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# Successful long-term management with toceranib phosphate of a recurrent muzzle mast cell tumour in a dog

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**Abstract:** A 10-year-old spayed female Yorkshire terrier with a muzzle mass was presented. The ulcerated mass was surgically resected, but recurred and grew rapidly over the subsequent 7 months. The submandibular lymph nodes were remarkably enlarged (right: 9.5 × 7.8 cm; left, 4.7 × 4.0 cm). The cytological analysis of the mass and lymph nodes revealed a high-risk mast cell tumour characterised by high mitotic figures (> 5/oil immersion field), nuclear pleomorphism, multi-nucleation, karyomegaly, and anisokaryosis. A polymerase chain reaction analysis targeting the *c-kit* domain revealed an exon 8 mutation. The resection of the mass was not considered optional because of the location. Oral toceranib (3.25 mg/kg, every other day) and prednisolone (1.0 mg/kg to 0.5 mg/kg, once a day) were administered, and the mass disappeared within 1 month. Subsequently, the toceranib therapy was stopped for 4 months due to neutropenia ($0.7 \times 10^9$/l). However, the mass recurred and the toceranib was resumed at the original dose, without affecting the neutropenia. No recurrence has been observed at the 16 months' follow up. Despite the persistent neutropenia, no clinical symptoms have presented. This is another report of the successful treatment of a recurrent muzzle MCT with toceranib in a dog.

**Keywords:** canine; *c-kit* mutation; inoperable; non-resectable; tyrosine kinase inhibitor

A mast cell tumour (MCT) is one of the most common canine cutaneous tumours and accounts for 16–21% of all canine skin neoplasms (Blackwood et al. 2012). The prognosis of MCT is highly variable and depends on a range of morphologic and molecular biology criteria (Thompson et al. 2016). Histological grading is considered a reliable negative prognostic tool (Blackwood et al. 2012). Furthermore, differential labelling patterns of KIT and phosphorylated KIT by immunohistochemistry (IHC) in MCTs are indicators of the biological behaviour associated with mutations in the *c-kit* proto-oncogene (Halsey et al. 2017; Thamm et al. 2019). High-grade MCTs are significantly associated with an aggressive systemic progression and a poor prognosis. The reported median survival times are between 4 months for a high-grade MCT and 2 years for a low-grade MCT (London et al. 2009; Blackwood et al. 2012).

The location and gross appearance of the MCTs are associated with their course (Takeuchi et al. 2013). Specifically, MCTs in the oral region, and particularly on the muzzle, are generally aggressive and go along with a high risk of lymph node metastasis (Hillman et al. 2010). In addition, surgical options in these locations are very limited because

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a lateral margin of at least 2 cm on the fascial plane deep margin, recommended for the curative surgical excision of an MCT, can hardly be achieved (Blackwood et al. 2012). Recently detected genetic factors have been suggested as prognostic indicators in MCTs (Thompson et al. 2016) and approximately 15–40% of canine MCTs involve a mutation of the proto-oncogene *c-kit* (Blackwood et al. 2012; Thompson et al. 2016). Such mutations are associated with intermediate to high-grade tumours, with uncontrolled cell proliferation, migration, maturation, and tissue invasion without *c-kit* ligand-binding (Welle et al. 2008; Blackwood et al. 2012; Takeuchi et al. 2013).

Tyrosine kinase inhibitor (TKI) therapy is currently the therapy of choice for certain tumours (Kim et al. 2016), and toceranib is one of the TKIs documented for the successful treatment of an inoperable or a recurrent canine MCT (London et al. 2009; Carlsten et al. 2012). Although toceranib phosphate is only approved for the treatment of mast cell tumours in dogs, recently the efficacy has been reported against other tumours, particularly neuroendocrine tumours (Flesner et al. 2019). Herein, we describe a case of successful treatment of a dog with toceranib with a recurrent ulcerative, and a *c-kit* mutation-positive MCT on the muzzle.

## Case description

A 10-year-old spayed female Yorkshire terrier was admitted to the Veterinary Medical Teaching Hospital at Konkuk University (Seoul, Republic of Korea) due to the recurrence of a previously excised cutaneous MCT. The physical examination revealed a soft round mass approximately 5.7 × 7.0 × 6.7 cm in size, covering the entire dorsal muzzle and nasal planum and exhibiting ulceration and erosion (Figure 1A). The dog had previously a mass diagnosed as an MCT, based on the cytologic analysis following the surgical resection, but it recurred rapidly after the surgical excision and, unfortunately, there was no histological diagnosis at that time.

A complete blood count revealed a leukopenia of unknown aetiology, with a white blood cell (WBC) count of 1.6 × $10^9$/l (reference interval 5.05–

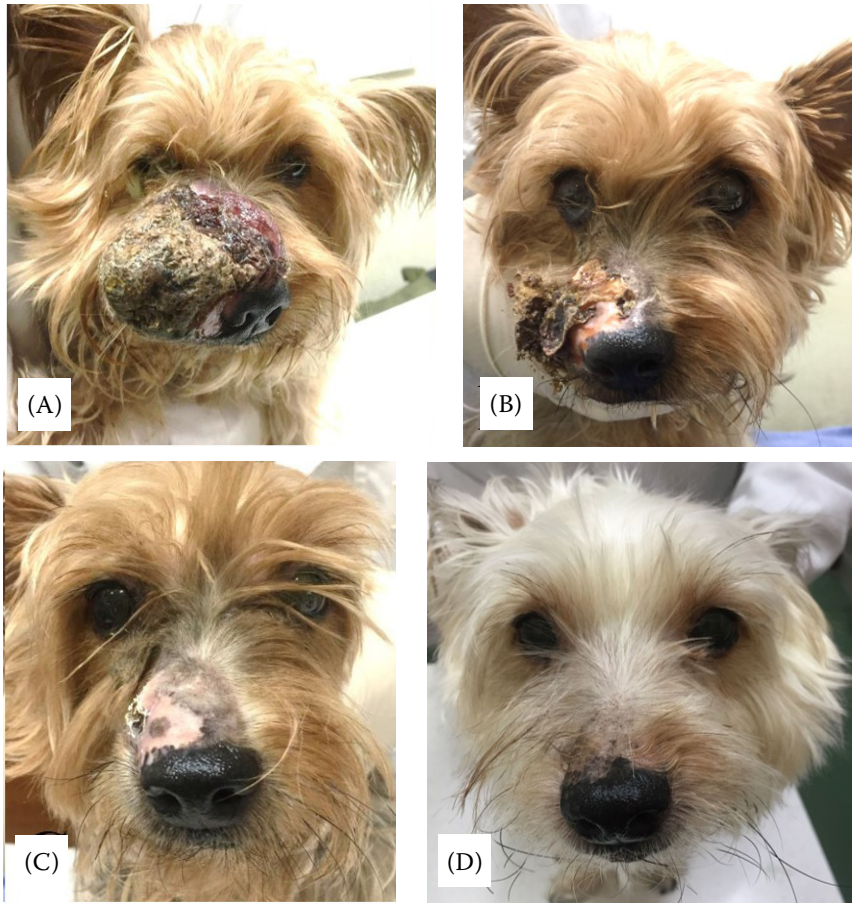


Figure 1. Photographs of the clinical features of a dog with a muzzle mast cell tumour before and after the toceranib therapy. (A) On the day of presentation (pre-treatment), the mass was ulcerative and 5.7 × 7.0 × 6.7 cm in size. (B) One week after the initiation of toceranib, the size of the mass had decreased significantly. (C) One month after the initiation of toceranib, a complete remission had been achieved. (D) After 1 year of the toceranib therapy, the dog remains in complete remission

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16.76 × $10^9$/l). The serum biochemical profile was within the reference range. In coagulation tests, the activated partial thromboplastin time was prolonged at 130 s (reference interval 72 s to 102 s), and the prothrombin time was normal at 14 s (reference interval 11 s to 17 s). The radiography revealed a dense soft-tissue mass without evidence of bone lysis. The ultrasonography was unremarkable. Following the wishes of the dog's owners, considering the presence of the partial coagulopathy, risk of infection, and location of the tumour, a further histologic examination was not performed. A fine needle aspiration (FNA) of the mass was performed and revealed numerous poorly granulated round cells with fine purple granules, the prevalence of mitotic figures (> 5/oil immersion field), nuclear pleomorphism, multinucleation, karyomegaly, and anisokaryosis, indicating a malignant MCT (Figure 2A). The aspiration cytology of the enlarged submandibular lymph nodes revealed large numbers of mast cells, suggesting a metastatic disease (Figure 2B). The polymerase chain reaction analysis of the material derived from the FNA to investigate the specific potential mutations revealed the mutation of the exon 8 of the *c-kit* gene (Figure 2C). To screen for mutations in the *c-kit* exons 8, the following primer set developed by Kobayashi et al. (2012) was used for amplifying the *c-kit* exons 8: the forward primer was 5'-AGCCTTGGTGAGGTGTTCCA-3' and the reverse primer was 5'-CTACCCTGCT GTCCTTCCCT-3'. Based on the history, cytology, and PCR analysis a high-risk MCT was diagnosed.

Medical management including toceranib (Palladia®; Pfizer Inc., Ascoli, Italy) and prednisolone (Solondo®; Yuhan Co., Seoul, Republic of Korea) was deemed the most appropriate course of treatment due to the inoperable location of the mass and lymph node metastasis. The dog was treated orally with toceranib (Palladia®; Pfizer Inc., Ascoli, Italy) at a dose of 3.25 mg/kg once every 2 days. Prednisolone (Solondo®; Yuhan Co., Seoul, Republic of Korea; 1.0 mg/kg to 0.5 mg/kg orally once a day), chlorpheniramine (Peniramin®; Yuhan Co., Seoul, Republic of Korea; 0.5 mg/kg orally twice a day), and famotidine (Famotidine®; Hanmi Pharm., Seoul, Republic of Korea; 0.5 mg/kg orally twice a day) were also administered to reduce the inflammation and inhibit the histamine secretion from the tumour. A complete remission of the mass was observed after 3 weeks of therapy, and the metastatic lymph nodes were also reduced to normal size. At the 25-day follow-up time-point, a complete blood cell count revealed the grade III neutropenia as defined by the Veterinary Cooperative Oncology Group – Common Terminology Criteria for Adverse Events (VCOG-CTCAE) criteria (VCOG 2016) (WBC 1.77 × $10^9$/l; neutrophils 0.7 × $10^9$/l, reference interval 2.95–11.64 × $10^9$/l). The toceranib phosphate therapy was stopped, but the neutropenia persisted without any clinical signs such as

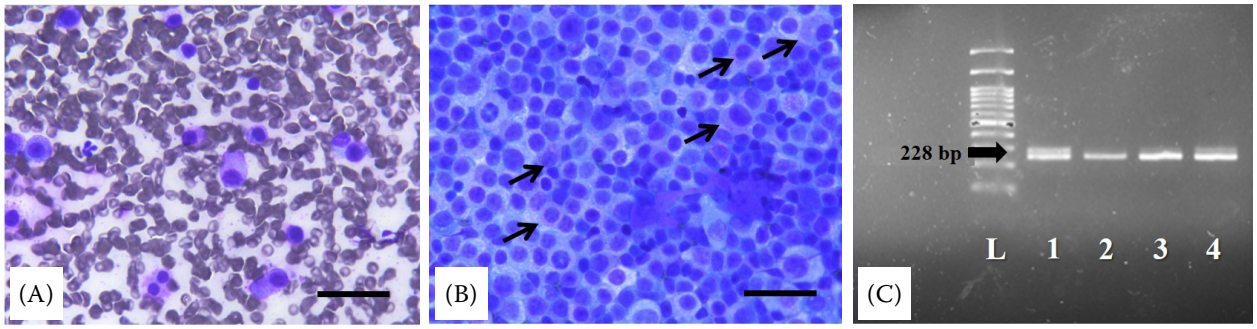


Figure 2. The fine needle aspiration (FNA) and polymerase chain reaction analysis targeting the *ckit* domain in a dog with a mast cell tumour (MCT). (A) The cytology revealed the proliferation of granulated mast cells with binucleation and mitotic figures (Diff-Quik stain, × 400, scale bar = 50 µm). (B) The FNA of the submandibular lymph node revealed the proliferation of medium sized lymphocytes and plasma cells with variously granulated mast cells (arrows) consistent with metastasis (Diff-Quik stain, × 400, scale bar = 50 µm). (C) The electrophoresis of the polymerase chain reaction products of the exon 8 of the *c-kit* gene from the FNA samples of a dog with an MCT. An aberrant banding pattern was detected at the target size region of the exon 8 (228 base pairs)

L = 100 base pair (bp) ladder; 1 = medial aspect of the mass; 2 = lateral aspect of the mass; 3 = submandibular lymph node; 4 = prescapular lymph node

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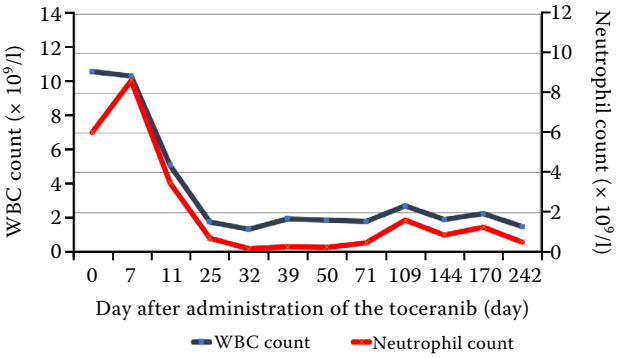


Figure 3. The white blood cell (WBC) and neutrophil counts following the administration of toceranib. Eleven days after the initiation of toceranib, the neutropenia occurred and gradually worsened. Although toceranib was discontinued on day 25, the neutropenia persisted, but there were no associated clinical signs. On day 170 (145 days after discontinuation), the toceranib treatment was resumed due to the mast cell tumour recurrence

hyperthermia or infections (Figure 3). Four months after the toceranib was stopped, the mass recurred (1.0 × 0.8 cm), and the toceranib treatment was then resumed at the original dose. The mass again shrunk rapidly thereafter, and no recurrence has been observed to date (16 months later). Although the neutropenia persists, no corresponding clinical signs have been observed.

## DISCUSSION AND CONCLUSIONS

In canine MCTs, wherever possible, surgery is considered the optimal treatment (Blackwood et al. 2012). For the excision of high-grade MCTs, a 3-cm lateral margin on the fascial plane deep margin is recommended, whereas for low-grade MCTs, a 2-cm lateral margin is generally considered sufficient (Blackwood et al. 2012). However, some MCT locations, such as the lip, the periocular region, and the muzzle, as in the present case, render surgery challenging. In the present case, the initial excision of the tumour was followed by the recurrence of the tumour within 7 months. The recurrence after surgery is associated with a worse prognosis (Blackwood et al. 2012). As a result, the subsequent medical management was changed to an oral systemic toceranib therapy instead of a repeat surgery.

In the present case, the mutation of the exon 8 of the *c-kit* gene was detected. Several TKIs, one of which is an anti-cancer drug, target the KIT domain. Toceranib phosphate, which is a TKI that targets the KIT as well as the vascular endothelial growth factor receptor and platelet-derived growth factor receptor, has been approved by the US Food and Drug Administration in veterinary medicine for treating recurrent high-grade and non-resectable MCTs (Bernabe et al. 2013; Thompson et al. 2016). Previous reports suggest that a KIT mutation is associated with an aggressive phenotype and has a tendency to respond well to TKIs targeting the *c-kit* (Thompson et al. 2016). However, there are several reports that show that the responses to TKIs cannot be predicted based on the presence of the mutations in the exons 8 and/or 11 of the *c-kit* gene (Kim et al. 2016). In the present case, toceranib was administrated for the medical management at 3.25 mg/kg once every 2 days, a generally prescribed dosage (Bernabe et al. 2013). In addition, prednisolone, famotidine, and chlorpheniramine were administered to inhibit the inflammation and histamine secretion (Welle et al. 2008). Notably, the recurrence of the mass after the toceranib was discontinued due to neutropenia, was halted after the toceranib reinstatement and the lack of subsequent MCT recurrence thereafter clearly demonstrate that toceranib was effective in the present case.

The possible side effects of toceranib include gastrointestinal disruption, bone marrow suppression, hepatotoxicity, protein-losing nephropathy, and cutaneous reactions (Bernabe et al. 2013; Cavalcanti et al. 2017). Toceranib can also cause minor neutropenia (Carlsten et al. 2012).

In humans, cutaneous adverse effects of tyrosine kinase inhibitors have been reported frequently and are clinically described as hand-foot skin reactions, including non-characterised skin eruptions, alopecia, and hair colour changes among others (Al Enazi et al. 2009; Giacchero et al. 2012). Similarly, the skin depigmentation associated with toceranib was reported in a dog (Cavalcanti et al. 2017). Although the pathogenesis of these adverse effects has not been established, activation of the *c-kit* is primarily responsible for the transmission of pro-migration signals in mammalian melanocytes has been implicated (Alexeev and Yoon 2006). In a previous canine case report (Cavalcanti et al. 2017), the first skin abnormalities were present after 2 weeks of the toceranib administration and worsened with the continuation of the treatment. However, in the present case, the hair colour did not change for the

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first 4 weeks of the toceranib treatment, although the coat gradually became lighter; however, there was no skin eruption or alopecia. In the present case, the cause of the coat colour change is unclear, as it might have occurred due to aging or the cutaneous skin adverse effect of toceranib.

Additionally, toceranib has been reported to cause minor neutropenia of grade I or II as determined by the VCOG (2016). In the present case, however, grade III neutropenia was detected. According to the manufacturer's documentation, the temporary discontinuation of toceranib can resolve this problem. In the present case, subclinical severe neutropenia occurred, and it did not improve despite the long-term toceranib discontinuation. Furthermore, the neutropenia did not worsen after the re-initiation of the toceranib due to the MCT recurrence 4 months after the toceranib discontinuation. Accordingly, mastocytosis or bone marrow infiltration of neoplastic mast cells were a concern in the present case; however, a bone marrow evaluation could not be performed due to the refusal of the client. Although the efficacy of the toceranib as a treatment for dogs with disseminated mastocytosis has not been determined, a previous study has revealed that imatinib, a KIT inhibitor, resulted in the complete remission in three dogs with a mast cell bone marrow infiltration (Marconato et al. 2008; Moirano et al. 2018).

The clinical signs of canine MCT are associated with the release of inflammatory mediators such as histamine, proteases, serotonin, and heparin from tumour cells. The clinical signs include ulceration, swelling, and irritation at the tumour site, coagulopathy, and delayed wound healing (Blackwood et al. 2012), as was evident in the present case. In addition, the histamine receptor stimulation in the stomach followed by excessive histamine secretion can cause gastrointestinal disturbances such as ulceration (Welle et al. 2008).

A fine needle aspirate and cytologic analysis is a standard method of diagnosing and grading MCTs, as it is rapid and non-invasive (Scarpa et al. 2016). In the present case, this approach was used when the histologic evaluation was not available. In cases of MCT, an FNA assessment is characterised by round cells (Welle et al. 2008). In one comparative study of a histologically confirmed MCT, the cytologic diagnosis by the Romanovsky type stain was correct in 92–96% cases (Welle et al. 2008). Another study on the cytological grading of canine MCTs has recently been reported (Scarpa et al. 2016). In those studies, the cytologic diagnosis and grading of canine MCTs reached a sensitivity of 84.6% to 88.2%, and a specificity of 97.3% to 94.8% (Scarpa et al. 2016).

We conclude that an anti-TKI therapy, such as the toceranib administration, is a treatment of choice in a case of a high-risk MCT, in particular, if the KIT mutations are identified and surgery cannot be performed. As the side effects of toceranib were significant in the present case, including grade III neutropenia, further studies on the side effects associated with toceranib are needed. At the time of this writing, 1.5 years after the treatment with toceranib, the patient has not suffered a recurrence nor has it developed any clinical signs associated with neutropenia, remaining healthy despite the persistent neutropenia.

## Conflict of interest

The authors declare no conflict of interest.

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