA* genes. KIT-negative extragastrointestinal stromal tumor is characterized by extra-gastrointestinal (GI) primary, epithelioid cell morphology and *PDGFRA* gene mutation.

**Table 1.** Risk groups and clinical course of KIT-negative EGIST

| <b>Group</b> | <b>Mitoses<br/>(HPF)</b> | <b>Size<br/>(cm)</b> | <b>No. of cases<br/>(n=)</b> | <b>Adverse outcome<sup>#</sup></b> | <b>Local recurrence</b> | <b>Distant metastasis</b> |
|--------------|--------------------------|----------------------|------------------------------|------------------------------------|-------------------------|---------------------------|
| 1            | ≤5/50                    | ≤2                   | 0                            | -                                  | -                       | -                         |
| 2            |                          | >2≤5                 | 1                            | 0/1(0%)                            | 0/1(0%)                 | 0/1(0%)                   |
| 3a           |                          | >5≤10                | 2                            | 0/2(0%)                            | 0/2(0%)                 | 0/2(0%)                   |
| 3b           |                          | >10                  | 5                            | 1/5(20%)                           | 0/5(0%)                 | 0/5(0%)                   |
| 4            | >5/50                    | ≤2                   | 0                            | -                                  | -                       | -                         |
| 5            |                          | >2≤5                 | 0                            | -                                  | -                       | -                         |
| 6a           |                          | >5≤10                | 0                            | -                                  | -                       | -                         |
| 6b           |                          | >10                  | 2                            | 2/2 (100%)                         | 2/2(100%)               | 1/2(50%)                  |
| Total        |                          |                      | 10                           | 3/10(30%)                          | 2/10(20%)               | 1/10(10%)                 |

<sup>#</sup> Adverse outcome is defined as either of recurrence, metastasis and death related with tumor

**Table 2.** Clinicopathological and histological findings of KIT-negative EGIST

| Case | Age | Sex | Site                | Size<br>(cm) | Mitosis<br>(/50HPF) | Risk group | MIB-1<br>(%) | Cell type   | Histological subtype                 | Follow-up information   |
|------|-----|-----|---------------------|--------------|---------------------|------------|--------------|-------------|--------------------------------------|---|
| 1    | 64  | M   | Pelvic cavity       | 11           | 15                  | 6b         | 10.8         | epithelioid | sarcomatous<br>>sclerosing           | lung metastasis at 7mo, local recurrence at 9 mo,<br>DOD at 22 mo |
| 2    | 84  | F   | Mesentery           | 25           | 4                   | 3b         | 3.3          | epithelioid | discohesive myxoid                   | died of other cause at 5 mo                                       |
| 3    | 52  | M   | Abdominal<br>cavity | 30           | 4                   | 3b         | 2.3          | epithelioid | sclerosing<br>>discohesive myxoid    | NED at 60 mo  |
| 4    | 46  | F   | Retroperitoneum     | 7            | 1                   | 3a         | 2.3          | mixed       | cellular spindle and<br>epithelioid  | NED at 86 mo  |
| 5    | 64  | F   | Mesentery           | 4            | 4                   | 2          | 1            | epithelioid | sclerosing                           | NED at 192 mo   |
| 6    | 62  | F   | Omentum             | 11           | 3                   | 3b         | 1.2          | epithelioid | sclerosing<br>>discohesive myxoid    | NED at 87 mo  |
| 7    | 41  | M   | Omentum             | 19           | 0                   | 3b         | 2            | epithelioid | discohesive myxoid<br>>hypercellular | NED at 27 mo  |
| 8    | 69  | M   | Omentum             | 8.5          | 1                   | 3a         | 2.6          | epithelioid | cohesive                             | NED at 20 mo  |
| 9    | 54  | F   | Omentum             | 20           | 18                  | 6b         | 15.4         | epithelioid | hypercellular<br>>discohesive myxoid | local recurrence at 1 mo, DOD at 14 mo                            |
| 10   | 59  | M   | Omentum             | 33           | 1                   | 3b         | 2.1          | epithelioid | hypercellular                        | DOD at 2 mo (autopsy case. no surgery when<br>alive)              |

mo, months; NED, no evidence of disease; DOD, died of disease

**Table 3.** Immunohistochemical and genetic findings of KIT-negative EGIST

| Case | Immunohistochemistry |      |              |       | KIT/PDGFR $\alpha$ mutation analysis     |
|------|----------------------|------|--------------|-------|--|
|      | KIT                  | CD34 | PKC $\theta$ | DOG1  |  |
| 1    | -                    | +    | -            | weak+ | Not examined                             |
| 2    | -                    | +    | +            | +     | PDGFR $\alpha$ exon 12 (V561D)           |
| 3    | -                    | +    | +            | +     | PDGFR $\alpha$ exon 18 (D842V)           |
| 4    | -                    | -    | +            | -     | KIT exon 11 (P577S)                      |
| 5    | -                    | +    | +            | weak+ | Wild                                     |
| 6    | -                    | +    | +            | +     | PDGFR $\alpha$ exon 18 (D842V)           |
| 7    | -                    | +    | +            | +     | PDGFR $\alpha$ exon 18 (D842V)           |
| 8    | -                    | -    | +            | +     | PDGFR $\alpha$ exon 18 (del IMHD843-846) |
| 9    | -                    | +    | +            | +     | PDGFR $\alpha$ exon 18 (del DIMH842-845) |
| 10   | -                    | +    | +            | +     | PDGFR $\alpha$ exon 18 (D842Y)           |

Figure 1

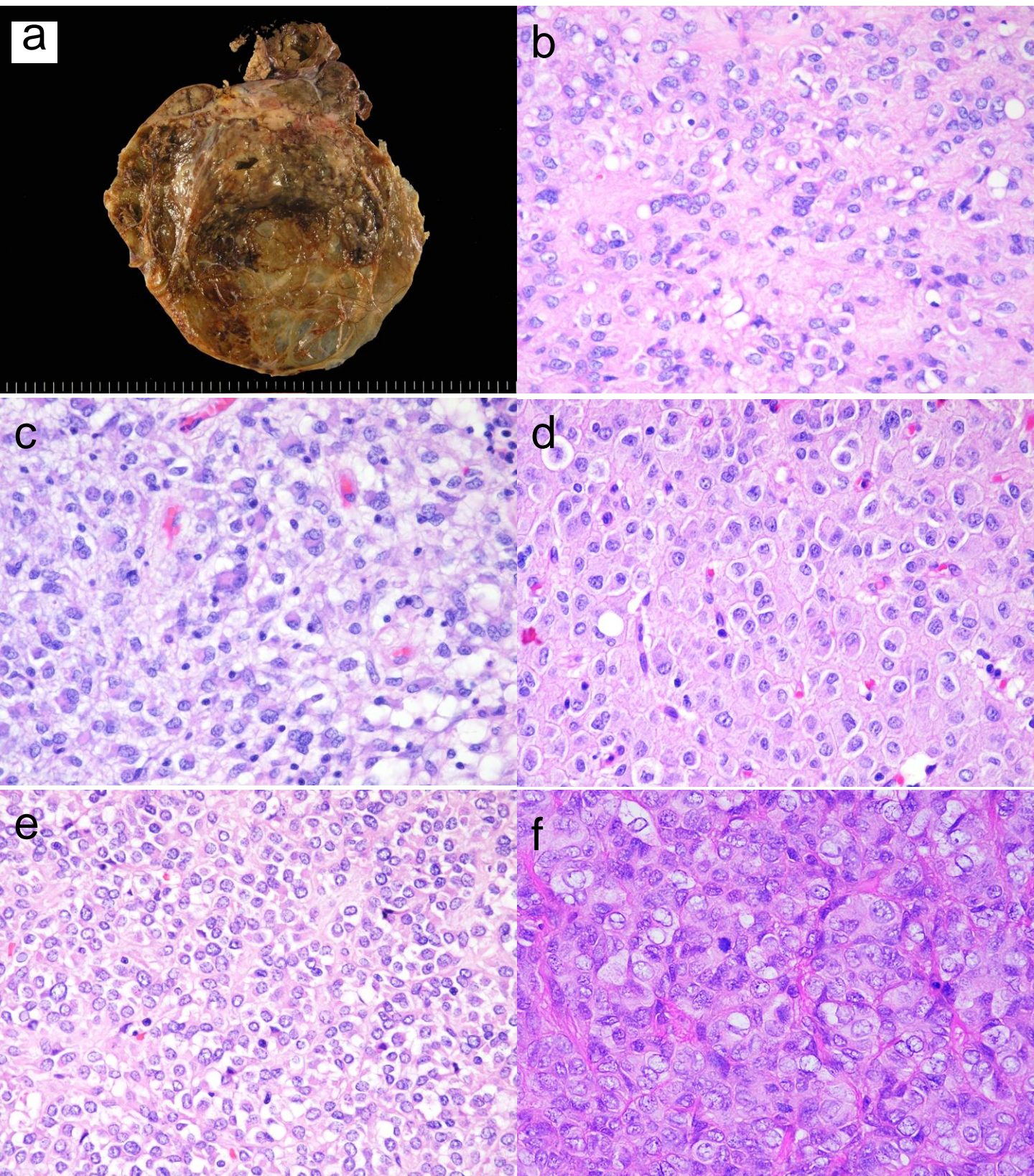




Figure2

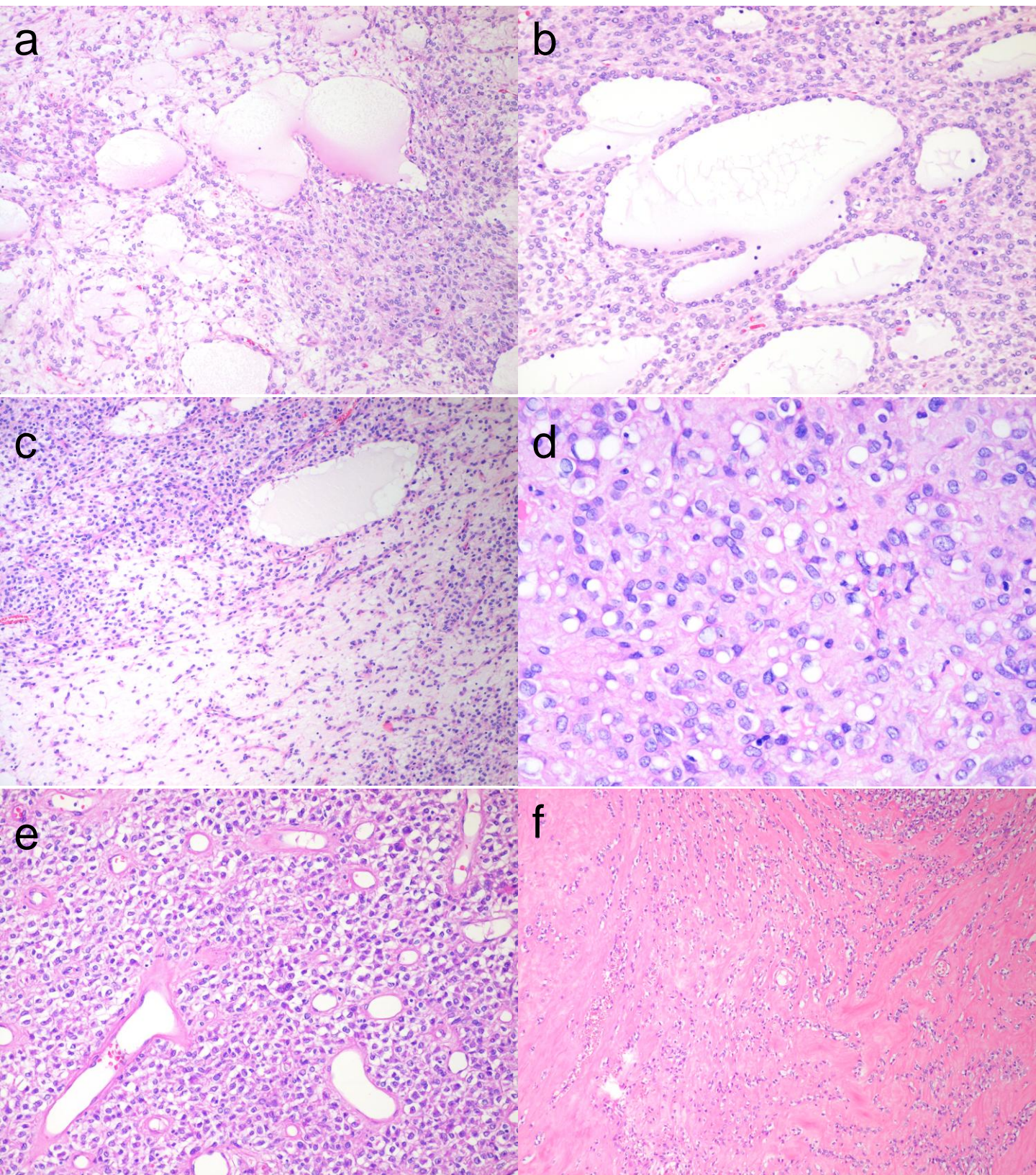


Figure3

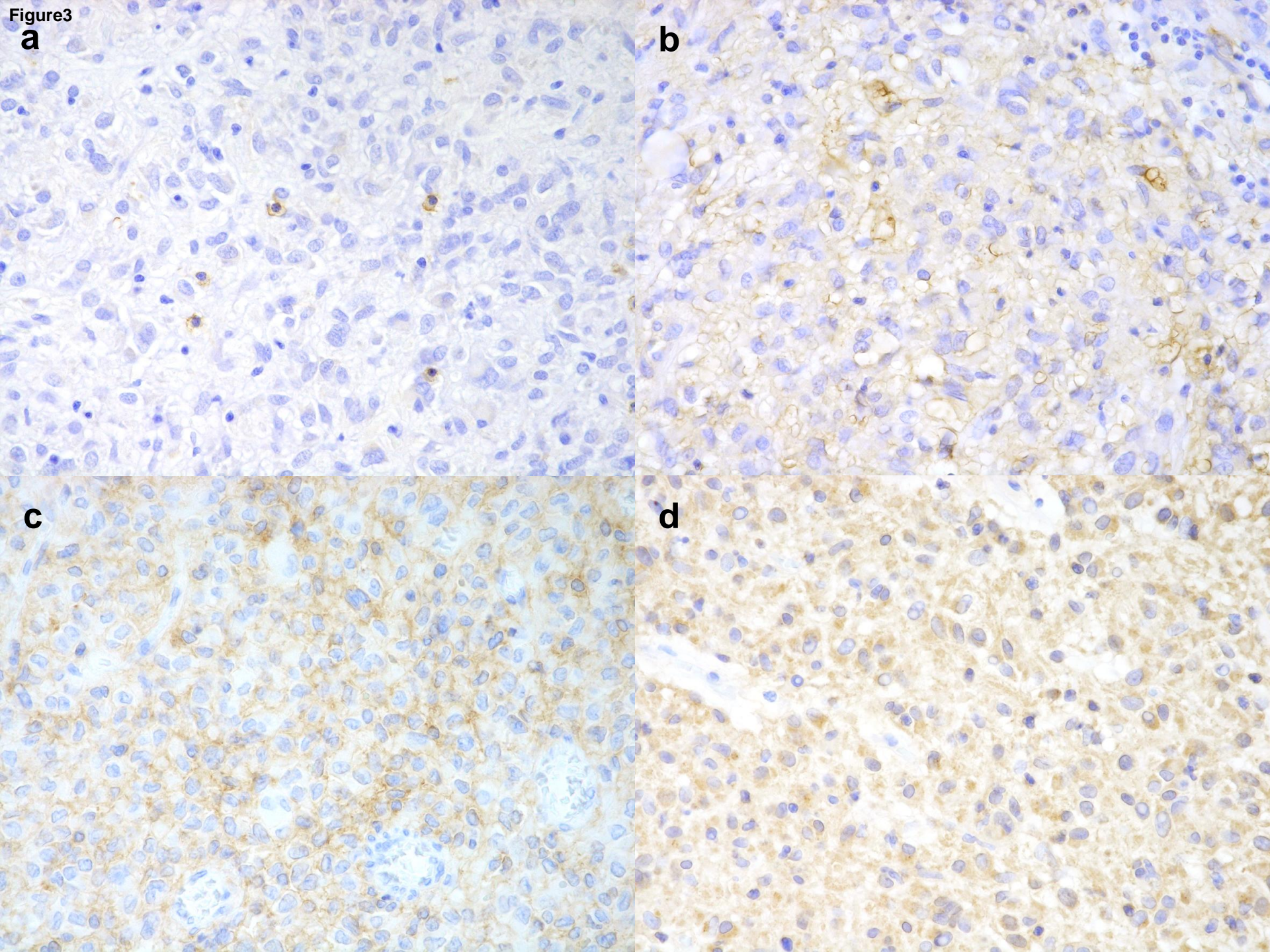


Figure4

