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Mast Cell Tumors

Yosuke Amagai and Akane Tanaka

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Abstract

Mast cell tumor is one of the major cutaneous tumors in dogs. Though the etiology of MCTs is not completely understood, it becomes clear that approximately 10–20% MCTs express mutant KIT receptors with ligand-independent phosphorylation. Tyrosine kinase inhibitors targeting KIT exert antitumor effects on malignant proliferation of mast cells with or without gene mutations. However, the efficacy of KIT inhibitors on dogs with MCTs has been limited. In this chapter, we would like to outline the general understandings of mast cells such as the process of its differentiation and proliferation, and what has been revealed regarding the mechanism of tumorigenesis and therapeutic approaches. In particular, KIT mutation-related evidences and therapeutic approaches in the future are discussed.

Keywords: mast cell tumor, stem cell factor, KIT, tyrosine kinase inhibitor

1. Introduction

Mast cells are originated from hematopoietic stem cells in bone marrow as similar to other granulocytes. However, mast cells are very unique because they are released from bone marrow as undifferentiated progenitor cells to the circulation, and their final maturation is completed in the peripheral tissues. We find mast cells in most of the tissues and organs in our body, particularly in interstitial connective tissues of each organ being close to blood vessels. Mast cells play crucial roles in innate immunity against parasites and microbes that is essential for host defense in humans and animals. In acquired immunity, activation of mast cells is induced by cross-linkage of IgE that binds to high-affinity IgE receptors after antigen exposure. Moreover, some chemicals and toxins as well as physical (i.e., scratching and heat) and neurogenic stimuli trigger activation of mast cells. Various chemical mediators and cytokines are released from mast cells after their degranulation, and sometimes initiate allergic inflammation and itch sensation. In canine medicine, serious involvement of mast cells in allergic

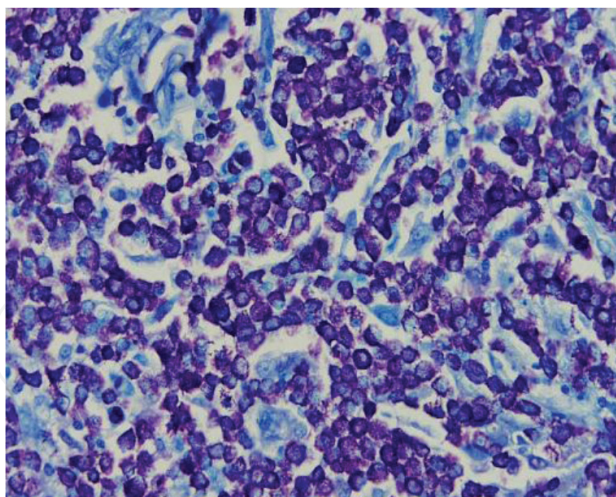


Figure 1. Typical histologic features of surgically removed canine MCTs stained with toluidine blue solution. A mass is consisted with numbers of differenced mast cells that show metachromasia. Connective tissues colored in light blue can be identified.

diseases has been identified, and mast cells are estimated as the most important target in medical treatment of allergy. Although malignancies of mast cell are uncommon disorders in humans, veterinary clinicians frequently encounter mast cell tumors (MCTs) in dogs and cats. The frequency of cutaneous MCTs has been reported to reach 20% of all tumors raised in the skin of dogs. Most of the malignant mast cells existed in a mass have granules in their cytosol, containing pruritogens, inflammatory factors, and various proteases (**Figure 1**). In this chapter, recent information on both basics in mast cell biology and clinical approaches for canine MCTs is outlined.

2. Differentiation and proliferation of mast cells

2.1. Differentiation of mast cells

Mast cell progenitors are differentiated from pluripotent hematopoietic stem cells in bone marrow. Being different from other granulocytes, mast cells are transported by blood stream to peripheral tissues as mononuclear immature cells without granules in their cytosol [1]. In peripheral tissues, mast cell precursors complete their differentiation and distribution being ready for the host defense (**Figure 2**). According to characters of microenvironment of each peripheral tissue, final types of mature mast cells are altered. In the skin, they differentiated into connective tissue-type mast cells that include heparin proteoglycan and abundant granules in cytosol. Various kinds of proteases and chemical mediators are in granules of connective tissue-type mast cells by which severe inflammation is induced when they are released at the affected sites. Heparin proteoglycan-positive mast cells can be detected with not only toluidine blue but also berberine sulfate and safranin O. In contrast, mast cells that reach to and invade in mucosal tissues differentiate into mucosal-type mast cells. Mucosal-type mast cells include chondroitin sulfate as proteoglycan, and cytosolic granules are very few. Chondroitin

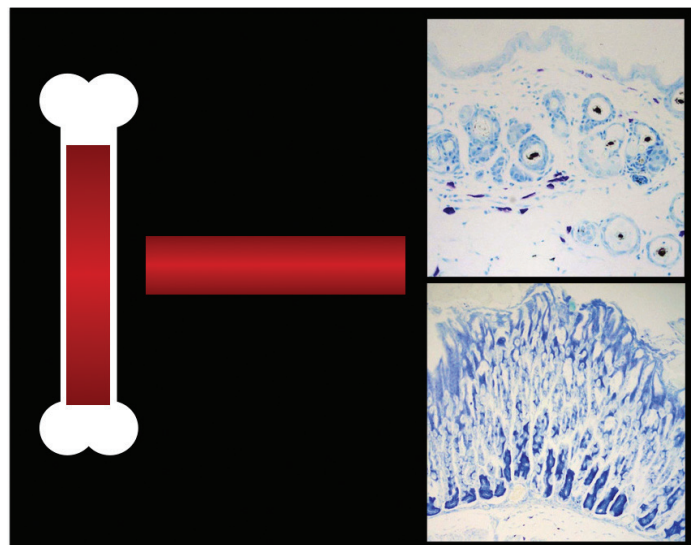


Figure 2. Origin and transportation of mast cell precursors. Immature mast cells without cytosolic granules are released from bone marrow and distributed to peripheral tissues via circulation. Final maturation of mast cells depends on factors and molecules expressed in microenvironment of each tissue. In the skin (the upper panel), connective tissue-type mast cells with rich cytosolic granules are differentiated. In mucosa of the gastrointestinal tract (the lower panel), mucosal-type mast cells with few cytosolic granules are differentiated.

sulfate-positive mast cells are identified with alcian blue staining, but not with toluidine blue, berberine sulfate, or safranin O. Connective tissue-type mast cells are found in the skin and connective tissues in various organs. On the other hand, mucosal-type mast cells are differentiated in mucosa of the gastrointestinal tract.

2.2. Proliferation of mast cells

Factors that involve in mast cell proliferation are not fully understood. In mice, mast cells proliferate in response to interleukin (IL)-3, IL-9, and stem cell factor (SCF). Both IL-6 and SCF are essential factors for proliferation and survival of human mast cells. Though less is known regarding development of canine mast cells, SCF has been reported to induce proliferation and survival, as well as migration and activation of canine mast cells [2, 3]. The IL dependency varies according to a species from which mast cells are derived. However, SCF is the most important factor for proliferation, differentiation, and survival of mast cells in humans and animals. SCF is the ligand for KIT receptors expressed on cell surface of mast cells. Since mast cells are differentiated from KIT-positive cell lineage and KIT is retained on the surface of not only immature precursors but also mature mast cells, influence of SCF on mast cells must be crucial for their proliferation and differentiation. In fact, gain-of-function mutations in the *KIT* gene have been identified in mast cells with factor-independent tumorigenic proliferation in humans, rodents, and dogs. Meanwhile, over 50% of canine MCTs and most of the feline MCTs show *KIT*gen mutation-independent development. Recent observations have revealed that not only SCF but also other cytokines and growth factors including epidermal growth factor, vascular endothelial growth factor, and nerve growth factor may facilitate proliferation, differentiation, and survival of mast cells. Unfortunately, investigation on development of canine mast cells has been insufficient.

3. Cellular biology of mast cells

3.1. Inflammatory responses of mast cells

Mast cells play key roles for inflammatory responses through degranulation and cytokine/chemokine production and secretion [4]. However, proteases, chemical mediators, and cytokines included in mast cell granules are different according to types of mast cells. Heterogeneity among mast cells is important to precisely understand the pathophysiological roles of mast cells in each tissue. Connective tissue-type mast cells include chemical mediators such as histamine that has strong biologic activity on nerve fibers and blood vessels. Histamine is known as a pruritogen that induces itch sensation resulting in scratching behavior in humans and animals. Vasoactive effects of histamine initiate inflammation at the affected sites. Scratching behavior stimulates physical degranulation of mast cells leading to exacerbation of swelling and inflammation. Exact roles of mucosal-type mast cells are not clearly demonstrated. Since numbers of mucosal-type mast cells are increased in the gut with parasite infection, roles in host reaction against parasite exclusion have been proposed. However, factors and mechanisms that involve in antiparasite effects of mucosal-type mast cells are not fully explored.

3.2. Heterogeneity of MCTs

Most MCTs in dogs are consisted with histamine-rich connective tissue-type mast cells. Steps responsible for transformation of mast cells are summarized in **Figure 3**. Since receptors for histamine are expressed in mucosal cells of the stomach, progression of MCTs has been suggested to induce gastric ulcer and serious damage on gastric function. However, the exact and direct association of MCTs with gastric ulcer remains unclear [5, 6]. Connective tissue-type mast cells contain tryptase and chymase that possess broad protease activities. Particularly, tryptase has been reported to regulate neovascularization. Connective tissue-type mast cells can also produce growth factors for vascular endothelial cells; therefore,

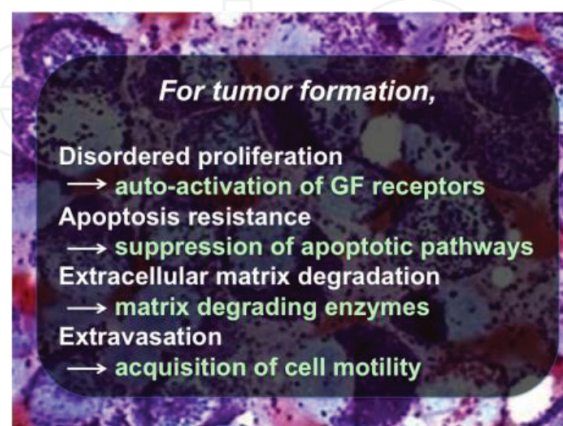


Figure 3. Malignant transformation of mast cells. For tumor formation, mast cell proliferation must be promoted by activation of ligand-independent activation on growth factor receptors. Also, acquisition of resistance against apoptosis pathways and invasive characters may facilitate malignant expansion of MCTs.

most of the MCTs must be rich with blood vessels. Moreover, dogs with serious MCTs show deficiency in blood coagulation possibly because connective tissue-type mast cells have plenty of heparin in their cytosol. Canine mast cells have unique chymase whose name is dog mast cell protease-3; however, its specific role has not been fully understood [7]. A mass formed with mucosal-type mast cells rarely develops in the gastrointestinal tract, which induces dysfunction of the gut. Influence of mucosal mast cell tumor on other organs except the gut has not been well documented.

4. Pathology and diagnosis of MCTs

4.1. Clinical presentation, incidence, and risk factors

MCTs are characterized by the aberrant proliferation of mast cells, accounting for approximately 20% of cutaneous tumors in dogs [8, 9]. They develop in the subcutaneous tissue and dermis in most cases, and other types of mast cell malignancies such as mast cell leukemia and visceral MCTs are rarely observed. They usually occur in the trunk or limbs and sometimes observed in the head and neck (**Figure 4**) [9, 10]. Because mast cells release proinflammatory mediators, erythema and wheal called Darier's sign are sometimes observed. However, MCT-specific symptoms that can distinguish MCTs from other tumors are rare.

Risk factors for MCTs, age, sex, breed, spay/castration, and tumor grading have been reported [8, 11]. Among these, most factors except sex are deeply related to its incidence [11]. Recently, genetic characteristics have been also investigated, showing the high correlation of KIT mutations with prognosis of dogs with MCTs [12–14]. As Mochizuki et al. [11] presented nice summary on each factor except genetic one, we would like to focus on the genetic characteristics of MCTs in the following sections.

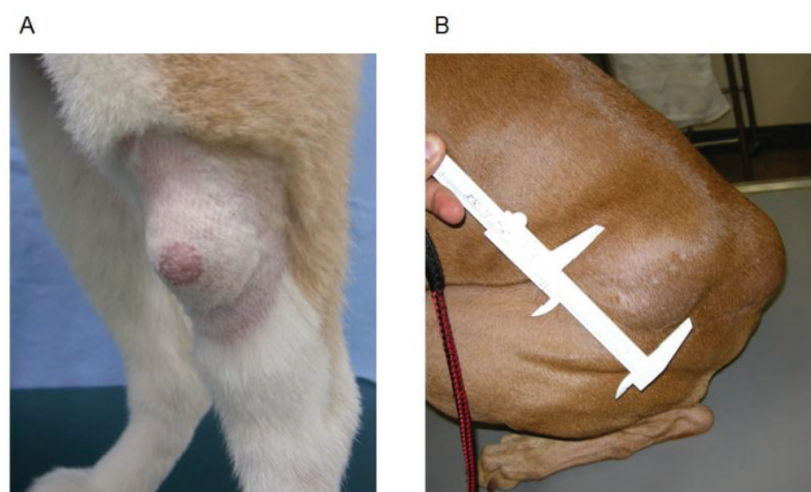


Figure 4. Representative photograph of MCT-diagnosed dog. (A) MCT occurred in the left leg in 11-year-old female, Shiba. (B) MCT occurred in the left waist in 8-year-old female, Pharaoh Hound.

4.2. Diagnosis of MCTs

Cytological or histological analyses through a fine needle aspiration or biopsy are required for the diagnosis of MCTs. Typically, round-shape cells with round nuclei and with rich cytosol are observed. Mast cells have abundant cytosolic granules, and specific staining methods with toluidine blue or safranin O can identify them. However, MCTs with undifferentiated more malignant mast cells possess few granules. Confirmation of the swelling of draining lymph nodes and sometimes fine needle aspiration of the lymph node may help to determine the presence of metastasis. Patnaik grading is mainly used pathological grading in the veterinary field because it is recognized as a good prognostic marker [15]. There are three pathological grades (grades I, II, and III), and higher grade indicates that the tumor is more malignant [15]. Several analyses have been revealed: the correlation between tumor grading and the *c-kit* gene mutation, clearly showing that *c-kit* mutations are more frequently observed in high-grade tumor [12–14]. Therefore, analyzing *c-kit* sequence can also be a prognostic marker for MCTs. In addition, analyzing *c-kit* gene is important in terms of selecting proper treatments because several molecular target inhibitors against KIT protein, a receptor encoded in the *c-kit* gene, are currently available for the treatment of MCTs. Polymerase chain reaction that amplifies exon 11 and intron 11 region using tumor genome enables the detection of internal tandem duplications (ITDs) in the juxtamembrane domain, which is the most frequent type of *c-kit* mutation. Recently, however, whole sequence of *c-kit* mRNA is more common because the proportion of mutations in other region of KIT domain or other type of mutations in the juxtamembrane domain are not negligible. Recent reduction in the cost for sequencing analysis will probably boost this trend (see Section 5).

5. Neoplastic transformation of mast cells

5.1. Overview

Mast cell malignancies are observed among species, though the incidence of mast cell malignancies is much lower in human and rodents than in dogs [8, 9]. One of the well-investigated mechanisms of mast cell tumorigenesis is mutations in the *c-kit* gene [9, 10]. We would like to overview the current understandings of the mutant KIT contribution on mast cell tumorigenesis as well as other tumor-related transformations of mast cells that may correlate with their tumorigenesis in mast cells.

5.2. KIT mutation-dependent neoplastic transformation

5.2.1. KIT mutations in human and rodents

KIT is a type III receptor tyrosine kinase of which ligand is stem cell factor (SCF) (**Figure 5**). It is consisted of the extracellular domain, transmembrane domain, juxtamembrane domain, and tyrosine kinase domain [16] (**Figure 5**). In human, aberrant proliferation of mast cell is observed in the patients of systemic/cutaneous mastocytosis, mast cell sarcoma, and mast cell leukemia [17]. Among them, most systemic mastocytosis occurs due to the mutations in the tyrosine kinase domain of KIT, especially a point mutation in Asp816 [12]. In general, SCF binding to KIT triggers the conformational changes in KIT and leads to the dimerization of

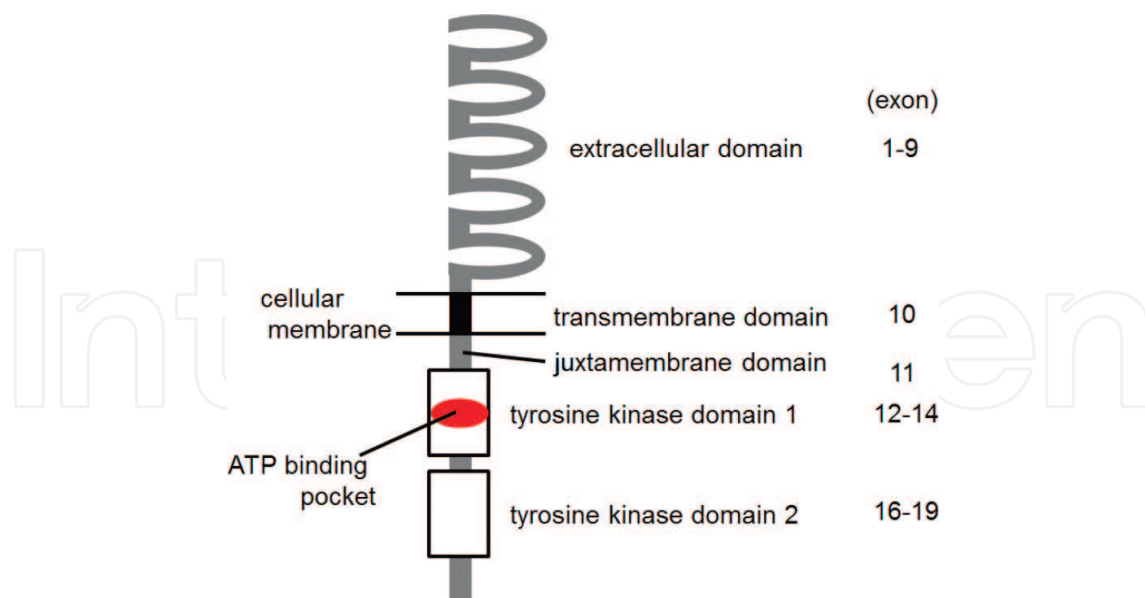


Figure 5. Schematic diagram of KIT protein. The numbers of exon correspond to the ones in dog KIT.

the protein, allowing the binding of adenosine triphosphate (ATP) and phosphorylation of tyrosine kinase domain [18]. Mutations in the tyrosine kinase domain alter the conformation to the one similar to its active form, thus resulting in the constitutive KIT activation even in the absence of either SCF binding, KIT dimerization, or ATP binding [19]. Neoplastic growth of mast cells is rarely observed in rodents, through currently available rodent-derived mast cell lines. For example, RBL-2H3 cells (derived from a Wistar rat) and P815 cells (derived from a DBA/2 mouse) express Asp817Tyr and Asp814Tyr, respectively, which correspond to the Asp816 mutation in human KIT [20]. As far as we know, other mechanisms that trigger rodent mast cell tumorigenesis have not been reported.

5.2.2. KIT mutations in dog

In contrast to human and rodents, KIT mutations in dog MCT are frequently observed in the juxtamembrane domain (**Table 1**). ITDs in the domain are the first discovered and the most frequent mutations in canine MCTs [21] (**Table 1**). Besides ITDs, other mutations in the juxtamembrane domain or extracellular domain have been also reported [21]. We recently demonstrated that most of these mutations in the extracellular or juxtamembrane domain cause aberrant KIT activation and neoplastic proliferation of mast cells by triggering ligand-independent dimerization (Ref. [22] and unpublished data). In contrast to the mutations in the tyrosine kinase domain, these mutations require ATP for the phosphorylation of the tyrosine kinase domain, providing a rationale for using ATP-competitive small molecule inhibitors for suppressing the aberrant KIT activations [18, 23].

5.3. KIT mutation-independent neoplastic transformation

Few mechanisms of mast cell tumorigenesis except KIT mutations have been identified, but we recently demonstrated that MCT cells produce SCF and support their growth in a

Analyzed exons	Domain	Exon	Mutation	Frequency (%)	References
All	Extracellular	8	417-421ITD	8/202 (4.2%)	[24]
		8	Gln430Arg	1/202 (0.5%)	
		9	Ser479Ile	5/202 (2.5%)	
		9	Asn508Ile	3/202 (1.5%)	
	Juxtamembrane	11	555–557mutation	7/202 (3.5%)	
		11	ITD	25/202 (12.4%)	
	Tyrosine kinase	17	del826–828	1/202 (0.5%)	
All	Extracellular	6, 7, 8	del102AA, Thr414Ala	1/47 (2.1%)	[25]
		8	417-420ITD	3/47 (6.4%)	
	Extracellular, transmembrane	9,10	ins4AA	1/47 (2.1%)	
		11	del558	1/47 (2.1%)	
		11	Leu575Pro	1/47 (2.1%)	
	Tyrosine kinase	11	ITD	8/47 (17.0%)	
		15	del714	1/47 (2.1%)	
11	Juxtamembrane	11	ITD	8/88 (9.1%)	[12]
		11	del	4/88 (4.5%)	
11	Juxtamembrane	11	ITD	8/60 (13.3%)	[13]
11	Juxtamembrane	11	ITD	7/68 (10.3%)	[14]
11	Juxtamembrane	11	ITD	24/118 (20.3%)	[26]
8, 9, 11	Extracellular	8	421-429del, ins3AA	1/21 (4.8%)	[27]

AA, amino acid.

Table 1. KIT mutations that have been reported in dog MCTs.

paracrine/autocrine manner [28]. In the analyses, high SCF production was confirmed in multiple clinical MCT samples [29]. It may explain the high response of clinical MCTs to KIT-specific molecular inhibitors even when the tumor cells express wild-type KIT. This will be further discussed in the following section.

Recent approaches such as next-generation sequencing will reveal even minor mutations or single nucleotide polymorphisms in neoplastic mast cells. In fact, Spector et al. [30] and Youk et al. [31] discovered a human mast cell leukemia-specific mutation in several genes. As the cost of these approaches decreases, they will be introduced in the veterinary field, probably leading to the deep understanding of mast cell tumorigenesis among species. Another approach aiming at the control of tumor growth is modifying epigenetic status in tumor genome [32]. Regarding an epigenetic alteration in MCTs, Morimoto et al. [33] showed that DNA hypomethylation widely occurred in malignant, higher-grade MCTs. Moreover, antitumor effects of AR-42, a histone deacetylase inhibitor, on several MCT cell

lines as well as primary tumor cells have been demonstrated [34]. Thus, the characterization of epigenetic alteration is likely to be an effective approach to reveal MCT transformation.

6. Treatment and prognosis

6.1. Conventional therapies

Complete surgical removal with wide margin is the best way for solitary cutaneous MCTs. However, for dogs with multiple masses, metastasis, or severe invasive MCTs, surgical removal is sometimes incompetent. Chemotherapies have failed to overcome MCTs. However, to reduce tumor size, some combination chemotherapies have applied. Glucocorticoid is one of the most important drugs for treatment of MCTs. More than 70% of cutaneous MCTs in dogs respond well to oral administration of glucocorticoid [35–37]. Expression levels of glucocorticoid receptors in MCT cells have been reported to associate with glucocorticoid sensitivity [37]. Glucocorticoid shows strong antitumor effects on MCT cells with high expression of glucocorticoid receptors. Oral administration of glucocorticoid must be an easy and effective chemotherapy for canine MCTs. Since side effects induced by glucocorticoid administration will sometimes be concerned, clinicians must pay attention on blood chemistry data and general conditions of dog patients. Glucocorticoid is usually applied as a part of multidrug chemotherapies for MCTs. Anticancer drugs, such as vincristine, vinblastine, cyclophosphamide, and CCNU, have been tested for combination chemotherapies for MCTs with or without glucocorticoid [38–42]. However, very little information on the chemotherapeutic response of MCTs can be obtained. Since recent studies have been based on small numbers of cases and have often included MCTs of different pathologic grades and clinical stages, data must be carefully evaluated [43]. Recently, adjuvant chemotherapies have been proposed in treatment of various cancers and sarcomas. Since neo-adjuvant administration with glucocorticoid usually reduces mass size of MCTs, wide surgical margins will be obtained [37]. On the other hand, postoperative adjuvant chemotherapy is suggested to kill MCT cells that remain at the affected site after incomplete excision. Several trials on adjunctive chemotherapy have been reported. However, most of the adjunctive chemotherapy does not appear to increase survival times. Although surgical removal with radiation has been tested for MCTs, remarkable improvement is not provided. No difference in overall survival rate has been observed between dogs with MCTs receiving and not receiving prophylactic irradiation of the regional lymph node [44].

6.2. Molecular target therapies targeting KIT

Because aberrant activation of mutant KIT is one of the causes in mast cell tumorigenesis, anticancer effects of KIT inhibitors have been investigated. In fact, clinical trials that enrolled MCT-diagnosed dogs have been undertaken to evaluate the efficacy of molecular targeting agents against KIT. We would like to overview the history of the research on KIT inhibitors and discuss therapeutic perspective for MCTs.

	All MCTs		MCTs with mutant KIT		MCTs without mutant KIT	
	Cases	CR + PR	Cases	CR + PR	Cases	CR + PR
Toceranib	86	32 (37.2%)	20	12 (60.0%)	66	20 (30.3%)
Placebo	63	5 (7.9%)	9	1 (11.1%)	54	4 (7.4%)
Placebo>toceranib	58	24 (41.4%)	9	7 (77.8%)	49	17 (34.7%)

The number of the enrolled patients and the proportion of CR/PR cases are indicated.

Table 2. Summary of a clinical trial of toceranib phosphate [26].

The first molecular inhibitor applied to human was the imatinib mesylate, which repress activations of KIT, platelet-derived growth factor receptor (PDGFR), and Bcr-Abl [45]. It was first administered to the patient of gastrointestinal stromal tumor with a mutation in the juxtamembrane domain of KIT [46]. At around the same time, KIT mutations in canine MCT were first discovered [21], suggesting the possibility that KIT inhibitors can be applied to dog MCTs. Actually, there have been several reports that show the inhibitory effects of imatinib mesylate on MCTs, especially for the tumor cells with KIT mutations in either the extracellular domain or juxtamembrane domain [22, 47]. Based on these results from basic researches, some clinicians administered imatinib mesylate to MCT-diagnosed dogs and obtained partial response at least in some of them [47]. However, there was no rationale for the administration of imatinib mesylate to MCT-diagnosed dogs through the clinical trials. In contrast to that, both masitinib mesylate and toceranib phosphate are approved by either the Food and Drug Administration (FDA) in the United States or European Medicines Agency based on the results from the clinical trials enrolling MCT-diagnosed dogs [26, 48,49]. Basically, imatinib mesylate, masitinib mesylate, and toceranib phosphate are all ATP-competitive inhibitors, and they suppress the activation of mutant KITs that require ATP binding for their activation.

Results of clinical trials for toceranib phosphate, which is a random double-blind trials, are summarized in **Table 2** [26]. In this trial, more than 150 MCT patients were enrolled. After the six-week treatment, either complete response (CR) or partial response (PR) was obtained 32 in 86 (37.2%) patients in the treatment group, while the proportion of CR/PR was only 7.9% (5 in 63 patients) in the placebo group. In addition, a group treated with toceranib phosphate following placebo-escape, which administered toceranib phosphate after the placebo treatment, responded the agent, resulting in the CR/PR in 24 cases out of 58 (41.4%). At least in this trial, significant increase in severe adverse effects (grade III or IV) was not detected. In case of mastinib mesylate, a phase III trial was carried out in France, enrolling more than 130 MCT patients. One-year survival and two-year survival were 62.1 and 39.8%, respectively, in the treatment group, though the ones were 36.0 and 15.0%, respectively, in the placebo group [49].

Interestingly, both agents showed antitumor effects even on MCTs expressing wild-type KIT (**Tables 2** and **3**). Though the agents suppress the activation of other receptor tyrosine kinases

	All MCTs		MCTs with mutant KIT		MCTs without mutant KIT	
	1 year	2 year	1 year	2 year	1 year	2 year
Toceranib	59/95 (62.1%)	33/83 (39.8%)	17/27 (63.0%)	7/23 (30.4%)	38/62 (61.3%)	23/54 (42.6%)
Placebo	9/25 (36.0%)	3/20 (15.0%)	1/7 (14.3%)	0/6 (0%)	7/16 (43.8%)	2/12 (16.7%)

The number and proportion of the patients that survived more than 1 or 2 years are indicated.

Table 3. Summary of a clinical trial of masitinib phosphate [49].

such as platelet-derived growth factor receptor or vascular endothelial growth factor receptor 2 [50, 51], aberrant activations of principal targets except KIT were not observed in our study using more than 30 clinical MCT tissue samples (unpublished data). Thus, it is likely that the data in **Table 2** indicate that tumor growth in no more than 30% of MCT was dependent on KIT signaling even though they express wild-type KIT. We consider that these can be at least partly explained by SCF autoproductin from tumor cells as described above [28, 29]. Though further investigations are necessary, analyses to determine the KIT activation status will probably be a direct diagnostic agent to accurately predict the therapeutic efficacy of KIT targeting inhibitors.

7. Final remarks

As described, molecular biological approaches to MCTs have started, and new findings are accumulating. However, some clinical researches present very limited information obtained from few cases. Therefore, clinicians should carefully collect information and evaluate data before clinical application of anticancer drugs and molecular targeting drugs to dogs with MCTs. It is dangerous to trust all data reported in few research reports or on few clinical cases. Knowledge on basic biology of mast cells will help clinicians to understand the recent molecular approaches to MCTs.

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