Veterinary Surgery
36:302–313, 2007

Reclassification of Small Intestinal and Cecal Smooth Muscle Tumors in 72 Dogs: Clinical, Histologic, and Immunohistochemical Evaluation

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Objectives—To reclassify canine small intestinal and cecal leiomyoma (LM) and leiomyosarcoma (LMS) into smooth muscle and gastrointestinal stromal tumors (GIST) using histologic and immunohistochemical (IH) analysis and to report clinical findings and survival data.
Study Design—Retrospective review of cases.
Animals—Dogs (n = 47) with small intestinal (40 LMS; 7 LM) and 25 dogs with cecal tumors (23 LMS; 2 LM).
Methods—Clinical and survival data were reviewed. Tissue sections were reevaluated for light-microscopic malignancy criteria and examined for expression of SMA, desmin, vimentin, S-100, and CD117 (KIT) by immunohistochemistry.
Results—Reclassification resulted in 2 LM, 9 LMS, 19 GIST, and 17 GIST-like tumors in the small intestine and 23 GIST and 2 GIST-like tumors in the cecum. GIST-like tumors were morphologic and IH identical to GIST but lacked KIT expression. No significant difference in survival was observed for tumor type, location, histologic, or IH characteristics; however, dogs with cecal tumors were significantly older in age, presented more commonly with intestinal perforation and peritonitis, and less commonly with weight loss. Cecal tumors had more histologic malignancy criteria than small intestinal tumors. After excision, 1 and 2 year recurrence-free periods were 80.1% and 67.2% for small intestinal and 83.3% and 61.9% for cecal tumors.
Conclusion—Prognosis for intestinal tumors with histologic smooth muscle appearance is good after excision and not related to tumor type, location, histologic, or IH characteristics.
Clinical Relevance—Clinical importance could not be demonstrated for reclassification, but may be for future treatment, of intestinal smooth muscle or stromal tumors.

INTRODUCTION

PRIMARY INTESTINAL tumors can be of epithelial, neuroendocrine, hematopoietic, or mesenchymal origin.[1] Most canine gastrointestinal (GI) mesenchymal tumors with smooth muscle appearance have been diagnosed as leiomyoma (LM) or leiomyosarcoma (LMS) by light microscopic examination of hematoxylin and eosin (HE)-stained tissue sections.[2] Clinical characteristics of both tumors have been described for dogs.[1–10]

In veterinary studies a higher incidence of malignant tumors with smooth muscle appearance has been report-

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Financial support for this study was provided by Hill's Pet Nutrition Inc., the Department of Pathobiology and the DOG Foundation, Department of Clinical Sciences of Companion Animals, University of Utrecht, Utrecht, the Netherlands.

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Submitted September 2006; Accepted March 2007

0161-3499/07
doi:10.1111/j.1532-950X.2007.00271.x

ed for the small intestine and cecum in contrast to benign tumors, which were found predominantly in the stomach.[2,6,11,12] Histologic malignancy criteria and tumor location in the GI tract may have significant effect on clinical signs and survival data.

Using immunohistochemical (IH) analysis, tumors identified by histologic evaluation of HE-stained specimens as being of smooth muscle origin are a heterogeneous group of mesenchymal tumors that may not express smooth muscle markers.[1,11] In human and veterinary patients, IH and ultrastructural studies of these tumors demonstrate differentiation toward either smooth muscle (myogenic), or neural tissue (neurogenic), a combination of both (mixed), or undifferentiated tissue (anaplastic).[1,2,13] Based on IH analysis, tumors from true smooth muscle origin can be differentiated from stromal tumors.

Many of the ''smooth muscle tumors'' in human patients have now been reclassified as gastrointestinal stromal tumors (GIST), which by definition express CD117 (KIT).[14] KIT is a receptor tyrosine kinase that occurs in several tissues including the interstitial cells of Cajal (ICC), hematopoietic progenitor cells, mast cells, and melanocytes. GIST are thought to originate from the ICC which form a network that coordinates peristalsis in the GI tract.[15] Uncontrolled activity of KIT in the GI tract is an important step in development of GIST.[1,12,16–18] In addition to surgical removal of GIST, medical treatment with a selective receptor tyrosine kinase inhibitor is being explored in humans.[15]

In persons, GIST are defined as KIT expressing tumors; however, in veterinary medicine the diagnosis of GIST has been primarily based on a combination of histological similarity to these tumors in man and a variety of IH stains.[2,11,12,19–23] Two large canine studies have reported expression of desmin, SMA, S-100, and vimentin in tumors with smooth muscle appearance in the GI tract.[2,12] Expression of KIT was positive in 54% of the malignant small intestinal and cecal tumors,[12] and although the other malignant tumors did not express KIT, they were reclassified as GIST. The IH characteristics that have been reported for intestinal tumors in animals are variable and not fully consistent with each other and with definitions used in humans.[2,11–13,19–23]

We are unaware of any reports that combine clinical presentation, histologic appearance, IH data, and long-term survival after complete surgical excision of small intestinal and cecal tumors with smooth muscle appearance in dogs. Thus our purposes were (1) to compare signalment, clinical presentation, and survival data after surgical excision of tumors with smooth muscle appearance in the small intestine and cecum in dogs, (2) to score histologic malignancy criteria for these tumors, and (3) to examine tumor differentiation and reclassify these tumors using histologic and IH analysis.

MATERIALS AND METHODS

Dogs

Medical records (1984–2004) were reviewed for dogs with a diagnosis of a smooth muscle tumor of the small intestine or cecum by light microscopic evaluation. Only dogs with small intestinal or cecal tumors with smooth muscle appearance that had archived paraffin-embedded sections available for IH staining, and could be followed were included.

Clinical Data

Patient signalment (breed, weight, sex, age) was retrieved. From patient history, clinical, diagnostic imaging, and surgical findings the following variables were scored: anorexia, lethargy, vomiting, diarrhea, melena, painful abdomen, palpable abdominal mass, weight loss, anemia, hypoglycemia, location and diameter (cm) of the tumor and tumor-associated hemoabdomen, intestinal perforation, peritonitis, and intestinal intussusception. Tumor location was classified as small intestinal (including duodenum, jejunum, or ileum) or cecal.

Histologic Analysis

After surgical excision or biopsy, representative specimens were fixed in buffered 10% formalin, embedded in paraffin, sectioned (5 μm) and stained (HE). For this study, all original HE and newly prepared IH slides were (re)evaluated by one pathologist (I.G.) without knowledge of the original results.

Comparable with previous reports,[2,12,14,15,24,25] 8 histologic criteria suitable for scoring malignancies[26,27] were selected. Scoring was tissue differentiation (0 [good]–2 [poor]); nuclear pleomorphism (0 [absent]–2 [widely present]); cellularity (0 [low]–3 [high]); presence of nucleoli (0 [absent]–4 [widely present]); number of mitoses/ $\times$ 40 high power field (mitotic count) was determined in 10 fields ($0 = 0$ mitoses, $1 = 1$ mitosis, or $2 = > 1$ mitosis); and the amount of necrosis, bleeding, and inflammation (0 [absent]–4 [widely present]). Presence of neutrophilic or lymphocytic inflammation was noted.

We examined if certain combinations of these criteria had prognostic value for survival after surgical resection. As important criteria of malignancy for GI smooth muscle tumors, scores for nuclear pleomorphism, cellularity, and mitotic score were added.[2] To evaluate local tumor effect on tissue, scores for necrosis, bleeding, and neutrophilic inflammation were combined.

IH Analysis

Selection of IH stains was based on previous veterinary[2,11,12,19–23,28] and human reports.[13–15,17,24,29] Expression of desmin and SMA (markers of myogenic differentiation); S-100 (marker of neurogenic differentiation), and vimentin (indicator of mesenchymal origin of tissue), was investigated by use of specific antibodies with an avidin–biotin complex immunoperoxidase (ABC/PO) method (Table 1).[2,12] KIT expression was determined by means of the EnVision method.[30] In all immunostaining protocols, the chromagen diamino-

Table 1. Staining Method, Type, Dilution, and Source of Antibodies Used for Immunohistochemical Analysis

	Primary Antibody (Code)	Dilution	Source	Second Antibody (Code)	Dilution	Source
Desmin (ABC/PO method)	Rabbit anti-desmin (2203PDE)	1:80	Eurodiagnostics*	Goat anti-rabbit/biotin (BA-1000/PK-4000)	1:250	Vector Laboratories†
Smooth muscle actin (ABC/PO method)	Mouse anti-SMA (MU128-UC)	1:1200	Biogenex‡	Horse anti-mouse/biotin (BA-2000/PK-4000)	1:250	Vector Laboratories
S-100 (ABC/PO method)	Rabbit anti-S-100 (Z0311)	1:600	DakoCytomation§	Goat anti-rabbit/biotin (BA-1000/PK-4000)	1:250	Vector Laboratories
Vimentin (ABC/PO method)	Mouse anti-vimentin (MUO74-UC)	1:150	Biogenex	Horse anti-mouse/biotin (BA-2000/PK-4000)	1:125	Vector Laboratories
KIT (EnVision method)	Rabbit anti-KIT (A4502)	1:40	DakoCytomation	Goat anti-rabbit/envision (K4003)	—	Dako Cytomation

*Apeldoorn, the Netherlands. †Burlingame, CA. ‡San Ramon, CA. §Glostrup, Denmark.

benzidine tetrahydrochloride (DAB) was used to establish a brown staining of positive tissue. IH stains were performed using previously published protocols.[2,12,30]

The positive control for SMA and desmin was normal smooth muscle tissue in the vascular wall and muscularis mucosa. Vascular endothelial cells were used as a positive control for vimentin. For S-100, the myenteric plexus of the GI tract, and for KIT, mastocytoma were used as positive controls. Parallel-stained samples without incubation of the primary antibodies were used as negative controls.

IH slides were evaluated microscopically to determine the extent of positive-stained tumor tissue, which was scored 0 (negative), 1 (<25% positive), 2 (26–50% positive) or 3 (>50% positive).

Tumor Classification

LM. Tumors were classified as LM when histologic malignancy criteria were absent,[2,12] combined with expression of either or both SMA and desmin, with or without vimentin, but without expression of S-100 and KIT. A score of 2, both in the combined malignancy criteria (nuclear pleomorphism, cellularity, mitotic score) and local tumor effect (necrosis, bleeding, neutrophilic inflammation) was considered the maximum for classification as histologically benign. Tumors with higher scores for either or both combinations were considered histologically malignant and further classified according to IH staining characteristics.

LMS. Tumors were classified as LMS when positive for either or both SMA and desmin, with or without vimentin, combined with negative staining for S-100 and KIT.[1,17,27]

GIST. Tumors were classified as GIST if they expressed KIT and vimentin[1] combined with either positive or negative staining for SMA, desmin, and S-100 according to human protocols [13,17,24] even if they had a histologically benign appearance. In contrast with previous veterinary[12] and human[13,30,31]reports where tumors with histological and IH appearance of GIST but without expression of KIT were classified GIST, we choose to classify these tumors as GIST-like tumors.

GIST-like. Tumors were classified as GIST-like if they lacked KIT expression but expressed vimentin, combined with either positive or negative staining for SMA, desmin, and S-100, regardless of histologically benign or malignant appearance. GIST and GIST-like tumors were further categorized based on their IH differentiation toward myogenic (SMA and vimentin positive), neurogenic (S-100 and vimentin positive), mixed (SMA, S-100 and vimentin positive), or anaplastic (only vimentin positive) tissue according to WHO standards.[1]

Survival Data

Follow-up information (recurrence, survival) was obtained by telephone interviews with the referring veterinarian or owner. Recurrence-free period (RFP) and survival time (ST) were calculated after surgical removal of the tumor. Dogs without surgical removal of the tumor (accidental finding during necropsy or irresectable mass) were excluded from these calculations. RFP was defined as the number of days between surgical resection and recurrence, signs consistent with, or diagnosis of, metastases. Patients that died of surgery or tumor-related causes (determined by physical and laboratory examination or necropsy) within 15 days of surgery were excluded from RFP calculations because this was considered a consequence of the original tumor and not a recurrence. ST was defined as days between surgical resection and death.

Statistical Analysis

For comparison of small intestinal and cecal tumors, frequency distributions were calculated and categorical data were compared using χ^2 analysis (large samples), or a Fisher's exact test (small samples). For continuous data (age, weight, tumor diameter, histological, and IH variables), means ($\pm$ SD) and 2-tailed significance were calculated and a 1-way ANOVA was used to determine differences between small intestinal and cecal locations. To determine significance of sex, castration or spaying, a Pearson's χ^2 was used. A 1-way ANOVA, post hoc multiple comparisons was used to determine significant differences between the histological and IH tumor scores after reclassification. After a test for homogeneity of variances, the Bonferroni test was used for variables with equal variances and a Tamhane's T2 test for the remaining nonequal variances. Results were considered statistically significant if $P < .05$.

Survival data were obtained by the Kaplan–Meier product limit method and significance was tested with the log-rank

test. The contribution of variables to the RFP (prognostic variables) was calculated using Cox's proportional hazards multivariate analysis. Entry into this model was determined by performing a univariate Cox's regression analysis with the Score test using the Newton–Raphson algorithm and the probability for entry was set at 0.10. Then a backward stepwise regression using the Newton–Raphson algorithm was used to design a significant model ($P < .05$). Hazard ratios (HR) > 1 indicate an increase of risk and HR < 1 indicate a positive influence on prognosis.

RESULTS

Dogs

Seventy-two dogs met the inclusion criteria; 35 were admitted to our hospital and 37 dogs were seen in veterinary practices. There were 47 small intestinal and 25 cecal tumors.

Tumor Location

Small Intestine. Tumor distribution was 15—duodenum, 21—jejunum, and 1—ileum. In 10 tumors, small intestine location was not specified but cecal involvement was ruled out by the histologic characteristics of the mucosa. Of the 47 dogs with small intestinal tumors, 42 had resectable masses treated by partial enterectomy and end-to-end anastomosis. Two dogs were euthanized because of nonresectable masses and poor physical condition. In 1 dog the mass was only biopsied, 1 dog had a gastroduodenostomy because of a nonresectable mass in the proximal duodenum, and in 1 dog the tumor was an incidental finding at necropsy.

Cecum. In this group, 19 dogs were treated by typhlectomy or partial enterectomy and ileocolic end-to-end anastomosis. Three dogs were euthanized intraoperatively because of a nonresectable mass and poor physical condition and in 3 dogs the mass was an incidental finding at necropsy.

Tumor Reclassification

Small intestinal tumors were originally classified as LMS in 40 dogs and LM in 7 dogs and cecal tumors as LMS in 23 dogs and LM in 2 dogs. Based on the criteria we used, 6 small intestinal and 3 cecal tumors originally diagnosed as LM were reclassified as malignant tumors (Tables 2 and 3). Seven small intestinal tumors had a histologic benign appearance, based on IH appearance 2 tumors were considered GIST and 3 were reclassified GIST-like tumors (Table 2). One small intestinal LMS was reclassified as LM. Nine of the small intestine tumors with a histologic malignant appearance could be classified according to IH staining characteristics as LMS but none of the cecal tumors. Nineteen small intestinal and 23 cecal tumors were reclassified as GIST based on expression of KIT and vimentin. The remaining 17 small intestinal and 2 cecal tumors had no detectable expression of KIT and were reclassified as GIST-like tumors. For the GIST and GIST-like tumors the IH differentiation is presented in Tables 2 and 3, with the small intestinal GIST being mostly undifferentiated (7) and most small intestinal GIST-like tumors (10) and cecal GIST (11) had mixed myogenic and neurogenic differentiation.

Clinical Data (Tables 4 and 5)

There were 34 breeds represented, of which mixed breed constituted the largest group (14), followed by Boxer (5) and Dachshund, Jack Russell Terrier, and Golden Retriever (4 each). There was no significant breed or sex predisposition for tumor type or location. Mean age for dogs with small intestinal tumors (9.1 ± 2.3 years) was significantly ($P = .003$) lower than for dogs with cecal tumors (10.7 ± 1.9 years).

Clinical signs and intraoperative findings are summarized in Table 5. All dogs with small intestinal tumors had at least 1 clinical sign whereas 4 dogs with cecal tumors had no clinical signs ($P = .012$). The tumor was either an incidental finding during abdominal palpation during an annual physical examination (3) or was observed at necropsy (1). Weight loss was observed more often in dogs with small intestinal tumors ($P = .020$) whereas intestinal perforation and peritonitis were observed more commonly in dogs with cecal tumors ($P = .002$ and .017, respectively).

In 45 dogs, diagnostic imaging was used to detect an intestinal lesion or tumor which was confirmed during surgery. In 27 dogs (60%) abdominal ultrasonography was performed and in 13 dogs (29%) abdominal radiography was used. In 5 dogs (11%) the results of abdominal radiography were inconclusive so abdominal ultrasonography was used to detect the tumor. In 1 dog, with a small intestinal tumor, hepatic metastases were observed during ultrasonography. Thoracic radiographs were performed in 23 dogs (51%) and in 1 dog, with a cecal tumor, pulmonary metastases were discovered. In both dogs, metastases were confirmed at necropsy.

Histologic Analysis (Table 6)

A significantly higher score for necrosis ($P = .011$) and bleeding ($P = .006$) was observed in the cecal tumor group. Between the different reclassified tumors several significant differences were observed, with strikingly higher scores in the small intestinal LMS for most histologic criteria.

Histologically, tumors ranged from benign well-differentiated masses with inapparent nucleoli and absence of

Table 2. Summary of Individual Immunohistologic Findings of Small Intestine Tumors with Light-Microscopic Smooth Muscle Characteristics, Combined with Results of Reclassification and Survival Data

Dog	SMA (0–3)	Desmin (0–3)	Vimentin (0–3)	S-100 (0–3)	KIT (0–3)	IH Dif GIST*	Tumor†	RFP (Days)	ST (Days)
1	0	3	1	0	0		LM	30	30
2	2	3	0	0	0		LM (LMS)	366	366
3‡	3	3	3	3	1	MN	G (LMS)	—	43
4‡	3	3	1	3	1	MN	G (LM)	2	2
5‡	3	3	1	1	0	MN	g (LM)	—	58
6‡	0	3	1	3	0	MN	g (LM)	587	603
7‡	0	3	3	3	0	MN	g (LM)	784	784
8	0	3	2	0	0		LMS	—	—
9	0	3	0	0	0		LMS	—	—
10	3	3	0	0	0		LMS	104	118
11	0	3	1	0	0		LMS	181	181
12	2	3	2	0	0		LMS	189	211
13	0	1	0	0	0		LMS	529	529
14	1	0	0	0	0		LMS	1093	1093
15	1	1	1	0	0		LMS	1373	1390
16	3	1	0	0	0		LMS	3491	3491
17	0	0	3	0	3	U	G (LMS)	4	4
18	0	3	3	0	3	U	G (LMS)	5	5
19	0	3	2	3	2	N	G (LMS)	12	12
20	1	3	1	3	3	MN	G (LMS)	61	214
21	0	0	3	0	3	U	G (LMS)	160	160
22	0	3	3	0	1	U	G (LMS)	446	446
23	3	3	1	3	3	MN	G (LM)	461	461
24	0	0	3	0	3	U	G (LMS)	638	638
25	0	0	3	0	1	U	G (LMS)	701	701
26	1	3	3	3	1	MN	G (LMS)	747	747
27	0	1	3	2	3	N	G (LMS)	826	826
28	0	2	3	2	3	N	G (LMS)	965	965
29	0	1	3	1	3	N	G (LMS)	1013	1108
30	1	3	3	3	1	MN	G (LMS)	1032	1032
31	0	3	3	3	3	N	G (LMS)	1147	1147
32	0	0	3	0	3	U	G (LMS)	1184	1218
33	0	0	3	2	3	N	G (LMS)	1519	1519
34	3	3	3	3	0	MN	g (LMS)	—	—
35	0	3	1	1	0	N	g (LMS)	2	2
36	3	3	3	3	0	MN	g (LM)	3	3
37	0	1	1	3	0	N	g (LMS)	4	4
38	0	0	3	1	0	N	g (LMS)	6	6
39	1	3	1	3	0	MN	g (LMS)	8	8
40	0	3	3	3	0	N	g (LMS)	24	24
41	0	1	1	1	0	N	g (LMS)	25	25
42	1	3	3	3	0	MN	g (LMS)	104	104
43	2	2	2	1	0	MN	g (LMS)	117	149
44	0	0	1	0	0	U	g (LMS)	135	135
45	3	3	3	3	0	MN	g (LMS)	325	325
46	0	1	3	2	0	N	g (LMS)	409	447
47	1	3	3	3	0	MN	g (LMS)	647	647

(Based on Head et al[1])

*Immunohistochemic differentiation for GIST according to the World Health Organisation: myogenic (M); neurogenic (N); combined myogenic and neurogenic (MN), or undifferentiated (U).

†Diagnosis after reclassification of the tumors. Tumors were leiomyoma (LM); leiomyosarcoma (LMS); gastrointestinal stromal tumors (GIST) (G); GIST-like tumor (g). The original diagnosis is shown in parentheses in the table if this was different from the new diagnosis.

‡Dog 3–7: These tumors showed a histological benign appearance (absence of nuclear pleomorphism, low cellularity, and low mitotic score) but were reclassified GIST (G) or GIST-like (g) based on the results of immunohistology.

RFP, recurrence-free period in days; ST, survival time in days.

Table 3. Summary of Individual Immunohistologic Findings of Cecum Tumors with Light-Microscopic Smooth Muscle Characteristics, Combined with Results of Reclassification and Survival Data

Dog	SMA (0–3)	Desmin (0–3)	Vimentin (0–3)	S-100 (0–3)	KIT (0–3)	IH Dif GIST*	Tumor†	RFP (Days)	ST (Days)
1	0	3	3	3	3	N	G (LMS)	—	—
2	2	0	2	1	3	MN	G (LMS)	—	—
3	3	1	3	3	3	MN	G (LMS)	—	—
4	0	3	1	1	3	N	G (LMS)	—	—
5	3	3	1	3	3	MN	G (LMS)	—	—
6	0	0	3	3	3	N	G (LMS)	—	—
7	1	3	3	0	3	M	G (LMS)	2	2
8	1	3	3	3	3	MN	G (LMS)	245	245
9	0	3	3	3	3	N	G (LMS)	369	428
10	2	3	3	3	3	MN	G (LM)	470	470
11	0	0	3	0	3	U	G (LMS)	495	495
12	0	2	3	1	3	N	G (LM)	515	515
13	0	3	3	3	3	N	G (LMS)	548	548
14	3	1	1	3	2	MN	G (LMS)	566	804
15	2	3	3	3	3	MN	G (LMS)	651	681
16	3	3	3	3	3	MN	G (LMS)	813	813
17	1	3	3	3	3	MN	G (LMS)	823	823
18	1	3	3	3	3	MN	G (LMS)	828	828
19	0	3	3	0	3	U	G (LMS)	867	867
20	2	3	1	2	3	MN	G (LMS)	1099	1099
21	0	3	3	0	3	U	G (LMS)	1204	1435
22	0	3	3	3	2	N	G (LMS)	1283	1283
23	0	3	3	2	3	N	G (LMS)	1393	1393
24	1	2	3	2	0	MN	g (LMS)	178	184
25	0	0	3	1	0	N	g (LM)	548	548

(Based on Head et al[1])

*Immunohistochemic differentiation for GIST according to the World Health Organisation: myogenic (M); neurogenic (N); combined myogenic and neurogenic (MN), or undifferentiated (U).

†Diagnosis after reclassification of the tumors. The following tumors were observed: gastrointestinal stromal tumors (GIST) (G); GIST-like tumor (g). The original diagnosis (LM, leiomyoma; LMS, leiomyosarcoma) is shown in parentheses in the table if different from the new diagnosis.

RFP, recurrence-free period in days; ST, survival time in days.

mitotic figures, necrosis, bleeding, or inflammation to malignant and undifferentiated (Fig 1A–E). The latter were characterized by a high cell count, with nuclear pleomorphism combined with multiple nucleoli/nucleus, presence of mitotic figures (Fig 1B and C), and sometimes combined with massive necrosis, bleeding, and/or inflammation. The cells could be spindle shaped and either arranged in well-organized bundles (Fig 1A), or an elaborate whirling pattern (Fig 1D), or both. In contrast to these very cellular tumors with compact cell type, tumor cells with an extensive cytoplasmatic vacuolization (Fig 1B and C) were observed in 23 dogs. These cells were significantly more common ($P < .001$) in cecal (60%, $n = 15$) than in small intestinal tumors (17%, $n = 8$). In 7 small intestinal (8.5%) and in 2 cecal tumors (8%) venous thrombi (Fig 1E) were observed.

IH analysis (Tables 2, 3, and 6)

Staining intensity for all 5 IH staining protocols ranged from very intense with strong punctuated or fibrillar brown staining (Fig 1F and G) to a vaguely positive cytoplasmic background staining (Fig 1H). Differences in distribution of positive-stained tissue were observed within tumors for all protocols. Tumor regions that appeared histologically identical contained a variety in staining intensity ranging from absolutely negative to strongly positive (Fig 1H). Positive internal controls were present in all sections and staining intensity was strong especially in unaffected intestinal wall at the muscular layers (SMA and desmin), myenteric plexuses (S-100), and vascular walls (vimentin). Staining of other tissues (e.g., normal muscular tissue, inflammation, or necrosis) and tissue near the margins of the specimen was observed, but not considered positive.

Survival Data (Tables 2 and 3)

Follow-up was available for all 42 surgically treated patients with small intestinal tumors and 19 dogs with cecal tumors. At last follow-up 3 dogs from the small intestinal group were still alive (446, 1093, and 3491 days) and 4 from the cecal tumor group (470, 813, 823, and

Table 4. Summary of Clinical Data Categorized by Small Intestine and Cecum Tumors

	Small Intestine	LM	LMS	GIST	GIST-Like Tumor	Cecum	GIST	GIST-Like Tumor
Age, mean ± SD (range; number of dogs)	9.1 ± 2.3[A] (4.0–12.6; 47)	7.2 ± 2.8 (5.2–9.2; 2)	8.5 ± 2.5 (4.0–12.5; 9)	9.0 ± 2.0 (6.2–12.6; 19)	9.8 ± 2.3 (4.6–12.2; 17)	10.7 ± 1.9[A] (6.0–13.6; 25)	10.9 ± 1.8 (7.3–13.6; 23)	8.1 ± 3.0 (6.0–10.3; 2)
Bodyweight (kg), mean ± SD (range; number of dogs)	24.6 ± 14.4 (5–69; 38)	7 (–; 1)	25.4 ± 14.5 (5–40; 8)	27.6 ± 16.4 (6.3–69; 16)	21.7 ± 11.8 (6.5–40; 13)	23.1 ± 11.2 (8.7–48; 20)	23.3 ± 11.8 (5.7–48; 18)	27 ± 1.4 (26–28; 2)
Male intact	15	—	4	6	5	7	7	—
Male castrated	7	1	2	3	1	7	7	—
Female intact	9	—	—	3	6	5	4	1
Female spayed	16	1	3	7	5	6	5	1

[A] $P = .003$ (1-way ANOVA).
LM, leiomyoma; LMS, leiomyosarcoma; GIST, gastrointestinal stromal tumors.

1393 days). All 7 dogs were free of clinical signs associated with possible recurrence. In the small intestine group, 12 dogs (29%) had recurrence of clinical signs, tumor, or signs of metastases; 3 were euthanized. Median survival after recurrence in the other dogs was 32 days (range, 13–153 days). Recurrence occurred in 7 (37%) cecal tumor dogs; 2 were euthanized. Median survival after recurrence in the other dogs was 59 days (range, 6–238 days). Necropsy results were not available.

Ten dogs (9 [21.4%] small intestine, 1 [5.3%] cecal tumor) died or was euthanized within 15 days of surgery because of surgery or tumor-related complications determined by interpretation of results from physical, laboratory, or necropsy examinations (e.g., septic peritonitis, renal, or multiple organ failure). For the other 33 dogs with small intestinal and 18 with cecal tumor, 1- and 2-year RFP were calculated. For dogs with a small intestine tumor the 1- and 2-year RFP was 80.1% (95% confidence interval [CI]: 60.9–90.6%) and 67.2% (95% CI: 45.8–81.7%). For the dogs with cecal tumor this was 83.3% (95% CI: 56.8–94.3%) and 61.9% (95% CI: 33.2–81.1%), respectively (Fig 2).

Calculated 1- and 2-year ST for the 42 surgically treated dogs with a small intestine tumor was 62.6% (95% CI: 45.6–75.6%) and 52.3% (95% CI: 34.8–67.2%) whereas for the 19 dogs with a cecal tumor this was 84.2% (95% CI: 58.6–94.6%) and 66.0% (95% CI: 38.7–83.3%), respectively.

There was no significant difference between locations or between the different tumor groups after reclassification for RFP or ST. None of the single or combined histologic or IH variables had significant influence on RFP and ST.

Prognostic Variables

From the clinical data, histologic and IH variables used in the multivariate analysis, 4 variables had a significant ($P < .001$) influence on prognosis: weight loss, painful abdomen, tumor diameter, and castration or spaying. Tumor diameter was not reported in 8 surgically treated dogs, so only data from 53 dogs was used in the multivariate analysis. Increased HR were observed for weight loss (HR = 4.72, 95% CI: 1.25–17.89%; $P = .022$), painful abdomen (HR = 4.43, 95% CI: 1.24–15.75%; $P = .021$) and tumor diameter (cm) (HR = 1.3, 95% CI: 1.01–1.26%; $P = .030$). A decreased HR was observed for dogs that had already been castrated or spayed (HR = 0.27, 95% CI: 0.08−0.88%; $P = .029$). None of the single or combined histologic or IH variables had a significant influence on prognosis.

Table 5. Summary of Clinical Signs and Tumor Diameter for Small Intestine and Cecum Tumors

Clinical Signs	Small Intestine (47)	LM (2)	LMS (9)	GIST (19)	GIST-Like Tumor (17)	Cecum (25)	GIST (23)	GIST-Like Tumor (2)
Any sign	47 (100%)[A]	2 (100%)	9 (100%)	19 (100%)	17 (100%)	21 (84%)[A]	19 (82.6%)	2 (100%)
Anorexia	19 (40.4%)	0 (0%)	4 (44.4%)	6 (31.6%)	9 (52.9%)	12 (48%)	12 (52.2%)	0 (0%)
Lethargy	21 (44.7%)	1 (50%)	5 (55.6%)	6 (31.6%)	9 (52.9%)	15 (60%)	14 (60.9%)	1 (50%)
Vomiting	26 (55.3%)	1 (50%)	5 (55.6%)	9 (47.3%)	11 (64.7%)	12 (48%)	12 (52.2%)	0 (0%)
Diarrhea	15 (31.9%)	1 (50%)	2 (22.2%)	6 (31.6%)	6 (35.2%)	6 (24%)	5 (21.7%)	1 (50%)
Melena	15 (31.9%)	0 (0%)	3 (33.3%)	8 (42.1%)	4 (23.5%)	5 (20%)	4 (17.4%)	1 (50%)
Painful abdomen	12 (25.5%)	2 (100%)	2 (22.2%)	5 (26.3%)	3 (17.6%)	5 (20%)	4 (17.4%)	1 (50%)
Palpable abdominal mass	31 (66%)	1 (50%)	7 (77.8%)	13 (68.4%)	10 (58.8%)	18 (72%)	17 (73.9%)	1 (50%)
Weight loss	12 (25.5%)[B]	0 (0%)	3 (33.3%)	3 (15.7%)	6 (35.2%)	1 (4%)[B]	1 (4.3%)	0 (0%)
Anemia	18 (38.3%)	0 (0%)	4 (44.4%)	8 (42.1%)	6 (35.2%)	5 (20%)	4 (17.4%)	1 (50%)
Hypoglycemia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)	2 (9%)	0 (0%)
Hemoabdomen	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)	2 (8.7%)	0 (0%)
Intestinal perforation	2 (4.3%)[C]	1 (50%)	0 (0%)	1 (5.2%)	0 (0%)	8 (32%)[C]	7 (30.4%)	1 (50%)
Peritonitis	3 (6.4%)[D]	1 (50%)	1 (11.1%)	1 (5.2%)	0 (0%)	7 (28%)[D]	6 (26.1%)	1 (50%)
Intussusception	5 (10.6%)	0 (0%)	1 (11.1%)	0 (0%)	4 (23.5%)	0 (0%)	0 (0%)	0 (0%)
Tumor diameter (cm) Mean ± SD	6 ± 4.0	5 ± 1.4	6.1 ± 5.0	6.3 ± 4.7	5.6 ± 2.7	7.82 ± 3.18	7.8 ± 3.3	8.0 ± 0*
Range	1–18	4–6	3–18	1–15	1–10	2.5–15	3–15	—

Clinical signs were scored in all patients: 47 small intestine and 25 cecum tumors.

*Tumor diameter was available for 42 small intestinal tumors (2 LM, 8 LMS, 18 GIST, and 14 GIST-like tumors) and 17 cecal tumors (15 GIST and 2 GIST-like tumors both with the same diameter).

The P value is showed to identify statistically significant differences between the small intestinal and cecal group.

[A]$P = .012$ Fisher's exact test.

[B]$P = .020$ Fisher's exact test.

[C]$P = .002$ Fisher's exact test.

[D]$P = .017$ Fisher's exact test.

LM, leiomyoma; LMS, leiomyosarcoma; GIST, gastrointestinal stromal tumors.

DISCUSSION

One of the most striking conclusions of our study is that tumor type, and histologic and IH characteristics had no significant influence on prognosis after surgical removal. Dogs with tumors that have a very malignant histologic appearance can expect long-term survival if complete excision of the tumor is accomplished, regardless of diagnosis.

The signalment of affected dogs was consistent with earlier reports[5,7,10,32] except for age of dogs with small intestine tumors, which was less than previously reported. As a whole, the observed clinical signs were identical to previous reports.[7,10,12] The more proximal location of a tumor likely makes interference with normal GI activity more probable for small intestinal than cecal tumors and may explain the higher incidence of weight loss and earlier presentation. The more caudal location and indirect position of the cecum relative to passage of ingesta is thought to be responsible for the later occurrence of clinical signs in dogs with cecal tumors. This may also result in larger tumors before diagnosis and therefore a higher chance of intestinal perforation and peritonitis than occurred with small intestine tumors in this study. Histologically this was supported by higher scores for necrosis and bleeding in cecal tumors. Tumor diameter was an important prognostic variable and is consistent with findings in human patients, where increased tumor size combined with a high mitotic index are used as negative prognostic indicators.[13] No significant importance was observed for the mitotic count in our study.

Dogs that were castrated or spayed had better survival statistics than intact dogs. Neutering is known to influence tumor growth and prognosis in several neoplastic diseases including vulvar or vaginal LM,[33] malignant mammary tumors,[34,35] perianal gland neoplasia,[36] and prostate carcinoma,[37,38] but to our knowledge this effect has not been reported for these intestinal tumors types in dogs. Human GIST are reported to express transforming growth factor-α (TGF-α) and epidermal growth factor receptor (EGFR). Activation of EGFR by TGF-á is associated with cell growth and transformation,[39] and this activation can be influenced by sex hormones.[40] Elimination of sex hormones by castration or spaying may be an explanation for the positive effect on prognosis we observed.

If our IH results are compared with published reports in dogs[2,12] there are similarities but also major differences. The total percentage of KIT expressing small intestinal and cecal tumors in our dogs (58%; Tables 2 and 3) is comparable to Frost et al[12] (54%); however, the lack of expression of desmin and S-100 reported in that study

Table 6. Summary of Mean (± SD) Values for Histologic and Immunohistologic Findings for Small Intestine and Cecum and Individual Reclassified Tumor Groups

	Small Intestine	LM (2)	LMS (9)	GIST (19)	GIST-Like Tumor (17)	Cecum	GIST (23)	GIST-Like Tumor (2)
Nuclear pleomorphism (0–2)	0.94 (0.94)	0 (0)	1.56 (0.88)^{A}	0.53 (0.84)^{a}	1.06 (0.90)	1.08 (0.81)	1.17 (0.78)	0 (0)
Differentiation (0–2)	0.43 (0.65)	0 (0)	1.11 (0.78)^{B}	0.11 (0.32)^{b}	0.41 (0.62)	0.52 (0.59)	0.57 (0.59)	0 (0)
Mitotic count	2.09 (2.36)	0.5 (0.71)	4.56 (1.51)^{C}	1.42 (1.80)^{c}	1.65 (2.60)^{c}	1.24 (0.93)	1.30 (0.93)^{c}	0.50 (0.71)
Mitotic score (0–2)	1.08 (0.85)	0.5 (0.71)	1.89 (0.33)^{A}	0.89 (0.81)^{a}	0.88 (0.86)^{a}	1.08 (0.57)	1.09 (0.60)	1 (0)
Cellularity (0–3)	1.66 (1.22)	0 (0)	2.00 (1.12)	2.00 (1.20)	1.35 (1.22)	1.76 (0.66)	1.70 (0.63)	2.50 (0.71)
Nucleoli (0–4)	1.11 (1.07)	1.00 (0)	2.11 (1.27)^{AB}	0.63 (0.76)^{b}	0.94 (0.90)^{a}	1.12 (0.67)	1.13 (0.63)	1 (1.41)^{a}
Necrosis (0–4)	1.89 (1.42)^{E}	2.00 (1.41)	2.67 (1.32)	1.68 (1.42)	1.76 (1.39)	2.76 (1.20)^{E}	2.78 (1.24)	2.50 (0.71)
Bleeding (0–4)	1.09 (1.41)^{F}	0 (0)^{CD}	0.67 (1.12)	1.37 (1.50)^{c}	1.12 (1.50)	2.04 (1.24)^{F}	2.17 (1.19)^{d}	0.50 (0.71)
Neutrophilic inflammation (0–4)	2.23 (1.40)	1.00 (0)^{CD}	3.00 (1.12)^{d}	1.79 (1.51)	2.35 (1.22)^{d}	2.00 (1.50)	2.00 (1.45)^{c}	2.00 (2.83)
Lymphocytic inflammation (0–4)	0.43 (0.74)	1.00 (1.41)	0 (0)	0.58 (0.84)	0.41 (0.71)	0.56 (0.82)	0.52 (0.85)	1 (0)
Nuclear pleomorphism + cellularity + mitotic score (0–7)	3.64 (1.94)	0.50 (0.71)^{C}	5.44 (1.88)^{c}	3.42 (1.46)	3.29 (1.83)	3.92 (1.29)	4.04 (1.19)	2.50 (2.12)
Necrosis + bleeding + neutrophilic inflammation (0–12)	5.19 (3.23)^{G}	3.00 (1.41)	6.33 (2.55)	4.84 (3.85)	5.24 (2.91)	6.80 (2.81)^{G}	6.83 (2.74)	6.50 (4.95)
SMA (0–3)	0.87 (1.19)	1.00 (1.41)	1.11 (1.27)	0.63 (1.12)	1.00 (1.27)	1.00 (1.16)	1.04 (1.19)	0.50 (0.71)
Desmin (0–3)	2.02 (1.28)	3.00 (0)	2.00 (1.23)	1.79 (1.40)	2.18 (1.24)	2.28 (1.17)	2.39 (1.12)	1.00 (1.41)
Vimentin (0–3)	2.00 (1.18)^{H}	0.50 (0.71)	0.67 (0.87)^{CD}	2.63 (0.76)^{d}	2.18 (1.07)^{c}	2.60 (0.87)^{H}	2.57 (0.90)^{d}	3.00 (0)^{d}
S-100 (0–3)	1.38 (1.36)^{I}	0 (0)	0 (0)^{AB}	1.63 (1.38)^{a}	2.00 (1.17)^{b}	2.08 (1.19)^{I}	2.13 (1.22)^{b}	1.50 (0.71)
KIT (0–3)	1.00 (1.32)^{J}	0 (0)^{B1}	0 (0)^{B2}	2.32 (0.95)^{B3b1b2}	0.18 (0.73)^{B4b3}	2.68 (0.85)^{J}	2.91 (0.29)^{B5b1b2b4}	0 (0)^{b3b5}

The range of possible scores for each variable is shown in parentheses in the first column.

1-way ANOVA, post hoc multiple comparison was performed to determine significant differences between the reclassified tumors, after a test for homogeneity of variances (Bonferroni's test for equal variances and Tamhane's T2 test for non-equal variances). Significant findings are consecutively numbered if more than one combination is present per variable (e.g. B1 versus b1 and B2 versus b2):

$^{A}P < .05$ for A versus a, Bonferroni's test.

$^{B}P \leq .01$ for B versus b, Bonferroni's test.

$^{C}P < .05$ for C versus c, Tamhane's T2 test.

$^{D}P \leq .01$ for D versus d, Tamhane's T2 test.

The P value is showed to identify statistically significant differences between the small intestinal and cecal group using a 1-way ANOVA:

$^{E}P = .011$ 1-way ANOVA.

$^{F}P = .006$ 1-way ANOVA.

$^{G}P = .039$ 1-way ANOVA.

$^{H}P = .028$ 1-way ANOVA.

$^{I}P = .034$ 1-way ANOVA.

$^{J}P < .001$ 1-way ANOVA.

LM, leiomyoma; LMS, leiomyosarcoma; GIST, gastrointestinal stromal tumors.

contrasts sharply with our results (82% expression of desmin, 68% of S-100; Tables 2 and 3) and LaRock and Ginn[2] (80% expression of desmin, 73% of S-100). Possible explanations for these differences may be: (1) different antibody manufacturers and production methods; (2) antibody (in)compatibility with canine tissue; (3) suboptimal antigen preservation[12]; (4) suboptimal or abnormal antigen expression in tumors; and (5) different populations (a population of domestic dogs in our study versus a population with 50% military working dogs).[12] In our study IH findings were given priority over histological appearance if tumors had a histological benign appearance combined with IH characteristics suggestive of differentiation other than strictly myogenic.

From the IH analysis we report, and from other studies, we conclude that GIST[1,2,12] and GIST-like tumors can display differentiation in several directions. Evidence of mixed myogenic and neurogenic differentiation is most suggestive of a relationship with primitive mesenchymal cells capable of pluripotential differentiation.[1] ICC are part of the intestinal pacemaker system placing them in close contact with smooth muscle cells and nerve fibers. ICC, like GIST express KIT, which makes involvement of ICC in KIT-expressing tumors likely.[15,17] The close relationship of ICC with smooth muscle and neural tissue may also explain the mixed myogenic and neurogenic differentiation of these tumors.[1,15] Differentiation only in myogenic direction without KIT expression is an indication of smooth muscle origin (e.g., LM or LMS) and solitary differentiation in a neurogenic direction without KIT expression can be suggestive of a tumor of neural origin (e.g., peripheral nerve sheath tumor).[1]

Tumors that histologically resemble GIST but lack KIT expression were observed in our dogs and have been

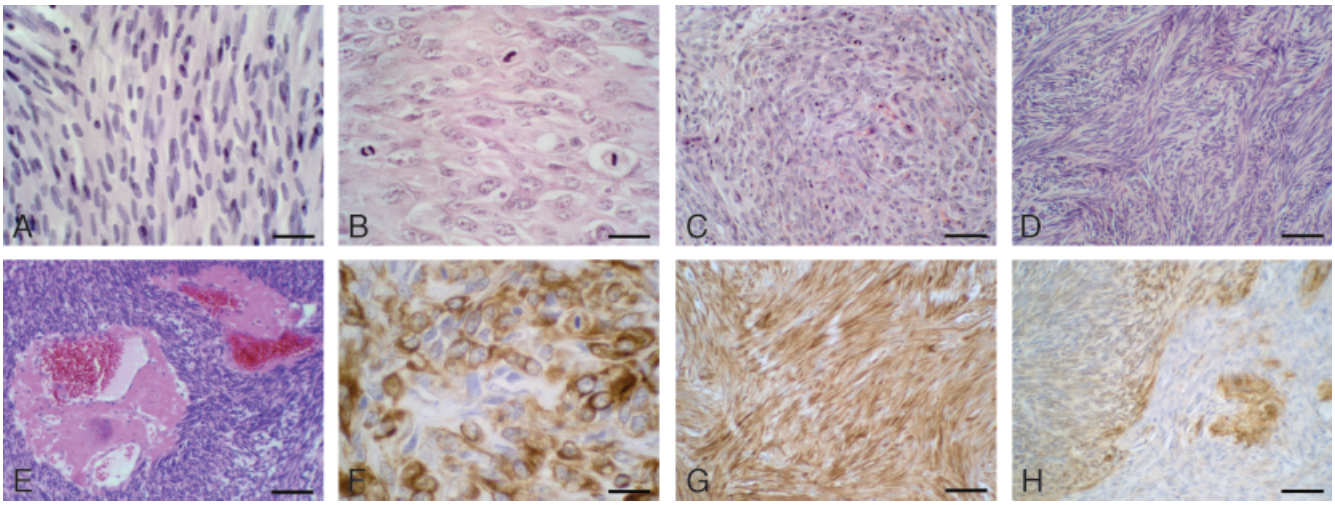

Fig 1. (A) Small intestinal gastrointestinal stromal tumors (GIST)-like tumor. The tumor is highly cellular and well differentiated with spindle-shaped cells organized in bundles. Hematoxylin and eosin (HE) × 500, bar = 25 µm. (B) Small intestinal GIST-like tumor. The tumor has poor differentiation, nuclear pleomorphism with multiple nucleoli, cytoplasmatic vacuolization, and several mitoses. HE × 500, bar = 25 µm. (C) Small intestinal leiomyosarcoma. The poorly differentiated tumor demonstrates nuclear pleomorphism with prominent nucleoli, mitoses, and vacuolization. HE × 200, bar = 62.5 µm. (D) Small intestinal GIST-like tumor. The highly cellular, well-differentiated tumor has spindle-shaped cells arranged in bundles with extensive whirling pattern. HE × 200, bar = 62.5 µm. (E) Small intestinal GIST-like tumor. The highly cellular tumor has venous thrombi showing recanalization. HE × 100, bar = 200 µm. (F) Small intestinal GIST-like tumor. The tumor has nuclear pleomorphism with stromal vacuolization and mitotic figures. The intensity of the punctuated cytoplasmatic staining varies per individual cell. Vimentin × 500, bar = 25 µm. (G) Small intestinal GIST-like tumor. The highly cellular tumor has spindle-shaped cells displaying strong fibrillar, cytoplasmatic staining. Desmin × 200, bar = 62.5 µm. (H) Small intestinal tumor GIST-like tumor. The highly cellular tumor has a whirling pattern. The intensity of the stain in this section changes between low, moderate, and high. S-100 × 200, bar = 62.5 µm.

reported in veterinary[12] and human patients.[30,31] These tumors were classified as GIST-like tumors in our study to distinguish them from "true" GIST. Because no effect on survival could be attributed to the histologic and IH features seemingly differentiation of tumors as LM, LMS, GIST, or GIST-like tumor for clinical use is of less importance. This is not to suggest that (re)classification is of not important but rather that a widely accepted system should be developed to facilitate comparison of previous and future studies. Thus we suggest the following method for (re)classification of intestinal tumors with light microscopic appearance of smooth muscle.

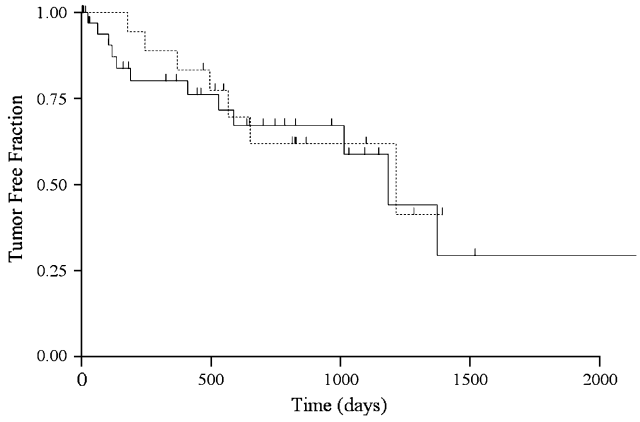

Fig 2. Kaplan-Meier cumulative curve of the recurrence-free period showing the tumor free fraction for the group of patients with a tumor in the small intestine (straight line) and in the cecum (dotted line).

Recommended Classification Scheme

A tumor is classified LM if it has a histologically benign appearance, combined with expression of either or both SMA and desmin, with or without expression of vimentin, and lack of S-100 or KIT expression. If this tumor with histological benign appearance is expressing KIT and vimentin, or S-100 and vimentin it is reclassified, respectively, as GIST or GIST-like. LMS are defined as tumors with histological malignant appearance combined with expression of either or both SMA and desmin, with or without expression of vimentin, and without expression of S-100 and KIT.[17,27] GIST are characterized by expression of KIT and vimentin, either with or without expression of SMA, desmin, and S-100.[2,12,16,18] GIST-like tumors are histologically identical to GIST and are char-

acterized by expression of vimentin, either with or without expression of SMA, desmin, and S-100, but lack expression of KIT.

Determining whether or not a tumor is KIT positive is essential in human patients because of the possibility of medical treatment with imatinib mesylate (Gleevec®, Novartis, Basel, Switzerland) when the tumor is nonresectable, or there is recurrence after surgery or metastases.[15] Although complete gross resection of primary, localized GIST appears to be possible in $\sim$ 85% of human cases, with negative microscopic margins in 70–95% of patients, at least 50% of the patients have recurrence.[41] Results of trials with imatinib mesylate seem promising with partial responses ranging between 48% and 67% and stable disease in 15–32% of treated patients.[41] For dogs with nonresectable masses, recurrences, and/or metastases, this additional therapy may be of importance in the future. To select candidates (dogs with tumors expressing KIT) we recommend use of the IH classification scheme we describe. Studies investigating whether this drug can be efficacious in dogs are needed.

ACKNOWLEDGMENTS

We thank the sponsors for their contributions, Dr. E. Teske for his assistance with the statistical analysis and A.M. van Ederen and coworkers from the Department of Pathobiology for their assistance with the IH staining.

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