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CASE REPORT
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Canine Mast Cell Tumour Lomustine's Sensitization by Tyrosine Kinase Inhibitors

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ABSTRACT

***Background*:** Tyrosine kinase inhibitors (TKIs) may sensitize neoplasms to conventional antineoplastic agents, however such studies are scarse in the veterinary literature and there is no *in vivo* study about this subject. Although the literature recommend consensual about the use of masitinib for unresectable or metastatic MCTs, the potential of tumour sensitization to chemotherapeutic agents exerted by the drug is poorly explored in veterinary medicine. The objective of this paper was to report, for the first time, the sensitization of 2 canine mast cell tumours (MCTs) to lomustine, with the use of 2 tyrosine kinase inhibitors: masitinib and toceranib.

***Cases*:** Two dogs were referred due tumour recurrence in the left pelvic limb (dog 1), and unilateral mass in the right nasal mucocutaneous region (dog 2). The first case was a 8-year-old female Pinscher, and the second case refers to a 8-year-old male mixed-breed dog. Fine needle aspiration of both lesions was performed, and the cytological analysis were compatible with high grade canine MCT. In the first case, it was started a chemotherapeutic treatment with intravenous vinblastine (2 mg/m^2), associated with prednisolone (40 mg/m^2, every 24 h for 7 days), followed by 25 mg/m^2 every 24 h, for more 30 days, tramadol (4 mg/kg every 8 h, until new recommendations) and gabapentin (3 mg/kg every 12 h, until new recommendations). However, there was no objective response, and vinblastine was substituted by lomustine (60 mg/m^2 every 21 days), however there was also no response after 2 doses. After masitinib importation, the same was started at 12.5 mg/kg orally every 24 h, but there was also no objective response. However, after new lomustine administration the lesion showed complete remission. The second dog initiated its treatment with toceranib, recently licensed in Brazil, at a dosage of 2.7 mg/kg every 48 h, and after 30 days, there was partial remission. However, the remaining lesion still deemed unresectable, and systemic chemotherapy with lomustine (50 mg/m^2) was initiated along with continuous toceranib. After 3 weeks of the first chemotherapy complete remission was noted and a second dose was administered. Once the patient remained in complete clinical remission, only toceranib was maintained at the same dose. After 11 months using the toceranib, there was sign of disease recurrence and lomustine was re-initiated resulting in complete remission.

***Discussion*:** The TKIs masitinib and toceranib might be considered the first-line therapy for unresectable and/or metastatic canine MCT, but also for those cases with confirmed internal tandem duplications in the exon 11 of the *c-KIT* proto-oncogene. Masitinib appears to be more selective than others TKI, such as toceranib, imatinib, dasatinib and sunitinib, because it causes weak inhibition of BCR/ABL (breakpoint cluster region-Abelson), Fms (macrophage colony-stimulating factor receptor), Flt-3 (FMS-like tyrosine kinase-3) and VEGFR (vascular endothelial growth factor receptor), which may partially explains its increased safety and lower risk of cardiotoxicity. In the first case, the animal has been treated with lomustine associated to masitinib and showed a progression-free interval of 33 days, however, the response reported may have been lower, due previously exposition to chemotherapeutic agents, which might compromise the response to TKI. The second case, with the association of lomustine and toceranib, was followed up for 365 days, presenting only one recurrence in the final third of the follow-up, however, with subsequent new complete remission. Sensitization of canine MCT to lomustine with TKIs increases the therapeutic possibilities for this neoplasm, mainly in patients with advanced stage and high-grade tumours.

Keywords: dog, mast cell, chemotherapy, masitinib, toceranib.

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INTRODUCTION

Canine mast cell tumour (MCT) is usually effectively treated by surgical resection; however it might be presented as advanced and/or unresectable disease associated with poor outcome and poor response to local and/or systemic therapy. In those cases, a broad spectrum of antineoplastic drugs may be used including vinblastine, lomustine and cyclophosphamide, but also tyrosine kinase inhibitors (TKIs), as masitinib mesylate and toceranib phosphate, which can increase the overall survival and occasionally prevent its fatal outcome [5].

The combination of TKI and conventional chemotherapeutic drugs might be useful in preventing tumour resistance or promoting sensitization to chemotherapeutic agents, nevertheless optimized protocols of such combinations are not yet fully established [9-11].

This paper aims to report 2 cases of tyrosine kinase inhibitors promoting *in vivo* sensitization of canine MCT to lomustine.

CASE

Case 1. A 8-year-old female spayed Pinscher, weighing 6.60 kg, with a body score 8/9, was presented to the Veterinary Hospital Prof. Ricardo Alexandre Hippler, at Universidade Vila-Velha - ES, Brazil, with a previous history of surgical excision of a skin nodule in the left pelvic limb, 2 months before, without any cytological or histopathological diagnosis. During physical examination it was observed a dermoepidermic mass affecting the inguinal region and posterior region of the left pelvic limb, compromising the physical exam of the superficial inguinal lymph node.

Fine needle aspiration of the neoplastic mass was performed, and the material obtained was stained with Diff-Quick[1] and evaluated by light microscopy. The cytological analysis revealed round cells with few basophilic granules in the cytoplasm, important anisokaryosis and 6 mitotic figures in 2.37 mm^2 (12 field of 400 x), compatible with a high grade MCT [2,11] (Figure 1A & B). Complete blood count, biochemical analysis and abdominal ultrasound was performed, and they were unremarkable.

The tumour was deemed unresectable (Figure 2A) and it was started a chemotherapeutic treatment with vinblastine[2] [2 mg/m^2 - intravenous (IV)], associated with prednisolone[3] [40 mg/m^2- IV, every 24 h, for 7 days, followed by 25 mg/m2 every 24 h], with ranitidine[4] [2 mg/kg - every 12 h, for 30 days], amoxicillin-clavulanic acid[5] [25 mg/kg - every 12 h, for 30 days], tramadol[5] [4 mg/kg - every 8 h, until new recommendations] and gabapentin[6] [3 mg/kg - every 12 h], until new recommendations. Once there was no objective response, vinblastine was substituted by lomustine[7] [60 mg/ m^2], however there was no response after 2 doses with 21-day interval. By this time, the dog's responsible imported masitinib[8] [12.5 mg/kg - every 24 h], and started its use [12.5 mg/kg - every 24 h]. The dog returned 10 days after the treatment onset, but there was also no measurable response, and the disease was still stable (Figure 2B). Despite the risk of possible side effects, it was chosen to perform an association of masitinib and lomustine, at the same

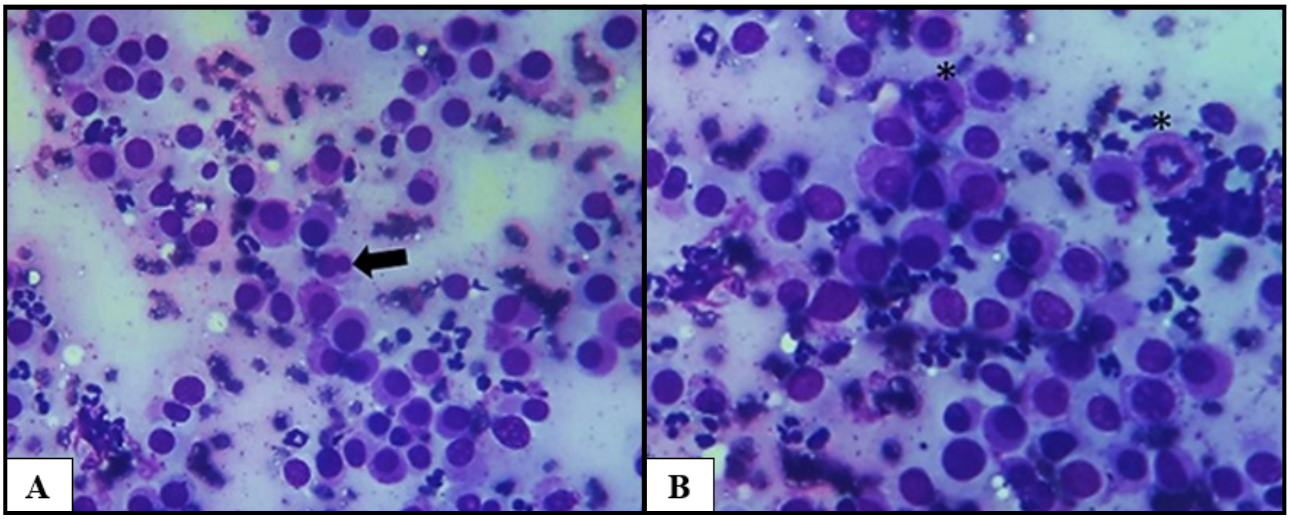

Figure 1. Cytological features from a 8-year-old female spayed Pinscher dog with with high grade mast cell tumour. A- Large cellularity of poorly granulated mast cell with binucleation (arrow). B- Prominent anisokaryosis and mitotic figures (*). [Diff-Quick Staining; 40x].

previous dosage. Surprisingly, response was noted within 7 days, with 80-95% of partial remission in 14 days (Figure 2C) and complete remission at 21 days (Figure 2D). The dog had a grade I neutropenia (1,512 x 10^3/mm^3; Ref: 3,000-11,500 x 10^3/mm^3) [14], but no other side effects. However, despite the satisfactory results, it was observed a disease-free interval of only 12 days, and even with a novel administration of lomustine, disease progression was noted, and the dog died spontaneously 25 days after it, resulting in a progression-free interval of 33 days and a survival time of 117 days since diagnosis of recurrent disease. Autopsy was not authorized by the pet's responsible.

***Case 2*.** A 8-year-old male breed mixed dog, weighing 5.45 kg, was referred to the same Veterinary Hospital with an unilateral mass in right nasal mucocutaneous region, measuring 1.5 cm. The dog did not present any other clinical disorder. The dog's owner reported approximately 4 years of slow-progressive growth, however, during the last 5 months a fast progression was observed. Fine needle aspiration of the lesion, complete blood count, biochemical profile and abdominal ultrasound were performed. The cytological examination allowed the identification of many round cells with few cytoplasmatic metachromatic granules. Although there was no mitotic figure, it was compatible with a high-grade mast cell tumour. Complete blood count, biochemical profile and abdominal ultrasound were unremarkable.

It was decided to start the treatment with toceranib[9], recently licensed in Brazil, at a dosage of 2.7 mg/kg every 48 h, due the unfavorable tumour's location for surgical resection (Figure 3A). After 15 days, it was noted stable disease (remission less than 30%), however, after 30 days there was partial remission, of approximately 60% (Figure 3B). Nevertheless, the remaining lesion still deemed unresectable, and systemic chemotherapy with lomustine (50 mg/m^2) was initiated along with continuous toceranib. After 3 weeks of the first chemotherapy complete remission was noted and a second dose was administered (Figure 3C). Once the

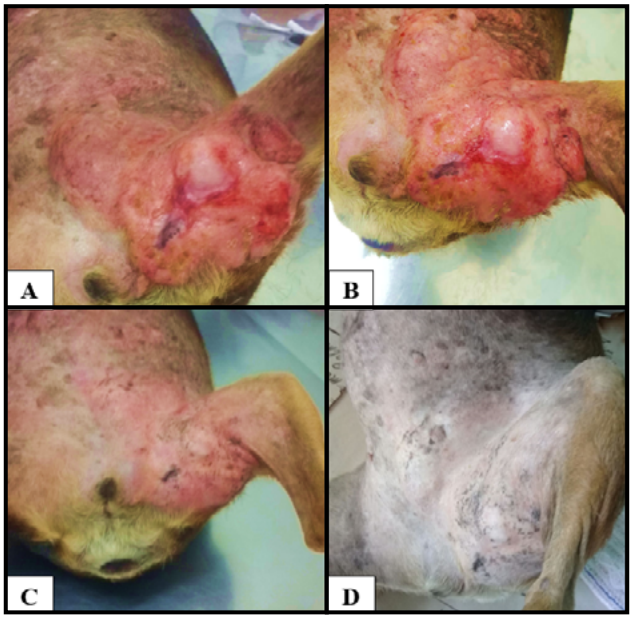

Figure 2. High-grade mast cell tumour in a eight-year-old Pinscher dog. A- With no treatment. B- After treatment with vinblastine, lomustine and masitinib, separately. C- 14 days after mastinib and lomustine combination, showing 80-95% of remission. D- 21 days after mastinib and lomustine combination showing complete remission.

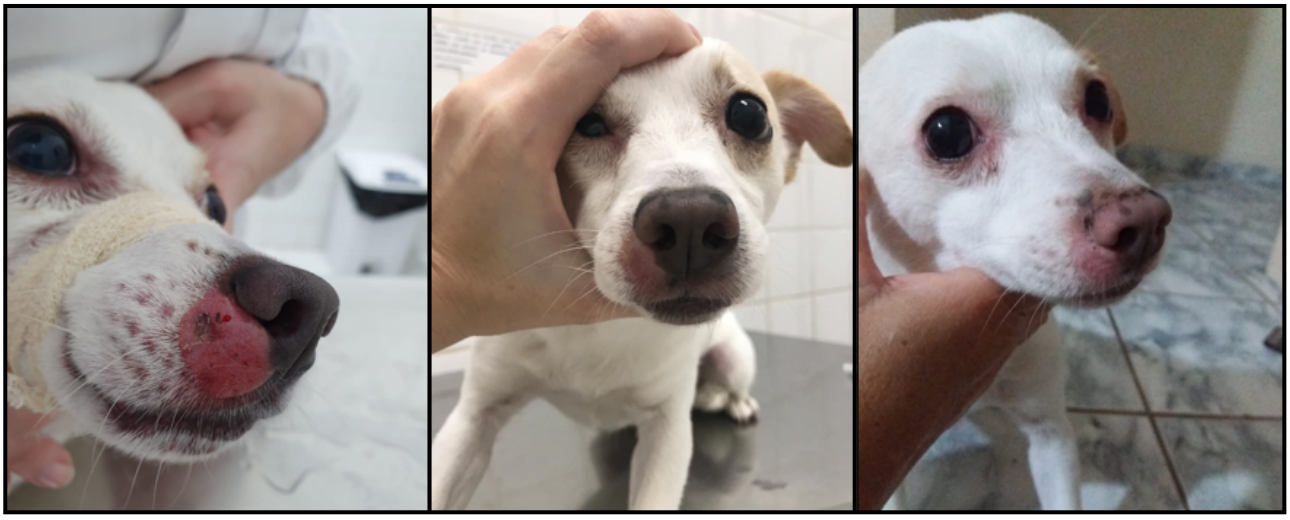

Figure 3. A 8-year-old male breed mixed dog, diagnosed with high-grade mast cell tumour in right nasal mucocutaneous region. A- First day with no treatment. B- Partial remission of the lesion after 30 days of treatment with toceranib. C- Complete remission after 3 weeks of inclusion of systemic chemotherapy with lomustine.

patient remained in complete clinical remission, only toceranib was maintained at the same dose.

The dog was monthly followed-up with clinical examination, complete blood count and biochemistry profile, due to the use of TKI. After 8 months using toceranib, the neutrophil count was 1,890/μL (2,500-12,500/μL) representing a grade I neutropenia, and the toceranib frequency was reduced for three times a week (Monday-Wednesday-Friday), with normalization of the leukocyte count after 60 days.

After 11 months using toceranib there was a sign of disease recurrence and lomustine was re-initiated. Only 1 dose was administered while maintaining toceranib, resulting in novel complete remission. The patient was followed for more 4 months with no signs of tumour recurrence or new lesions.

DISCUSSION

Tyrosine kinase inhibitors may be used as first-line therapy for unresectable and/or metastatic canine MCT, but also for those cases with confirmed internal tandem duplications in the exon 11 of the *c-KIT* proto-oncogene [3,5].

Toceranib was approved by Food and Drug Administration (FDA) in 2009 for the treatment of MCT in dogs and by the Ministry of Agriculture in Brazil in 2018. In a study, 86 dogs presenting recurrent or metastatic MCT were treated with toceranib at a dosage of 3.25 mg/kg every 48 h, for 6 months, resulting in total or partial response in 37.2% of the treated dogs. There was 60% of response in patients with mutations on the *c-KIT* and 25% in dogs without *c-KIT* mutations [9]. Masitinib also proved to be effective to postpone disease progression, especially in dogs with mutations in the *c-KIT* gene and those treated with masitinib as first-line therapy [5]. Toceranib and masitinib are TKI small molecules able to regulate mast cell activity due to inhibition of the KIT tyrosine kinase receptor (KITr), but likewise other drugs of this class it has a multitarget effect, once it regulates other protein kinases, mainly PDGFR α and β (platelet-derived growth factor receptor). Toceranib may also inhibit VEGFR 1 and 2 (vascular endothelial growth factor receptor), Flt-3 and CSF1R at plasmatic concentrations of 1-10nM, and, at higher plasmatic concentrations (100 nM), VEGFR3, RET, ALK and AXL. An experimental study about primary characterization of the activity of masitinib, demonstrated i*n vitro* inhibition of the recombinant human *c-KIT* gene product with half of its inhibitory concentration, with consequent blockage of stem cell factor-induced proliferation and KITr tyrosine phosphorylation. At the same study, an *in vivo* response was demonstrated for masitinib, once it significantly inhibited tumour growth in mices with subcutaneous grafts of Ba/F3 cells lines, expressing mutations in the KITr juxtamembrane domain [4]. Masitinib also inhibits the SRC family including Fyn, Lyn-B, Lck and Yes tyrosine kinease proteins, and, to a lesser extent FGFR-3 (fibroblast growth factor receptor), and it has a dose dependent activity in inhibiting mast cell histamine and TNF-α's release [3]. However, masitinib appears to be more selective than others TKI, such as toceranib, imatinib, dasatinib and sunitinib, because masitinib cause weak inhibition in BCR/ABL (breakpoint cluster region-Abelson), Fms (macrophage colony-stimulating factor receptor), Flt-3 (FMS-like tyrosine kinase-3) and VEGFR (vascular endothelial growth factor receptor), which may partially explain its increased safety and lower risk of cardiotoxicity [3,8].

Tyrosine kinase inhibitors (TKIs) may sensitize neoplasms to conventional antineoplastic agents, however such studies are rare in the veterinary literature and there is no *in vivo* study about this subject. *In vitro* sensitization was demonstrated for histiocytic sarcoma cell lines to vinblastine (70x reduction of mean inhibitory concentration, with 5 μm of masitinib), osteosarcoma and mammary carcinoma cell lines to gemcitabine (70x reduction of mean inhibitory concentration, with 5-10 μm of masitinib), as well as B-cell lymphoma, hemangiosarcoma, urinary bladder carcinoma and melanoma to doxorubicin (2-10x reduction of mean inhibitory concentration, with less than 10 μm of masitinib) [13]. The authors of such study concluded that the combination of masitinib and chemotherapeutic agents can be effective in the treatment of various canine neoplasm, possibly through chemosensitization [13].

Despite of those encouraging results, this is the first report of MCT's lomustine sensitization after the use of masitinib [12.5 mg/kg - every 24 h] and toceranib [2.7 mg/kg- every 48 h], showing, for the first time *in vivo*, the efficacy of such drugs as chemosensitizers.

Studies in humans have demonstrated that the use of TKIs agents as first-line therapy followed by chemotherapy, increased the overall survival [1,8]. A phase I study performed in dogs with measurable MCT, with the simultaneous combination of vinblastine and

toceranib revealed an objective response in 71.4% of dogs, however vinblastine was used with a 54% reduction in dose-intensity [1.6 mg/m^2 - intravenously, every 2 weeks] to ensure the safety and acceptable levels of neutropenia when combined with toceranib [3.25 mg/kg- PO every 48 h] [12].

A retrospective study was performed posteriorly [10]. In this same study, medical records of 40 dogs with MCT, treated with vinblastine [1.6 mg/m^2 - IV every 2 weeks, at a total of 8 doses], associated with prednisolone [1 mg/kg - PO every 24 h, for the duration of the treatment] and toceranib phosphate [2.5 mg/kg - PO on Monday-Wednesday-Friday], at 12 months [10]. These dogs were separated into 3 groups: neoadjuvant (disease poorly operable, with metastasis in 50% of these patients), adjuvant (Kiupel high grade, with metastasis in 27%) and palliative treatment (gross metastatic disease). An objective response of 88% was found in the neoadjuvant group (14/16; 6 with complete remission) and 92% in the palliative arm (12/13; 3 with complete remission), revealing the efficacy of such combination. The median for progression-free interval was reached only for patients in the palliative group [10].

In the first described case, the dog has been treated with lomustine associated with masitinib and showed a progression-free interval of 33 days, however, the response reported may have been lower due to previously exposition to chemotherapeutic agents, that can compromise the response to TKI, as previously reported [5]. Even though the glucocorticoids do not represent a modern therapy in the canine MCT treatment, they have been historically used to prevent mast cell degranulation and measurable responses can occur in up to 75% of cases [4]. However, the concomitant use with TKI is discouraged, once it can increases P-glycoprotein expression, which have, as substrates, drugs such as masitinib, imatinib, dasatinib and sutinib, but not toceranib [6]. Thus, this drug was suspended from the start of masitinib use, however it might still have favored the development of tumour resistance and new studies are necessary to confirm this hypothesis. Sensitization of canine MCT to lomustine with TKIs increases the therapeutic possibilities for this neoplasm, mainly in patients with advanced staging and high-grade tumours.

In the second described case, toceranib was used as first-line therapy and treatment response was apparently enhanced due to the use of lomustine, resulting in a survival time higher than 14 months. Despite the rapid response after lomustine initiation it is not possible to know if a similar response would also be obtained with longer use of toceranib or lomustine monotherapy and further clinical studies are needed to standardize protocols with optimized results and acceptable toxicity.

Although the literature recommend the use of masitinib for unresectable or metastatic MCTs, the potential of tumour sensitization to chemotherapeutic agents exerted by the drug is poorly explored in veterinary. To the best of our knowledge, those are the first reports of *in vivo* canine MCT sensitization to lomustine by tyrosine kinase inhibitors.

Declaration of interest. The authors report that they have no conflict of interest. The authors are responsible for the content and writing of the paper.

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