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Review

C-kit as a prognostic and therapeutic marker in canine cutaneous mast cell tumours: from lab to clinic.

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Abstract

Cutaneous mast cell tumours (MCTs) are some of the most common canine neoplasms and their variable and often aggressive biological behaviour makes them particularly challenging for the veterinary practitioner. Over the years, scientists accumulated a wealth of knowledge on these tumours, which originated better prognostic markers and targeted therapies. These are mostly focused on inhibiting c-kit, a protein that plays a major role in the biopathology of MCTs. In fact, masitinib and toceranib, targeted inhibitors of c-kit and other receptor tyrosine-kinases (RTKs), bear the promise of improving the outcome of patients with aggressive MCTs. However, the bulk of available knowledge on MCTs is mostly dispersed, making it difficult for practitioners to take advantage of it, when consulting the pathologist or making therapeutic decisions. This work brings together current knowledge on the biopathology of MCTs, reviewing prognostic markers and their applications, as well as the development of c-kit inhibitors in the context of the basic cellular, molecular and pathologic features of MCTs. Future perspectives opened by recent biopathological data or experimental therapeutic approaches are also addressed.

Keywords: C-kit; Immunohistochemistry; Masitinib; Mast cell tumours; Mutation; Toracenib

Introduction

Among canine diseases, cancer occupies a prominent place, due to its high incidence and associated distress and mortality. Many dog breeds, with their specific genetic backgrounds, show particularly high incidences of certain tumours. This is typically the case with cutaneous mast cell tumours (MCTs), actually the most common skin neoplasms in dogs. The present review is focused on cutaneous MCTs, that should not be confused with their visceral counterparts, since these display significantly different biological features. The c-kit protein called the attention of the veterinary research community due to its role in the oncogenesis of MCTs and, more recently, as a useful therapeutic target. The recent development and application of c-kit inhibitors to treat canine MCTs represents an important example of how translational research generated a new targeted therapy for use in veterinary oncology. The present review deals with both research papers and reviews concerning biopathological and clinical information on canine MCTs published online up to December 2014. Although the work is focused on the role of c-kit and the development of c-kit inhibitors, some works providing essential background knowledge were also included, as well as a few studies representing new trends in the therapy of canine MCTs.

Canine MCTs: signalment and pathological features

The annual incidence rate of MCTs has been estimated at as much as 129 per 100 000 insured dogs in the UK (Dobson et al., 2002). According to different authors, MCTs account for about 7% to 21% (Macy, 1986; Thamm and Vail, 2007) of all canine skin tumours, which represent about a third of all canine neoplasms (de las Mulas et al., 1999). MCTs usually occur in older animals, with an average age of 9 years (Blackwood et al., 2012), but can also occasionally be identified in dogs as young as 3-

1 weeks-old (Davis et al., 1992). There is a strong breed predisposition (e.g. for boxer
2 dogs) but no gender predilection for developing MCTs (Dobson and Scase, 2007;
3 Warland and Dobson, 2013), although Kiupel et al. (2005) reported a worsened post-
4 surgical prognosis in males, compared with females. Multiple tumours occur in about
5 10% to 21% of cases (London and Seguin, 2003; Murphy et al., 2006, Thamm and Vail,
6 2007) and an animal may develop as many as 20 nodules (Gil da Costa et al., 2005).

7
8 The biological behaviour of canine MCTs is highly variable, ranging between
9 that of a single surgically resectable nodule and that of a fatal systemic disease
10 (O'Connell et al., 2011). Over the years, the cytological and histological aspects of
11 MCTs have been organized to compose histological grading systems, and histological
12 grade remains the the single most important prognostic factor for MCTs (Blackwood et
13 al., 2012). The system proposed by Patnaik et al. (1984) divides MCTs into threee
14 histological grades. Histological grade results from a combination of cytological criteria
15 with tumour depth, assuming that MCTs arise in the superficial dermis and
16 progressively invade deeper tissues until reaching the subcutis. However, the system has
17 been criticized for ascribing excessive importance to tumour depth and for including in
18 grade II (moderately differentiated lesions) more that 40% of all MCTs, creating a vast
19 and heterogeneous group of lesions with different biological behaviour (Gross et al.,
20 2005). A new histological grading system was proposed by Kiupel et al. (2011), as a
21 way to overcome such limitations. This is two-tiered system, thereby avoiding the
22 problems related to Patnaik's intermediate grade. Also, all grading criteria are based on
23 nuclear morphology and mitotic activity, excluding issues like tumour depth. High-
24 grade MCTs are significantly associated with shorter average survival (6.3 versus 24.3
25 weeks) and disease-free survival compared with low-grade MCTs (6.3 versus 12.4

1 weeks). Kiupel et al. (2011) also report a higher inter-observer consistency compared
2 with Patnaik's system. These findings were later confirmed by Takeuchi et al. (2013).
3 All Patnaik's grade I MCTs fit in Kiupel's low grade, grade III MCTs are all high grade.
4 Most grade II MCTs are classified as low grade, and the new system has the advantage
5 of providing clearer prognostic information, compared with Patnaik's (Sabattini et al.,
6 2015). Since Kiupel's grading scheme is solely based on cytological criteria, Scarpa et
7 al. (2014) recently proposed that grading might be performed on cytological
8 preparations, allowing for better planning of subsequent surgery. The authors reported a
9 strong correlation between cytological and histological gradation, but some high-grade
10 MCTs were still missed.

11
12 Still, despite these advances, histological grading alone cannot predict the
13 behaviour of each specific MCT, and additional markers to predict prognosis and
14 response to chemotherapy keep being studied, including DNA mutational and ploidy
15 analysis (Ayl et al., 1992), morphometric (Strefezzi et al., 2003; Maiolino et al., 2005),
16 and receptor tyrosine kinases expression analysis, p53 (Ginn et al., 2000; Jaffe et al.,
17 2000), p21 and p27 (Wu et al., 2004), studies of proliferation and angiogenesis markers
18 (Ranieri et al., 2003; Preziosi et al., 2004a; Giantin et al., 2012a), cyclooxygenase-2
19 (Vascellari et al., 2012; Prada et al., 2012), matrix metalloproteinases (Leibman et al.,
20 2000; Giantin et al., 2012), interleukin-2 and its receptor (Meyer et al., 2012; Meyer et
21 al., 2013), some bcl-2 family proteins (Strefezzi et al., 2012; Vascellari et al., 2012).
22 Fenger et al. (2014) reported that the micro-RNA-9 (miR-9) is overexpressed in high-
23 grade MCTs and suggested it might enhance tumour invasion and metastasis. Giantin et
24 al. (2014) recently used a transcriptomic approach to identify a set of 13 differentially-
25 regulated genes that significantly correlated with survival time. Rich et al. (2014) found

that the p62/sequestosome-1 protein expression patterns were also of prognostic value. Among all studied markers, c-kit was identified as a key player in the development of MCTs, and to be a useful prognostic and therapeutic marker.

C-kit: structure and function

C-kit weights approximately 145 to 160 kDa and is a transmembrane protein. C-kit acts as a receptor tyrosine-kinase (RTK) and is structurally related to other RTKs, such as the platelet-derived growth factor receptor (PDGFR) (Lennartsson and Rönnstrand, 2012). C-kit is encoded by the proto-oncogene c-kit (Yarden et al., 1987), the cellular homologue of the viral oncogene v-kit, identified in 1986 in the Hardy-Zuckerman 4 feline sarcoma virus (Besmer et al., 1986), comprising 21 exons separated by intronic sequences. This RTK is composed of five extracellular immunoglobulin-like domains (encoded by exons 1 to 9), a transmembrane domain (exon 10), a juxtamembrane domain (exons 11 and 12) and a tyrosine-kinase domain divided into two separate parts (Fig. 1) (Longley et al., 2001; Webster et al., 2006a; Lennartsson and Rönnstrand, 2012). The c-kit ligand is a growth factor known as stem cell factor (SCF), also designated mast cell growth factor or Steel factor (Williams et al., 1990), which exists as a bivalent dimer. C-kit activation occurs physiologically when SCF binds to the extracellular domains of two c-kit proteins. These then associate to form a dimer and undergo trans-phosphorylation on multiple tyrosine residues on their intracellular domains (Fig. 1). Phosphorylation at these sites promotes c-kit's interaction with multiple signal transduction proteins (Blume-Jensen et al., 1991; Philo et al., 1996; Lemmon et al., 1997). In mast cells, c-kit activation by SCF promotes signalling through multiple pathways, such as the RAS/ mitogen-activated protein kinase (MAPK) pathway, the PI3-kinase (with its p65 and p85 subunits) and the Src family of kinases

(SFK) pathway. In this way, c-kit is involved in many of the hallmarks of cancer, such as cell survival, proliferation and motility (reviewed by Rönnstrand, 2004 and Lennartsson and Rönnstrand, 2012). RAS activation by c-kit is mediated through the SOS protein and activation of the RAS/MAPK pathway ultimately modifies gene transcription, promoting cell proliferation and survival (Kyriakis et al., 1992; Crews et al., 1992; Rönnstrand, 2004). C-kit-induced activation of Src kinases also leads to enhanced RAS/MAPK signalling (Timokhina et al., 1998). Activation of the PI3-kinase pathway leads to mast cell differentiation and degranulation, as well as to enhanced fibronectin adhesion and cell motility through cytoskeletal rearrangements (Serve et al, 1995; Vosseller et al., 1997). Additionally, activation of the PI3-kinase pathway results in activation of the anti-apoptotic factor AKT, thus promoting cell survival and resistance to apoptosis, a critical feature of cancer cells (Timokhina et al., 1998; Kissel et al., 2000).

The juxtamembrane domain is involved in regulating this receptor's tyrosine-kinase activity, although the regulatory mechanisms are not yet entirely clarified. Protein kinase C is also known to regulate c-kit activity through a negative feedback loop: c-kit activation leads to phospholipase D (PLD γ) activation, thus producing diacylglycerol, which activates protein kinase C. This enzyme then phosphorylates specific c-kit sites, thus inhibiting its activity (Blume-Jensen et al., 1991; Blume-Jensen et al., 1995; Kozawa et al., 1997). Additionally, protein kinase C promotes the degradation c-kit's extracellular domain, thus reducing its sensitivity to SCF (Yee et al., 1993; Yee et al., 1994). Impaired c-kit regulation (most commonly due to c-kit gene mutations affecting the juxtamembrane domain) results in enhanced mast cell proliferation, survival and invasion, driving carcinogenesis.

The role of c-kit in canine MCTs

In adult dogs, c-kit is expressed in Purkinje cells of the cerebellum, in Cajal cells of the digestive tract, in luminal epithelial cells of the mammary gland, endometrial epithelial cells and in mast cells (Morini et al., 2004). The immunoexpression of c-kit is well documented in canine neoplasms, including gastrointestinal stromal tumours (Bettini et al., 2003), ovarian (Morini et al., 2004), mammary gland (Morini et al., 2004) and testicular neoplasms (Morini et al., 2004; Reis-Filho et al., 2004), intestinal carcinoids (Gil da Costa et al., 2006), Merkel cell tumours (Gil da Costa et al., 2010) and renal cell tumours (Gil da Costa et al., 2011), cutaneous melanomas and melanocytomas (Gomes et al., 2012) and, especially, in MCTs (London et al., 1996; Reguera et al., 2000; Morini et al., 2004; Gil da Costa et al., 2007).

Recently, SCF has also been reported to be expressed by canine MCTs and a MCT murine xenograft (Amagai et al., 2013), suggesting the presence of an autocrine/paracrine loop in neoplastic mast cells, whereby local production of SCF may activate c-kit in the same cell or in neighbouring cells inside a mast cell tumour.

Mutations of the c-kit gene, most commonly located on exon 11, which encodes the critical regulatory juxtamembrane domain of c-kit, were identified in 8.3 % (Giantin et al., 2012a), 9.0% (Zemke et al., 2002), 15% (Webster et al., 2006b) or 17% of MCTs (Takeuchi et al., 2013), of MCTs of all histological grades. However, Downing et al. (2002) reported exon 11 mutations to be more frequent (30% to 50%) in grade II or III tumours. These numbers seem to be affected by the study population and are likely to increase as more sensitive methods for detecting c-kit mutations become available

(Gentilini et al., 2013). Importantly, c-kit mutations identified in primary MCTs match those found in their respective metastases (Marconato et al., 2014).

As previously mentioned, mutations on exon 11 impair the regulatory function of the juxtamembrane domain, causing constitutive c-kit activation, independently of SCF binding (London et al., 1999; Ma et al., 1999; Zemke et al., 2002). Canine MCTs also show other mutations, affecting namely the exons 2, 5, 6, 7, 8, 9, 10 and 15 (Letard et al., 2008; Takeuchi et al., 2013). Some mutations on exons 8 and 9 result in constitutive c-kit phosphorylation (Letard et al., 2008), but their full biological, prognostic and therapeutic implications remain to be clarified. Still, the available knowledge on c-kit originated a number of useful prognostic markers that are now commonly use to predict the biological behaviour of MCTs.

Zemke et al. (2002) correlated the presence of c-kit mutations with higher histological grade, suggesting their association with an aggressive phenotype. These findings were later confirmed by Webster et al. (2006b) in a study involving 60 dogs treated with surgery alone. The authors showed that presence of exon 11 c-kit mutations correlated with increased risk of dying from MCTs (odds ratio = 15.0) and with increased risk of developing local and systemic recurrence (odds ratio of 5.4 and 6.1, respectively). Takeuchi et al. (2013), recently reported that the presence of activating c-kit mutations (whether located in exon 11 or elsewhere) correlated with shorter median overall survival (341 vs. 553 days) and progression-free survival (181 vs. 324 days) in canine MCTs treated according to Thamm and Vail (2007). These results had no statistical significance, which may be due to the adjuvant therapy employed (which in some cases included imatinib, a known c-kit inhibitor). However, when the impact of

1 exon 11 internal tandem duplications apart from other mutations was evaluated, the
2 authors were able to identify a significant correlation with reduced progression-free
3 survival (130 vs. 345 days, $p=0.01$). More recent studies using proteomic approaches
4 confirm that c-kit exon 11 mutations correlate with changes on proteins involved in
5 cytoskeleton structure and cell motility, possibly explaining why these mutations induce
6 an aggressive and invasive phenotype (Schlieben et al., 2012; Schlieben et al., 2013).
7 The presence of c-kit gene mutations also correlates with aberrant c-kit
8 immunoexpression patterns (Webster et al., 2006b). Immunohistochemically, the c-kit
9 protein may present different expression patterns, namely membrane-associated (pattern
10 I), focal cytoplasmic (paranuclear or Golgi-like, pattern II) or diffuse cytoplasmic
11 (pattern III) patterns (Reguera et al., 2000; Morini et al., 2004). The membrane-
12 associated pattern is the one observed in normal mast cells and the presence of
13 cytoplasmic c-kit immunoexpression correlates with reduced post-surgical survival
14 (Kiupel et al., 2004; Preziosi et al., 2004; Abadie et al., 2005), higher histological grade
15 and increased cell proliferation (Gil da Costa et al., 2007). Giantin et al. (2012a)
16 reported that c-kit mRNA levels were increased in MCTs compared with normal skin,
17 but were not correlated with the immunohistochemical staining pattern, suggesting that
18 cytoplasmic c-kit patterns may be due to post-translational modifications rather than to
19 increased gene expression. In a large study involving 100 dogs treated with surgery
20 only, Kiupel et al. (2004) associated patterns I, II and III with 16.7%, 45.0% and 61.6%
21 recurrence rates (including both local and distant recurrence) and with 2.4%, 25.6% and
22 38.5% MCT-related death rates, respectively. Abadie et al., (2005) also reported that c-
23 kit cytoplasmic expression correlated with reduced overall survival compared with
24 membrane-associated expression (33 ± 4 months versus 9 ± 8 months) and with a reduced
25 2-years post-surgical survival (20% versus 100%). In a recent study, Giantin et al.

(2012a) found only a weak, statistically non-significant correlation between the c-kit staining pattern and local recurrence and survival, which may be due to the relatively small population involved in the study.

Given c-kit's role in driving cell proliferation and cancer invasion, it seems natural that changes in c-kit expression correlate with increased cell proliferation and microvessel density (Patrino et al., 2014). In fact, enhanced cell proliferation, assessed through the mitotic index, has long been an important factor when grading MCTs. Romansik et al. (2007) reported that dogs whose MCTs presented mitotic indices (mitotic figures per 10 high-power fields) of 5 or less had a median survival of 70 months, compared with 2 months for mitotic indices greater than 5. More recently, a different cut-off point of 7 was proposed by Kiupel et al. (2011) in their new histological grading system. Other methods used to quantify cell proliferation include the counting of nucleolar organizer regions (NORs) detected on the basis of their argyrophilic properties (AgNORs) (Hung et al., 2000; Scase et al., 2006), in vivo bromodeoxyuridine (BrdU) incorporation (Sakai et al., 2002) and the immunohistochemical detection of the proliferating cell nuclear antigen (PCNA) (Hung et al., 2000; Scase et al., 2006) and the proliferation marker Ki-67 (Sakai et al., 2002; Scase et al., 2006). All these methods provide some indications concerning the biological behaviour of canine MCTs, but the use of BrdU is precluded by the need for in vivo administration and AgNOR counts are difficult to standardize and show great variability between researchers. Immunohistochemical markers (Ki-67 and PCNA) are more practical and widely adopted., Ki67 is a more sensitive proliferation marker than PCNA, since PCNA is present only in cells during the S phase of the cell cycle, while Ki-67 is detected in all proliferating cells, excluding only resting (i.e., G0) cells (Peña et

al., 1998; Roels et al., 1999). As a prognostic factor for canine MCTs, Ki-67 has the additional advantage of being independent of tumour grade (Scase et al., 2006). The three c-kit expression patterns are significantly correlated with both the AgNOR and Ki-67 indices (Gil da Costa et al., 2007; Webster et al., 2007) and in subcutaneous MCTs, c-kit expression patterns and the Ki-67 labelling index were also found to significantly correlate with prognosis (Thompson et al., 2011).

Overall, the current view concerning the oncogenesis of MCTs is that c-kit gene mutations lead to the expression of a constitutively active c-kit protein. This abnormal protein accumulates in the cytoplasm, and this altered expression pattern can be detected immunohistochemically. Aberrant c-kit drives cell transformation promoting several of the cancer hallmarks, in particular unchecked cell proliferation, most often assessed in the context of histological grading through the mitotic index, or immunohistochemically by determining the Ki-67 labelling index. Accordingly, the most relevant and commonly used prognostic markers for MCTs are: the detection of c-kit gene mutations (using gel electrophoresis and DNA sequencing techniques), histological grading by a pathologist, the c-kit protein immunohistochemical expression pattern, and the assessment of cell proliferation, most commonly using Ki-67. Apart from validating such prognostic markers, perhaps the most valuable contribution of all the research done on MCTs was the identification of c-kit as a therapeutic target and of c-kit mutations as indicators of response to therapy. Therapeutic advances based on c-kit inhibition will be dealt with in the following sections.

Treating canine MCTs

Given the highly variable biological behaviour of MCTs, treating these tumours is a major challenge for clinicians. The therapeutic approach for MCTs largely depends on whether complete surgical excision is possible and whether local therapy is sufficient or systemic therapy is required. Tumour grading is critical and lymph node status is predictive of prognosis (Weishaar et al., 2014). Surgery is the first and best option for localized, non-metastatic MCTs, including cases of multiple cutaneous MCTs (Weisse et al., 2002; Govier, 2003; Blackwood et al., 2012). Both the histological grade and the status of the surgical margins are important factors for surgical cure (Schultheiss et al., 2011; Donnelly et al., 2013) and the current guidelines for treating grade I and II (Patnaik) MCTs are largely based on the status of tumour margins (Blackwood et al., 2012). Radiotherapy is also an option, while chemotherapy is most commonly used as an adjuvant or neo-adjuvant approach for treating systemic disease. This is often the case with aggressive MCTs, where chemotherapy is used to prevent or delay the development of metastases (Matsuda et al., 2011; Blackwood et al., 2012; Lejeune et al., 2013; Miller et al., 2014). First-line chemotherapy is often based on prednisolone and vinblastine and second-line approaches on lomustine, although protocols alternating vinblastine and lomustine and other drugs are also in use (Thamm et al., 2006; Stanclift et al., 2008; Taylor et al., 2009; Rivera et al., 2013). In face of the challenging nature of canine MCTs, several innovative therapies have recently been proposed, including electrochemotherapy, aiming to increase the cellular uptake of cisplatin or bleomycin (Kodre et al., 2009; Spugnini et al., 2011; Suzuki et al., 2014), immunotherapy based on IL-2 administration (Ziekman et al., 2013), as well as an experimental approach based on oncolytic virotherapy (Hwang et al., 2013). While these and other proposals are developed and tested, targetted therapies based on inhibiting c-kit and other related receptor tyrosine-kinases (RTKs) are being increasingly adopted in clinical practice.

Targeted therapies: c-kit inhibitors

Imatinib was the first RTK inhibitor approved for use in human patients. Although it was initially directed against the BCR-ABL fusion protein in chronic myeloid leukaemia, imatinib was quickly used to inhibit c-kit too. Over recent years, several small-molecule inhibitors of c-kit and other related RTKs became available to treat human and animal cancers (reviewed by Takeuchi and Ito, 2011 and London, 2013). Such RTK inhibitors most often compete with ATP for binding sites on RTKs, thus blocking the phosphorylation of their catalytic domain and preventing their activation. While some drugs are relatively specific, others target a number of different RTKs, which often results in higher efficacy but also in a broader range of toxicities that may limit their application. A number of RTK inhibitors has been screened for their efficacy against MCT cell lines in vitro (Takeuchi et al., 2011a, b) and some studies show that imatinib may have some efficacy against MCTs (Isotani et al., 2008; Marconato et al., 2008; Yamada et al., 2011; Nakano et al., 2014).

Recently, two orally bioavailable RTK inhibitors were approved by the European Medicines Agency and the United States Food and Drug Administration for treating canine MCTs (Blackwood et al., 2012; Barnabe et al., 2013). Toceranib (Pfizer Animal Health) was approved for treating recurrent, nonresectable grade II and III MCTs, while masitinib (AB Science) has been approved for the same group of tumours, when these confirmedly harbour c-kit mutations. Both toceranib and masitinib target c-kit. Toceranib also inhibits the vascular endothelial growth factor receptor 2 (VEGFR2) and PDGFR, both involved in tumour angiogenesis and metastasis (Pryer et al., 2003; London et al., 2009), while masitinib also targets PDGFR, the lymphocyte-specific

kinase, fibroblast growth factor receptor 3 and focal adhesion kinase (Dubreuil et al., 2009; Marech et al., 2014). Proteomic and transcriptomic studies of a masitinib-treated MCT-derived cell line confirmed that c-kit inhibition result in profound transcriptional changes in treated cells (Klopfleisch et al., 2012). Approximately 16% of the canine genes were transcriptionally regulated, largely resulting in reduced cell metabolism and proliferation. However, several alternative pro-proliferative pathways were also found to be up-regulated, which may allow treated cells to keep proliferating, and may partly explain masitinib resistance in the clinical setting. In fact, although RTK inhibitors often provide excellent initial results, resistance frequently develops during long-term treatment (London, 2013). Halsey et al. (2014) recently provided some additional clues on this matter, reporting that toceranib-resistant MCT cell lines develop secondary c-kit mutations affecting the juxtamembrane and tyrosine-kinase domains and also up-regulate c-kit expression but do not express the chemoresistance-associated P-glycoprotein. Importantly, sensitivity to vinblastine and lomustine was maintained, and resistance to other RTK inhibitors was variable, opening potential alternatives to deal with RTK-resistant MCTs. Another important issue that should not be ignored when using RTK inhibitors is their associated toxicity. This is usually mild, and for toceranib, the most commonly described side-effects are diarrhoea, anorexia, lethargy, vomiting, lameness and weight loss (London et al., 2009). For masitinib, frequently described toxicities were diarrhoea, vomiting, oedema and neutropaenia. Protein losing nephropathy and haemolytic anaemia were less commonly described in connection with masitinib administration (Hahn et al., 2008).

Both toceranib and masitinib show significant efficacy as single agents against canine MCTs. Toceranib's safety and efficacy were first studied in dogs against a

1 range of malignancies, including various sarcomas and carcinomas, MCTs, melanomas
2 and myeloma (London et al., 2003). The highest response rate was observed among
3 dogs with MCTs showing c-kit mutations. Adverse reactions were mainly
4 gastrointestinal and the maximum tolerated dose was found to be 3.25mg/kg every other
5 day. Following these results, toceranib was then tested in dogs with grade II or III
6 MCTs with or without lymph node metastases, in a placebo-controlled double-blinded
7 study (London et al., 2009). During the blinded phase, toceranib-treated animals showed
8 a 37.2% response rate vs. 7.9 among placebo treated animals. However, if all dogs that
9 received toceranib following placebo escape are also included, the overall response rate
10 rises to 42.8% (n=145). Interestingly, MCTs with c-kit mutations showed a better
11 response rate (69.0%) than those with wild-type c-kit (37.0%). Robat et al., (2012)
12 addressed the combination of toceranib with vinblastine in dogs. The authors found that
13 the dose-limiting toxicity was neutropaenia, and the maximum tolerated doses were 1.6
14 mg/kg vinblastine every other week with 3.25 mg/kg toceranib every other day. Thus,
15 the enhanced myelosuppression of this combination led to a 50% reduction in the
16 vinblastine dose intensity. Still, the overall response rate was 71.0%, which is superior
17 to any of the drugs when used as single agents. A study combining toceranib with
18 prednisone and radiotherapy (6 Gy once per week for 4 weeks) for treating
19 nonresectable MCTs, achieved a 76.4% overall response rate with no added toxicity
20 compared with radiotherapy alone (Carlsten et al., 2012).

21
22 The safety and efficacy of masitinib, the second approved RTK inhibitor, were
23 investigated in a large double-blind, placebo-controlled study involving 200 dogs with
24 grade II or III, non-metastatic, recurrent or non-resectable MCTs (Hahn et al., 2008).
25 Masitinib increased the time to tumour progression compared with placebo (118 vs. 75

days, $p=0.38$) at an oral daily dose of 12.5 mg/kg. This effect was more pronounced when masitinib was used as first-line rather therapy (253 vs 75 days, $p=0.001$). As with toceranib, the presence of c-kit mutations improved response to masitinib. A long-term follow-up study showed that masitinib increased the survival rates at 12 and 24 months compared with placebo (40.0% vs. 15.0% at 24 months) (Hahn et al., 2010). At 24 months, 9.0 of masitinib-treated dogs still showed complete responses. Interestingly, the short-term response to masitinib (at 6 weeks) had no correlation with long-term survival, contrary to the more mid-term response at 6 months, which was highly predictive. The more recent results of Smrkovski et al., (2013) show that masitinib, when administered as a rescue to 12 dogs (7 of which with metastatic disease) which failed to respond to previous chemotherapy, resulted in a 25% overall response rate. As could be expected in light of previous findings, first-line masitinib administration (to 14 dogs, 8 of which with metastatic disease), resulted in a higher, 57% overall response rate (Smrkovski et al., 2013).

Conclusions

Long years of basic research into the role of c-kit in canine MCTs originated improved prognostic markers and a group of targetted therapies. Despite these advances, and largely because of them, MCTs remain an active and exciting field of research. In particular, the development of masitinib for treating human malignancies benefits from this spontaneous animal model of mast cell neoplasia (Marech et al., 2014). New developments concerning the biopathology of canine MCTs, as well as translational and clinical experiments are continuously reported, making this a fascinating theme for veterinary practitioners and researchers alike.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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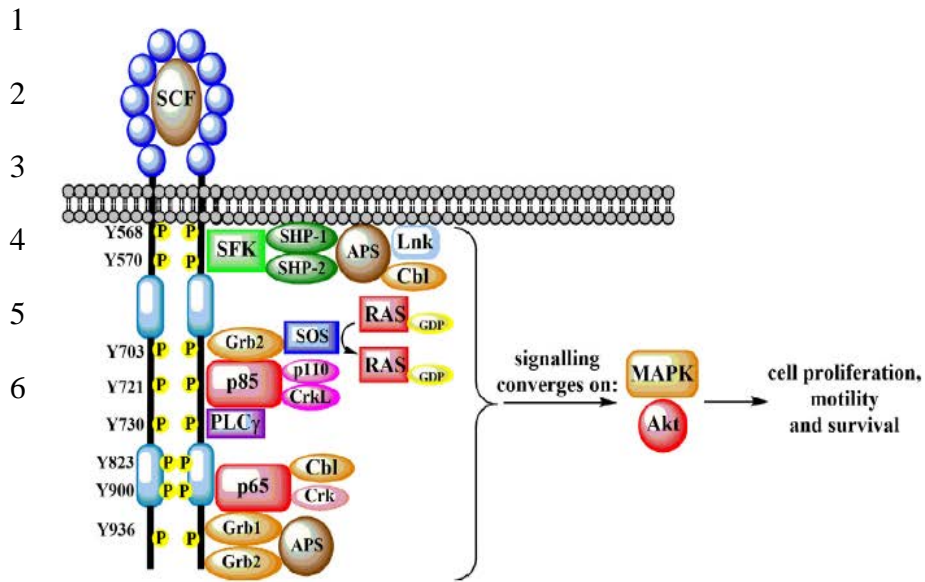


Fig. 1. A schematic overview of the receptor tyrosine kinase c-kit, its activation by SCF and some of the main signal transduction pathways leading to c-kit-induced cell proliferation and survival.