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Dysregulation of tyrosine kinases and use of imatinib in small animal practice

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ABSTRACT

Imatinib inhibits the activity of several tyrosine kinases, including BCR-ABL, KIT and platelet-derived growth factor receptor (PDGFR). Dysregulation of KIT is found in mast cell tumours (MCTs) and *KIT* is mutated in approximately 30% and 70% of canine and feline MCTs, respectively. *KIT* mutations have also been reported in canine and feline gastrointestinal stromal tumours (GISTs), canine acute myeloid leukaemia and canine melanoma. In addition, *BCR-ABL* and *PDGFR* mutations have been found in canine leukaemia and haemangiosarcoma, respectively. Imatinib has anti-tumour activity with tolerable toxicity towards a certain subset of MCTs in dogs and cats. Favourable clinical responses are likely to be associated with the presence of *KIT* mutation. Anti-tumour activity of imatinib has also been demonstrated in canine GISTs with a *KIT* mutation and in feline hypereosinophilic syndrome; however, to date only one of each of these cases has been reported. In conclusion, analysis of *KIT* mutations appears to provide valuable data for individual treatment with imatinib in dogs and cats.

Introduction

Since the discovery of KIT dysregulation due to mutations in canine mast cell tumours (MCTs) (London et al., 1999; Ma et al., 1999), veterinary oncology research with a focus on targeted therapy using tyrosine kinase inhibitors (TKIs) has dramatically increased. Two TKIs (toceranib and masitinib) have entered the clinic (Hahn et al., 2008; London et al., 2009) and are currently changing the therapeutic approach for malignancies in dogs and cats.

The small-molecule TKI, imatinib (Novartis; also known as Gleevec or Glivec), is a prototype of target-oriented drugs in humans. It has sparked a revolution in cancer therapy by dramatically improving treatment for chronic myeloid leukaemia (CML) in humans (Druker et al., 2001). Imatinib targets BCR-ABL, KIT, platelet-derived growth factor receptor (PDGFR), colony stimulating factor 1 receptor, ABL1, ABL2, discoidin domain receptor 1/2 and lymphocyte-specific protein tyrosine kinase (Deininger et al., 2005; Dewar et al., 2005; Manley et al., 2010).

Some tumours in dogs and cats possess mutations in these tyrosine kinases; therefore, imatinib is one of a number of potential target-oriented therapeutic approaches for canine and feline neoplasms. The aim of this article is to review the current knowledge regarding the dysregulation of kinases and the therapeutic effects of imatinib in canine and feline neoplasms, particularly in MCTs.

KIT and other potential targets for imatinib in canine and feline tumours

Among the potential targets for imatinib, KIT, which is expressed in MCTs, has been the most extensively studied in dogs and cats. To date, there is only limited information available regarding other potential targets in canine and feline tumours.

KIT mutations in canine mast cell tumours

The reported location and frequency of *KIT* mutations in canine MCTs are summarised in Table 1. *KIT* mutations have been observed in approximately 30% of canine MCTs (Letard et al., 2008; Takeuchi et al., 2013). *KIT* mutations are most frequently found in exon 11 (~14–21%) and primarily consist of internal tandem duplication (ITD) mutations (9–17%) in randomly selected MCT cases (Zemke et al., 2002; Webster et al., 2006; Letard et al., 2008; Takeuchi et al., 2013). Other than exon 11 mutations, mutations have been found in exons 2, 6, 7, 8, 9, 15 and 17 (Letard et al., 2008; Takeuchi et al., 2013). Compared with mutations in exon 11, mutations in exons 8 and 9 are less frequent, although a significant number of mutations have been identified (Letard et al., 2008; Takeuchi et al., 2013). Mutations in other exons are generally infrequent (<3%).

A high incidence of exon 11 ITD mutations has been reported in higher grade MCTs classified according to Patnaik grades (Downing et al., 2002; Zemke et al., 2002; Webster et al., 2006). Although no significant differences in the frequency of the exon 11 ITD mutation were observed in MCTs across Patnaik grades, the frequency

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Table 1
Reported frequency of *KIT* mutation in randomly selected canine mast cell tumours.

Number of cases	Region of *KIT* examined	Frequency of mutation (%)					Reference
		Exon 8	Exon 9	Exon 11 (ITD)	Other exons	Total	
47	Entire *KIT*	6.4	0	21.3 (17.0)	<3	34.0	Takeuchi et al. (2013)
191	Exons 8–13 and 17–19	4.7	4.2	16.8 (13.1)	<3	26.2[a]	Letard et al. (2008)
88	Exon 11	NA	NA	13.6 (9.1)	NA	NA	Zemke et al. (2002)
60	Exon 11	NA	NA	NA (13.3)	NA	NA	Webster et al. (2006)

NA, not available; ITD, internal tandem duplication mutation.
[a] Value indicates the total frequency of mutations in exons 8–13 and 17–19.

of this mutation in Kiupel high grade MCTs was significantly higher than that in low grade tumours (Takeuchi et al., 2013). Moreover, the presence of exon 11 ITD mutation is associated with an increased incidence of recurrent disease, metastasis, death or shorter progression-free survival (Downing et al., 2002; Webster et al., 2006; Takeuchi et al., 2013). All these reports suggest an association between clinical aggressiveness and the presence of ITD mutations, suggesting that imatinib may have therapeutic utility for canine MCTs with aggressive behaviour.

Although mutated KIT is an important target for imatinib in canine MCTs, genetic heterogeneity within the same tumour and/or between different tumours from the same patient (primary tumour vs. metastasis) could exist. In humans, the presence of intratumoral genetic heterogeneity influences therapeutic response and contributes to resistance against kinase inhibitors (Bedard et al., 2013). However, in dogs, Marconato et al. (2014) observed no difference in the mutation status of *KIT* between primary canine MCTs and their corresponding metastases. In contrast, Amagai et al. (2013) reported that two cases of canine MCT had an ITD mutation in the primary lesion but not in the secondary lesion. The latter finding implies the presence of heterogeneity in *KIT* mutational status among different geographical regions of MCTs, similar to that reported for various human tumours (Bedard et al., 2013). Genetic heterogeneity could therefore be an issue when using imatinib therapy for canine MCTs.

KIT mutation in feline mast cell tumours

The mutational status of *KIT* in feline MCT is summarised in Table 2. Isotani et al. (2010) found that 67.7% of feline MCTs had a mutation in *KIT*. The majority of the mutations were identified in exon 8, where they mostly consisted of an ITD mutation, and in exon 9. Sabattini et al. (2013) reported that 62.5% of cats with MCT had a mutation in at least one of their multiple nodules and that the majority of these mutations were found in exons 8 and 9. Although the most frequent mutation was reported to be in exon 8 (45.2% of cases) in the study of Isotani et al. (2010), it was reported to be in exon 9 (50.0% of cases) in the study of Sabattini et al. (2013). The reason for this difference is unclear, but could reflect a difference in the tumour population (e.g. visceral vs. cutaneous). Although Dank et al. (2002) investigated *KIT* mutation in exons 11, 12 and 17 in 10 cats with MCT, no mutation was identified. Thus, *KIT* mutations appear to be more frequent in feline MCTs than canine MCTs, and the 'hotspot' of mutation differs from that of canine MCTs. Furthermore, in contrast to canine MCTs, no significant relationship between *KIT* mutation status and tumour behaviour was observed for feline MCT (Sabattini et al., 2013).

Sabattini et al. (2013) demonstrated that multiple nodules from the same cat had different *KIT* mutational status; one cat had two tumour nodules that each showed a different mutation, while five cats had tumour nodules in the same subject with or without *KIT* mutations. As speculated for canine MCTs, some feline MCTs may have genetic heterogeneity in the same animal.

KIT mutations in canine and feline gastrointestinal stromal tumours

KIT mutations have been detected in canine and feline gastrointestinal stromal tumours (GISTs). In dogs, Frost et al. (2003) reported that 2/4 cases had a deletion/insertion or a substitution mutation (*KIT* exon 11 was examined) and Gregory-Bryson et al. (2010) reported that 6/17 cases had deletion mutations in *KIT* exon 11 (*KIT* exons 8, 9, 11, 13 and 17, and *PDGFRA* exons 12, 14 and 18, were examined). A deletion mutation in *KIT* exon 11 was also identified in a case of canine GIST (Kobayashi et al., 2013) and a deletion mutation in *KIT* exon 11 has been reported in a case of feline GIST (Morini et al., 2011). There was an overlap between canine and feline GIST mutations with one of the hotspots of driver mutations in human GIST (Corless et al., 2011). A substitution mutation reported by Frost et al. (2003) in canine GIST has also been reported in some human GISTs (Lasota et al., 1999). Although the number of cases examined is still small, these studies indicate that a certain subset of canine and possibly feline GISTs may share similar molecular features with human GISTs and that mutant KIT could be a potential therapeutic target of imatinib in these tumours.

KIT mutations in canine leukaemia and lymphoma

Usher et al. (2009) examined the mutation status of *KIT* exons 8, 10, 11 and 17 in canine acute leukaemias (myeloid, lymphoid and undifferentiated). In this study, 3/21 cases of acute myeloid leukaemia had *KIT* mutations (one mutation in each of exon 11 and 17, one mutation in exon 17 or two mutations in exon 17). In contrast, no *KIT* mutation was found in cases of acute lymphoid leukaemia ($n = 14$) or acute undifferentiated leukaemia ($n = 1$). Giantin et al. (2013a) also investigated the presence of mutations

Table 2
Reported frequency of *KIT* mutation in randomly selected feline mast cell tumours.

Number of cases	Region of *KIT* examined	Frequency of mutation (%)						Reference
		Exon 6	Exon 8 (ITD)	Exon 9	Exon 11	Other exons	Total	
62	Entire *KIT* or exons 8, 9, 11, 13, and 17	5.9[a]	45.2 (40.3)	24.2	1.6	0	67.7	Isotani et al. (2010)
24	Exons 8, 9, and 11	NA	16.7[b] (8.3[b])	50.0[b]	8.3[b]	NA	62.5[b]	Sabattini et al. (2013)

NA, not available; ITD, internal tandem duplication mutation.
[a] Mutation in exon 6 was examined in 17 cats and one cat had a mutation.
[b] The frequency of mutation was recalculated from the original report as the percentage of cats that had a mutation in at least one of their multiple nodules.