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# Tyrosine Kinase Inhibitor as Clinical Application Feasibility in Canine Intractable Tumor Diseases

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**Abstract :** A tyrosine kinase is an enzyme that can transfer a phosphate group from ATP to a protein in a cell. It functions as an "on" or "off" switch in many cellular functions. This study aims to show that the actions of growth factors associated with PDGFR-α, PDGFR-β, VEGFR-2, c-KIT, and c-ABL, which are used in veterinary medicine, are expressed in canine intractable tumors. This study used archival cases of canine paraganglioma, gastrointestinal adenocarcinoma, hepatocellular carcinoma, and renal cell carcinoma. Tissues had been immunohistochemical analysis. The antibodies used were PDGFR-α, PDGFR-β, c-kit, VEGFR-2, and c-Abl. PDGFR-α was expressed only in HCC, and PDGFR-β was expressed in all tumors. VEGFR was also only expressed in HCC, and c-KIT has been expressed in HCC, paraganglioma, and small intestinal adenocarcinoma. c-Abl was expressed in all cancers, but was weakly expressed in paraganglioma, while more than moderately expressed in other tissues. In conclusion, this study investigated how TKIs used in human medicine can be applied to canine intractable tumors, through immunohistochemistry. The results indicate that there may be an application for TKIs in treating canine intractable tumors.

***Key words*** **:** tumor, dog, tyrosine kinase inhibitor, immunohistochemistry.

## Introduction

A tyrosine kinase is an enzyme that can transfer a phosphate group from ATP to a protein in a cell. It functions as an "on" or "off" switch in many cellular functions. Tyrosine kinases are a subclass of protein kinase. Angiogenesis plays a pivotal role in the growth of most solid tumors and also contributes to the progression of tumor metastasis (7,18). Vascular endothelial growth factor (VEGF), its receptor tyrosine kinases VEGFR-2 and kinase insert domain receptor (KDR) are key regulators of angiogenesis. Knowledge about biochemical cellular dysfunctions has advanced the discovery of targeted therapy, such as masitinib, in several fields of research both in veterinarian and human medicine, from inflammatory (rheumatoid arthritis, inflammatory bowel disease, asthma, atopic dermatitis) or degenerative-chronic disease (Alzheimer's disease) to cancer.

TKIs with antiangiogenic and antitumor activity target the platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), KIT, and FLT3. PDGFRs and platelet-derived growth factors (PDGFs) are known to play a crucial role in the pathogenesis, invasion, and distant metastasis of human cancers, and recent studies have suggested their involvement in an autocrine or paracrine loop that causes tumor growth and progression in osteosarcomas (OSAs) (26,36,38).

Platelet-derived growth factor (PDGF) is a peptide regulatory growth factor. Dimeric PDGF isoforms are composed of four different PDGF chains (A, B, C, and D). The A and B chains may unite to form three possible isoforms (PDGF-AA, PDGF-AB, and PDGF-BB), whereas the PDGF-C and -D chains form homodimers (12). The PDGFRs consist of an α and an β subunit that dimerize on binding of the PDGF isoforms. PDGF-A and PDGF-C bind selectively to PDGFR-α, whereas the PDGF-B chain binds and dimerizes with both PDGFR-α and PDGFR-β (12).

Sunitinib (a TKI) also inhibits two additional receptor tyrosine kinases (RTKs) that are activated in multiple cell types within the pNF microenvironment, including: PDGFR and VEGFR (33,34). Targeting these two RTKs in other human cancers with similar molecular aberrations to pNF has shown some efficacy in both animal models and ear-ly human clinical trials (11,13). Tyrosine kinase dysfunction occurs frequently in human cancers, and recent studies have indicated that a similar pattern of dysfunction can be observed in canine and feline cancers (17,20). TKIs that are specific for the c-KIT receptor and others are currently used in the treatment of canine mast cell tumors with excellent results (22).

c-Abl was first discovered as the normal cellular counter-

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part to a virally encoded gene, v-Abl, which is found in the murine leukemia virus. c-Abl is a large protein (approximately 1150 residues). Human cells express two alternative splice variants of c-Abl (Abl 1a and Abl 1b), which differ only at the very N-terminal region; Abl 1b is myristoylated, whereas Abl 1a is not known to be. c-Abl is expressed in almost all types of cells and carries out functions in both the nucleus and the cytoplasm. Some of its normal cellular functions in the nucleus include mediating cell differentiation, cell division, apoptosis, and the stress response to DNA damage. In the cytoplasm, it is thought to mediate integrin binding (39).

The TKIs toceranib phosphate, masitinib mesylate, and imatinib mesylate have been successfully used in dogs and, more recently, imatinib mesylate has been used in cats.

Toceranib phosphate exhibits potent inhibitory activity against members of the split-kinase receptor family, including VEGFR, PDGFR, and KIT, and was therefore predict-ed to have both anti-angiogenic and direct antitumor activity. This study enrolled 57 dogs with a variety of cancers including carcinomas, sarcomas, mast cell tumors (MCTs), melanomas, and lymphomas, among others (21). Masitinib mesylate is another TKI that primarily targets KIT and, possibly, PDGFR. An open-label phase II study of masitinib mesylate was completed in dogs with grade II and III MCTs (9). A feline tumor type that may also benefit from imatinib mesylate is vaccine-associated sarcoma (VAS). As previously mentioned, VAS cell lines were shown to express PDGFR-β, and imatinib mesylate was shown to block PDGF-induced phosphorylation in these cells (16).

This study aims to show that the actions of growth factors associated with PDGFR-α, PDGFR-β, VEGFR-2, c-KIT, and c-ABL, which are used in veterinary medicine, are expressed in canine intractable tumors.

## Materials and Methods

### Sample collection and clinical follow-up

This study used archival cases of canine paraganglioma, gastrointestinal adenocarcinoma, hepatocellular carcinoma, and renal cell carcinoma from the Small Animal Tumor Diagnostic Center of Konkuk University (Seoul, Korea) between 2004 and 2014. The initial diagnoses had been made with hematoxylin and eosin (HE)-stained sections.

**Table 1.** Clinical and histological characteristics of the Tumors

| Antibody | Buffer | Method |
|---|---|---|
| PDGFR-α | Citric acid, pH 6.0 | HIER (microwave), 5min |
| PDGFR-β | Citric acid, pH 6.0 | HIER (microwave), 20min |
| c-Kit | Tris-EDTA, pH 9.0 | HIER (microwave), 15min |
| VEGFR-2 | Citric acid, pH 6.0 | HIER (microwave), 5min |
| c-Abl | Citric acid, pH 6.0 | HIER (microwave), 5min |

RT; Room temperature

### Immunohistochemistry

Core tissues (2 mm in diameter) were taken from representative formalin-fixed paraffin-embedded tissue blocks. Tissues underwent heat-induced epitope retrieval (HIER) for further immunohistochemical analysis.Immunostaining was evaluated semi-quantitatively for intensity (0 = negative; +1 = mild positive; +2 = moderate positive; +3 = strong positive) and distribution (0 = negative 0%; +1 = scant < 10%; +2 = moderate < 50%; +3 = widespread > 50%) by two experienced pathologists. The positive control tissues were the following: PDGFR-α = canine cerebellum; PDGFR-β = canine kidney; c-KIT = canine mast cell tumor; VEGFR-2 = canine mammary adenocarcinoma; and c-Ab = canine mammary adenocarcinoma. All samples had been fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Sections were dewaxed in xylene, hydrated through graded ethanol, and washed three times in phosphate-buffered saline (PBS; pH 7.4, 137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4). Endogenous peroxidase was blocked by incubating the sections in hydrogen peroxide 3% solution diluted in PBS for 20 min at room temperature. After three more washes in PBS, PDGFR-α antigens were retrieved by boiling the sections in a citric acid buffer (pH 6) for 5 min in a microwave oven (650 W, high power). After three further washes in PBS, PDGFR-β antigens were retrieved by boiling the sections in a citric acid buffer (pH 6) for 20 min in a microwave oven (650 W, high power). After three more washes in PBS, c-KIT antigens were retrieved by boiling the sections in Tris-EDTA buffer (pH 9) for 15 min in a microwave oven (650 W, high power). After three further washes in PBS, VEGFR-2 antigens were retrieved by boiling the sections in a citric acid buffer (pH 6) for 5 min in a microwave oven (650 W, high power). After another three washes

**Table 2.** Primary antibodies used for immunohistochemistry

| Antibody | Type | Cell line | Source | Dilution | Incubation time | Positive control tissue |
|---|---|---|---|---|---|---|
| PDGFR-α | Polyclonal-Rabbit IgG | LS-B6056 | LS-Bio | 1:2000 | 2h, RT | Canine cerebellum |
| PDGFR-β | Polyclonal-Rabbit IgG | ab107169 | Abcam | 1:700 | 2h, RT | Canine kidney |
| VEGFR-2 | Abcam (ab2349), Polyclonal- Rabbit IgG | ab2349 | Abcam | 1:100 | 1h 30min, RT | Canine mammary adenocarcinoma |
| c-Kit | Polyclonal-affinity isolated Rabbit antibody | A4502 | DAKO | 1:300 | 3h, RT | Canine mast cell tumor |
| c-Abl | Polyclonal-Rabbit IgG | sc-131 | Santa cruze biotechnology | 1:800 | 1h 30min, RT | Canine mammary adenocarcinoma |

RT, Room temperature

in PBS, c-Abl antigens were retrieved by boiling the sections in a citric acid buffer (pH 6) for 5 min in a microwave oven (650 W, high power) (Table 1). After cooling, slides were washed three times in PBS. Endogenous peroxidase was blocked by incubating the sections in hydrogen peroxide with 5% normal goat serum in PBS for 30 min at room temperature. Subsequently, sections were overlaid with the primary antibody (PDGFR-α [LS-B6056, 1:2000, LS-bio, Seattle, WA], PDGFR-β [ab107169, 1:700, Abcam, Cambridge, UK], c-KIT [A4502, 1:300, DAKO, Carpinteria, CA], VEGFR-2 [ab2349, 1:100, Abcam, Cambridge, UK], and c-Abl [SC-131, 1:800, Santa Cruz Biotechnology, Santa Cruz, CA]) diluted in PBS (Table 2). The sections were washed three more times in PBS and secondary antibody

**Table 3.** Clinical and histological characteristics of the Tumors

| Animal ID | Breed | sex | age | Category | diagnosis |
|---|---|---|---|---|---|
| 1 | Jindo | F | 8 | Paraganglioma | Aortic body carcinoma (Metastasized) |
| 2 | Boston terrier | CM | 7 | Paraganglioma | Tumor of the chemoreceptor organ |
| 3 | Shih tzu | IF | 12 | Paraganglioma | Carotid body carcinoma (Metastasized) |
| 4 | Pekingese | CM | 12 | Paraganglioma | Aortic body carcinoma (Metastasized) |
| 5 | NR | SF | NR | Gastrointestinal adenocarcinoma | Small intestinal adenocarcinoma |
| 6 | Schnauzer | SF | 9.1 | Gastrointestinal adenocarcinoma | Large intestinal(Colorectal) adenocarcinoma |
| 7 | Shih tzu | SF | 12 | Gastrointestinal adenocarcinoma | Gastric adenocarcinoma (Tubular type) |
| 8 | Shih tzu | Neu | 12 | Gastrointestinal adenocarcinoma | Intestinal adenocarcinoma (Ileocecal region) |
| 9 | Yorkshire terrier | IM | 13 | Hepatocellular carcinoma | Hepatocellular carcinoma |
| 10 | Poodle | F | 11 | Hepatocellular carcinoma | Hepatocellular carcinoma |
| 11 | Maltese | SF | 14 | Hepatocellular carcinoma | Hepatocellular carcinoma, Glycogen degeneration |
| 12 | Maltese | SF | 9 | Hepatocellular carcinoma | Hepatocellular carcinoma (Metastasized) |
| 13 | Dachshund | NR | > 8 | Renal cell carcinoma | Renal cell carcinoma (Papillary type) |
| 14 | Cocker spaniel | CM | 13 | Renal cell carcinoma | Renal cell carcinoma (Solid type) |
| 15 | Mixed | IM | 6.2 | Renal cell carcinoma | Renal cell carcinoma (Tubulopapillary type) |
| 16 | Cocker spaniel | Neu | 10 | Renal cell carcinoma | Renal cell carcinoma (Tubulopapillary type) |

CM, Castrated male; IM, Intact male; IF, Intact female; NR, not recorded.

**Table 4.** Expression of angiogenesis-related proteins

| ID | Category | PDGFR-α | | PDGFR-β | | VEGFR | | C-kit | | Abl | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | D | I | D | I | D | I | D | I | D |
| 1 | Paraganglioma | 0 | 0 | 3+ | 3+ | 0 | 0 | 0 | 0 | 1+ | 1+ |
| 2 | Paraganglioma | 0 | 0 | 3+ | 3+ | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | Paraganglioma | 0 | 0 | 2+ | 2+ | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | Paraganglioma | 0 | 0 | 3+ | 2+ | 0 | 0 | 1+ | 1+ | 0 | 0 |
| 5 | Gastrointestinal adenocarcinoma | 0 | 0 | 3+ | 3+ | 0 | 0 | 1+ | 1+ | 2+ | 2+ |
| 6 | Gastrointestinal adenocarcinoma | 0 | 0 | 1+ | 2+ | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | Gastrointestinal adenocarcinoma | 0 | 0 | 1+ | 3+ | 0 | 0 | 0 | 0 | 1+ | 1+ |
| 8 | Gastrointestinal adenocarcinoma | 0 | 0 | 1+ | 3+ | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | Hepatocellular carcinoma | 1+ | 1+ | 2+ | 2+ | 1+ | 1+ | 0 | 0 | 0 | 0 |
| 10 | Hepatocellular carcinoma | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 0 | 0 | 2+ | 3+ |
| 11 | Hepatocellular carcinoma | 0 | 0 | 3+ | 3+ | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | Hepatocellular carcinoma | 2+ | 1+ | 3+ | 3+ | 2+ | 1+ | 1+ | 1+ | 0 | 0 |
| 13 | Renal cell carcinoma | 0 | 0 | 3+ | 3+ | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | Renal cell carcinoma | 0 | 0 | 3+ | 3+ | 0 | 0 | 0 | 0 | 2+ | 3+ |
| 15 | Renal cell carcinoma | 0 | 0 | 2+ | 2+ | 0 | 0 | 0 | 0 | 1+ | 1+ |
| 16 | Renal cell carcinoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Intensity (0; negative, +1; Mild positive, +2; Moderate positive, and +3; strong positive)
Distribution (0; negative 0%, +1; Scant < 10%, +2; moderate < 50%, and +3; widespread > 50%

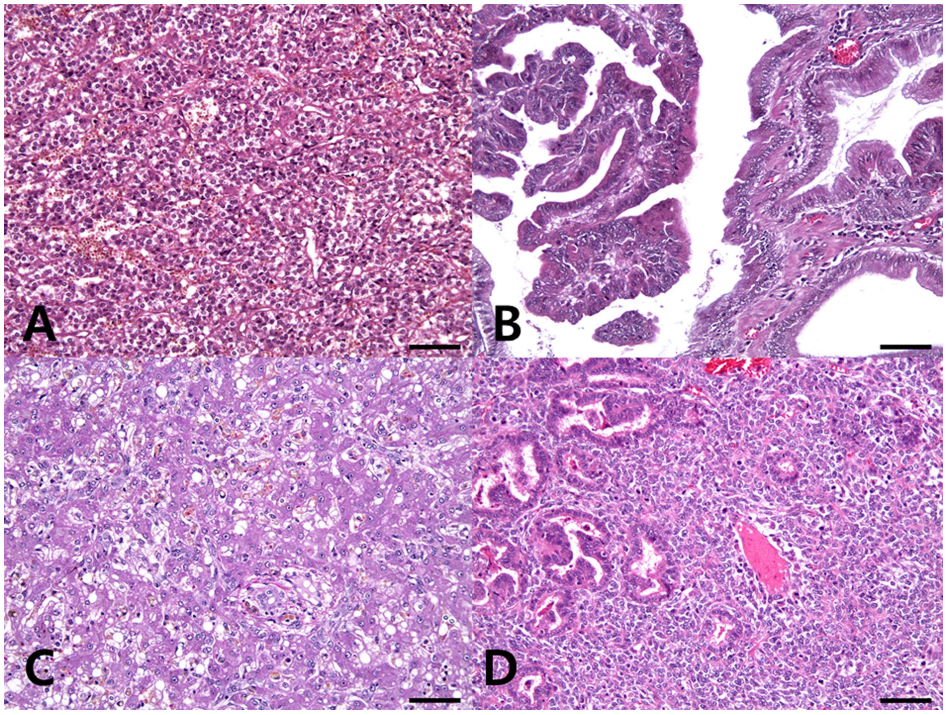


**Fig 1.** Canine intractable tumors. A. Aortic body carcinoma (Metastasized), B. Gastric adenocarcinoma (tubular type), C. Hepatocellular carcinoma, D. Renal cell carcinoma (papillary type). H&E stain. Scale Bar = 70 μm.

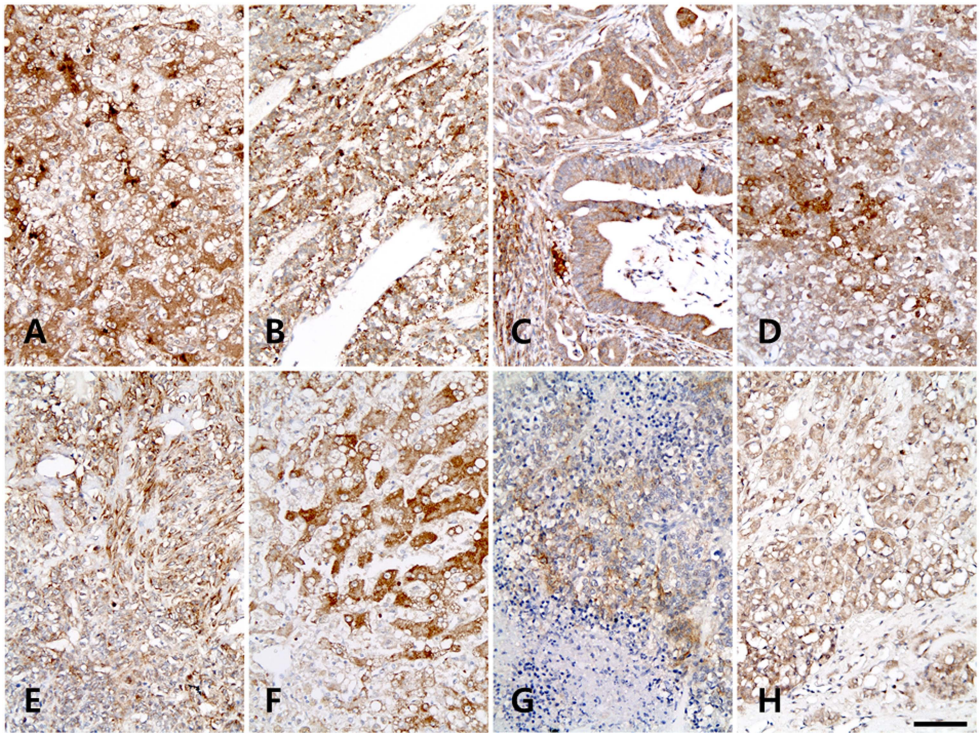


**Fig 2.** Canine intractable tumors. PDGFR-α expression in hepatocellular carcinoma (A), PDGFR-β expression in paraganglioma (B), small intestinal adenocarcinoma (C), hepatocellular carcinoma (D), renal cell carcinoma (E), VEGFR expression in hepatocellular carcinoma (F), c-KIT expression in paraganglioma (G), c-Abl expression in renal cell carcinoma (H). IHC. Hematoxylin counterstain. Scale bar = 70 μm.

(Dako EnVision Kit [ready-to-use]) for 40 min at room temperature. To "visualize" immunolabelling, by using DAB + substrate solution 3:200, each reaction time could be adjusted. The reaction was stopped by washing in distilled water. After two further washes in distilled water, sections were counterstained with Gill's hematoxylin for approximately 5 s, but the time was adjusted based on the situation. Sections were washed in distilled water twice to remove the remaining dye

and dehydration in 95% EtOH, and 100% EtOH. Stand slides were used to remove EtOH and clearing in xylene. Mounting was achieved by using a xylene-based medium.

## Results

### Histological classification of Canine tumors

Of the 16 dogs, eight were male and eight were female. The median age of the dogs was 10 years (range six to 14 years). All dogs were in one of four groups: paraganglioma (n = 4), gastrointestinal adenocarcinoma (n = 4), hepatocellular carcinoma (n = 4), and renal cell carcinoma (n = 4) (Fig 1). All subgroups are shown in Table 3.

### Expressions of angiogenesis-related proteins

The expression of angiogenesis-related proteins is shown in Table 4. Increased PDGFR-α expression was detected in canine hepatocellular carcinoma (HCC) compared with the positive control canine cerebellum (Fig 2A). HCC (75%; three positive signs in four cases) showed PDGFR-α-positive signals in tumor cells. PDGFR-α was not expressed in tumor cells of paraganglioma, gastrointestinal adenocarcinoma, or renal cell carcinoma (RCC). Their intensity was mild to strong and their distribution was scant to widespread (< 10% to > 50%).

Increased PDGFR-β expression was detected in canine paraganglioma, gastrointestinal adenocarcinoma, HCC, and RCC (Figs 2B-2E), compared with the positive control canine kidney.

Increased VEGFR expression was detected in canine HCC compared with the control canine mammary adenocarcinoma (Fig 2F). HCC (75%; three positive signs in four cases) showed VEGFR-positive signals in tumor cells. VEGFR was not expressed in tumor cells of paraganglioma, gastrointestinal adenocarcinoma, or RCC. Their intensity was mild to strong and their distribution was scant to widespread (< 10% to > 50%).

c-KIT overexpression was detected in canine aortic body carcinomas, small intestinal adenocarcinomas, and HCCs (metastasized) (Fig 2G). RCC did not express any c-KIT. Paragangliomas, gastrointestinal adenocarcinomas, and HCCs (25%; one positive sign in four cases) showed c-KIT-positive signals in tumor cells. Their intensity was mild and their distribution was scant (< 10%).

c-Abl expressed strong immune reactivity in canine paragangliomas and gastrointestinal adenocarcinomas (Fig 2H). Aortic body carcinomas, gastric adenocarcinomas, and small intestinal adenocarcinomas were expressed in c-Abl immunohistochemistry. Positive control canine mammary adenocarcinomas expressed c-Abl. Their intensity was mild to moderate and their distribution was scant to moderate (< 10% to < 50%). c-Abl expressed strong immune reactivity in HCC and RCC. HCC, RCC (solid type), and RCC (tubulopapillary type), were expressed in c-Abl immunohistochemistry. Positive control canine mammary adenocarcinomas expressed c-Abl. Their intensity was mild to moderate and their distribution was scant to widespread (< 10% to > 50%).

PDGFR-α was expressed only in HCC, and PDGFR-β was expressed in all tumors. VEGFR was also only expressed in HCC, and c-KIT has been expressed in HCC, paraganglioma, and small intestinal adenocarcinoma. c-Abl was expressed in all cancers, but was weakly expressed in paraganglioma, while more than moderately expressed in other tissues.

## Discussion

In human medicine, 177 chemotherapy-naive patients aged 75 years or younger and diagnosed with stage IIIB/IV non-small cell lung cancer or postoperative recurrence harboring EGFR mutations (either the exon 19 deletion or L858R point mutation) were randomly assigned. A minimization technique was used to determine whether the patients would receive gefitinib (250 mg/day orally; n = 88) or cisplatin (80 mg/m$^2$ intravenously) plus docetaxel (60 mg/m$^2$ intravenously; n = 89), administered every 21 days for three to six cycles. Five patients were excluded and 172 patients (86 in each group) were included in the survival analyses. The gefitinib group had significantly longer progression-free survival compared with the cisplatin plus docetaxel group, with a median progression-free survival time of 9.2 months (95% CI 8.0 to 13.9 months) versus 6.3 months (range 5.8 to 7.8 months; hazard ratio [HR] 0.489, 95% CI 0.336 to 0.710 months, log-rank P < 0.0001) (24).

The Sorafenib Hepatocellular carcinoma Assessment Randomized Protocol (SHARP) was a phase III, multicenter, randomized, placebo-controlled trial. From March 2005, 602 patients with advanced HCC, who did not receive prior systemic therapy at sites in the United States, Europe, Australia, or New Zealand, were randomized to receive either sorafenib 400 mg twice daily or placebo. The primary objective of the study was to compare overall survival between patients administered sorafenib versus placebo. The median survival time was 10.7 months in sorafenib-treated patients compared with 7.9 months in placebo-treated patients (HR 0.69; P = 0.0006) (19).

TKIs have anti-angiogenic and antitumor activities that target PDGFR, VEGFR-1, VEGFR-2, KIT, FLT3, and RET. TKIs might be one therapeutic strategy for patients with malignant pheochromocytomas and paragangliomas (1,28). In a previous study, VEGFR-1 score was higher in paragangliomas and VEGFR-2 was overexpressed in pheochromocytomas and paragangliomas. PDGFR-α score was higher in pheochromocytomas and paragangliomas, and PDGFR-β score was higher in paragangliomas (2). Positive immunohistochemical staining for VEGF was observed in five of nine surgical specimens and in six of eight archival paraganglioma specimens (11 of 17, or 65%) (15). In this study, PDGFR-β was expressed in all four paraganglioma tissues, and c-KIT and c-Abl were weakly expressed in one of four paraganglioma samples.

VEGF-A overexpression has been correlated to PDGF-B overexpression in both the intestinal-type (P < 0.005) and diffuse-type (P < 0.0001) groups. This result indicates that VEGF-A and PDGF-B are secreted simultaneously in human gastrointestinal adenocarcinoma and, therefore, may work together to play important roles in angiogenesis (37). In this study, PDGFR-β, c-KIT, and c-Abl were expressed in canine small intestine adenocarcinomas, while PDGFR-β and c-Abl

were expressed in canine gastric adenocarcinomas.

The presence of PDGFR-α, as well as activated PDGFR-α in HCCs in more than 70% of patients and its relationship to proliferation in liver development and other tumors, justifies its consideration as a valid therapeutic target in patients with HCC (35). In this study, PDGFR-β, c-KIT and c-Abl were expressed in canine small intestine adenocarcinomas, while PDGFR-β and c-Abl were expressed in canine gastric adenocarcinomas.

RCC is well known as a hypervascular tumor in which angiogenesis is promoted by tumor cells producing VEGF (5,27,31,32,40). Although VEGF is involved in angiogenesis and correlates with tumor stage, no correlation has been reported between VEGF and microvessel density (MVD) or prognosis (30). The results of this study also failed to indicate any correlation between VEGF and MVD. RCCs are remarkably resistant to chemotherapy, and development of drug resistance is common in humans (6,25). Expression of the multidrug-resistant transporter in tumor cells may be the mechanism by which drug resistance occurs in RCCs (3). The lack of response to chemotherapy in the RCC patient was corroborated in this study, where survival time was not extended for dogs treated with chemotherapy compared with those not treated. In human medicine, therapy is continuously evolving, and immunotherapy, TKIs, and mono-clonal antibody therapies are currently used in the management of human RCC, including high-dose interleukin-2, VEGF inhibitors, and mTOR inhibitors (6).

Previous studies have demonstrated overexpression of c-KIT in human chromophobe RCC and oncocytoma by gene expression and immunohistochemical analysis (29). Cytoplasmic, granular, generally moderate c-KIT immunoreactivity was observed in 12 tumors from 13 cases (8). However, the present study did not support these previous findings.

Immunohistochemical data showed that PDGF-A and PDGF-B are expressed in 42% and 60% of the OSAs analyzed, respectively, while PDGFR-α and PDGFR-β were expressed in 78% and 81% of cases, respectively. Quantitative PCR data showed that all canine OSA cell lines overexpressed PDGFR-α, while six of seven overexpressed PDGFR-β and PDGF-A relative to a normal osteoblastic cell line. Collectively, these data show that PDGFRs and PDGFs are co-expressed in canine OSAs, which suggests that an autocrine and/or paracrine loop is involved and that they play an important role in the etiology of OSAs. PDGFRs may be suitable targets for the treatment of canine OSA with a specific TKI (23).

In a previous study, canine patients with suspected cerebellar transitional meningioma were prescribed a combination of imatinib mesylate (a TKI) and hydroxyurea. After two weeks of this combination treatment, the mass size had reduced significantly. The mass continuously decreased in size until the patient died during anesthesia. Cerebellar transitional meningioma was confirmed by histopathologic examination (14).

Compared with a placebo, masitinib (a TKI) has been shown to significantly improve the survival rate of dogs with nonresectable mast cell tumors. Fifty-nine of 95 (62.1%) and nine of 25 (36.0%) dogs were alive at 12 months, and 33 of 83 (39.8%) and three of 20 (15.0%) dogs were alive at 24 months, for masitinib and placebo, respectively. Masitinib significantly increased survival rates at 12 and 24 months in dogs with nonresectable mast cell tumors (10).

Oclacitinib maleate inhibits the function of a variety of pro-inflammatory, pro-allergic, and pruritogenic cytokines that are dependent on Janus kinase enzyme activity. Oclacitinib maleate selectively inhibits Janus kinase-1 and produces a rapid onset of efficacy (within 24 h). Mean oclacitinib maleate pruritus visual analogue scale (VAS) scores given by owners were significantly better than placebo scores ($P < 0.0001$) on each assessment day. Pruritus scores decreased from 7.58 cm to 2.59 cm following oclacitinib maleate treatment. In this study, oclacitinib maleate provided rapid, effective, and safe control of pruritus associated with allergic dermatitis, with owners and veterinarians noting substantial improvements in pruritus and dermatitis VAS scores (4).

Limited data exist on the clinical efficacy of small molecule inhibitors in veterinary medicine. In part, this is due to the fact that targets for therapeutic intervention are not clearly defined for most canine or feline cancers. Additionally, many of the human TKIs are currently cost prohibitive, which prevents their widespread use (20).

This study examined how much of the growth factor expression in tumor tissues could be diagnosed through immunohistochemical staining for growth factors that inhibit TKIs. Growth factors involved in proliferation of the tumor can be expected to have a better response to the TKI block of growth factors because it is expressed in many tissues.

In conclusion, this study investigated how TKIs used in human medicine can be applied to canine intractable tumors, through immunohistochemistry. The results indicate that there may be an application for TKIs in treating canine intractable tumors.

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