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FROM HUMANS TO DOGS AND BACK: THE TRANSLATIONAL LESSON OF METRONOMIC CHEMOTHERAPY

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ABSTRACT

The main priority in veterinary oncology is to maintain patient quality of life. It is important that new chemotherapy strategies aim to minimize side effects, thus making the treatment attractive for owners as well as their pets. Metronomic chemotherapy has been shown to have an important stabilizing effect on human cancer (including chemotherapy-resistant disease) resulting in prolonged clinical benefit. In addition, this form of treatment has been shown to have positive effects on the quality of life of patients with various types of cancer. These positive effects are obtained without any indication of high grade toxicity. Moreover, low cost and oral administration (which reduces the need for hospitalization and enables patients to stay at home longer) are key characteristics of this schedule, offering important advantages in frail subgroups of patients (e.g., old patients) for whom new therapeutic options are greatly needed. From another perspective, use of metronomic chemotherapy in dogs could reveal new and innovative schedules that could be applied to humans. Veterinary oncology cases treated with metronomic schedules represent the unique opportunity to ethically investigate novel drugs or combination treatments that may be highly translatable to the human community. The aim of the present review was to describe how this new form of treatment has evolved in canine patients thus far.

Keywords: Metronomic Chemotherapy, Antiangiogenic Therapy, Oncology, Cancer

1. INTRODUCTION

The prevalence of cancer in dogs has increased in recent years. This may be the result of an actual increase in cancer incidence, an increase in the population of dogs at risk for the development of cancer and/or increased awareness and interest in the pet-owning community to pursue diagnostic and treatment options. Advances in the care of animals has allowed dogs to live longer due to better nutrition, vaccination for common infectious diseases, leash laws that limit automobile deaths and the availability of more sophisticated diagnostics and treatments for many ailments previously considered to be life-threatening. However, the improved general health of pets has resulted in an increase in age-related diseases, including cancer (Paoloni and Khanna, 2007; Giorgi, 2012).

As in normal tissues, tumors require nutrients and oxygen and the removal of carbon dioxide and catabolites, these functions are accomplished by the vasculature. During embryogenesis, blood vessels develop via two processes: Vasculogenesis, whereby endothelial cells differentiate from progenitor cell types; and angiogenesis, in which new capillaries sprout from existing vessels. The vasculature is quiescent in the normal adult mammal, except for the predictable changes

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that occur during the female reproductive cycle (ovulation, menstruation, implantation, pregnancy). In contrast, in tumor progression activation of angiogenesis is permanent with continuous sprouting of new blood vessels that can support and supply nutrients to the neoplastic growth (Hanahan and Folkman, 1996). Historically, angiogenesis was only considered important in the rapid growth phase of the tumor, however more recent surveys attribute great importance to angiogenesis in microscopic premalignant lesions (Hanahan and Weinberg, 2011). Hence, there is a need to develop antiangiogenic strategies to combine with conventional antineoplastic chemotherapy protocols. Such a multifaceted attack will increase the likelihood of success in the fight against malignant tumors.

The importance of angiogenesis in tumor growth was first described by Folkman (1971), however the efficacy of some forms of antiangiogenic chemotherapy was not described for another twenty years (Kerbel, 1991). The term angiogenesis refers to endothelial sprouting or Intussusceptive Microvascular Growth (IMG) and applies to the formation of capillaries starting from pre-existing vessels. Nutrients and oxygen delivered via blood vessels are crucial for the survival and function of cells and for this reason, cells further than 100 micron from a capillary will not be viable (Ribatti *et al*., 2009). Both solid and haematological tumors have some angiogenic capabilities and their growth, invasiveness and metastatic potential are angiogenesis-dependent (Ribatti *et al*., 2007).

The Vascular Endothelial Growth Factor (VEGF) is certainly one of the most studied of the proangiogenic factors. Different isoforms of its high affinity tyrosine-kinase receptor have been identified: VEGFR-1 (or Flt-1), VEGFR-2 (or KDR/Flk-1) and VEGFR-3 (or Flt-4) (Ribatti *et al*., 2007). VEGFR-2 is responsible for the majority of the angiogenic and permeability-enhancing effects of VEGF (Giovannini *et al*., 2010). Thus, both VEGF and VEGFR-2 have been logical candidates for new therapeutic approaches, based on their functional relevance to cancer pathophysiology (Ferrara *et al*., 2003). Another mechanism which contributes to the expansion of new blood vessels within a tumor involves incorporation of bone marrow derived, Endothelial Progenitor Cells (EPC) into capillaries, although it is not yet clear how this affects the entire process of angiogenesis. It has been demonstrated that increased serum VEGF promotes and enhances the differentiation of bone marrow stem cell lines towards the endothelial phenotype (Takahashi *et al*., 1999; Gill *et al*., 2001). Furthermore, it has also been shown that increased production of VEGF by tumor cells favors the mobilization of these progenitor cells from the bone marrow to the peripheral circulation and increases their recruitment into tumor vasculature (Asahara *et al*., 1999; Hattori *et al*., 2000). Even hypoxia, which is often present in areas within a tumor could increase the mobilization of EPCs from the bone marrow and thus contribute to the process of neoangiogenesis (Takahashi *et al*., 1999).

In veterinary medicine in the last decade, several studies have been designed to demonstrate increased proangiogenic factors during tumor expression.

Immunohistochemistry or real-Time Polymerase Chain Reaction (RT-PCR) have been used to observe expression of VEGF and/or their receptors and/or MMP in canine breast cancer (Queiroga *et al*., 2011; Al-Dissi *et al*., 2010; Qiu *et al*., 2008a; 2008b; Restucci *et al*., 2002), mast cell tumors (Giantin *et al*., 2012; Patruno *et al*., 2009; Rebuzzi *et al*., 2007), hemangiosarcoma (Yonemaru *et al*., 2006), intracranial tumors of various origins (Rossmeisl *et al*., 2007; Platt *et al*., 2006; Dickinson *et al*., 2008) nasal epithelial neoplasia (Shiomitsu *et al*., 2009), lymphomas (Wolfesberger *et al*., 2007; 2008; 2012), melanoma (Taylor *et al*., 2007) meningiomas (Matiasek *et al*., 2009), squamous cell carcinomas (Al-Dissi *et al*., 2007; Maiolino *et al*., 2000) and soft tissue sarcomas (Queiroz *et al*., 2010).

Other studies have tried to correlate this expression with Microvascular Density (MVD) (Queiroz *et al*., 2010; Wolfesberger *et al*., 2008; 2012), with expression of COX-2 and MVD (Queiroga *et al*., 2011) and with other factors (Yonemaru *et al*., 2006; Qiu *et al*., 2008b; Matiasek *et al*., 2009).

Other surveys have focused on observation of blood concentrations of VEGF and/or MMP in various malignancies (Troy *et al*., 2006; Wergin and Kaser-Hotz, 2004; Marchetti *et al*., 2012) or in well-defined tumors such as lymphoma (Aresu *et al*., 2012; Gentilini *et al*., 2005; Zizzo *et al*., 2010), intracranial cancer (Rossmeisl *et al*., 2007), melanoma (Taylor *et al*., 2007), hemangiosarcoma (Clifford *et al*., 2001), breast cancer (Kato *et al*., 2007), osteosarcoma (Thamm *et al*., 2008) and soft tissue sarcoma (Queiroz *et al*., 2012).

1.1. The Metronomic Chemotherapy Approach: From Humans to Dogs

In veterinary oncology where the highest priority is maintaining patient quality of life, it is important that new chemotherapy strategies aim to minimize side effects, so treatment is more attractive for owners as well as their pets.

Browder *et al*. (2000) and Klement *et al*. (2000) published two pioneering works demonstrating the effectiveness of some anti-angiogenic drugs administered continuously at low doses in subcutaneous tumors in mice chemoresistant for the same drugs. This mode of administration was termed "metronomic" (Hanahan *et al*., 2000). While preclinical studies have shown that the release of endothelial progenitor cells from the bone marrow is stimulated by high-dose chemotherapy but suppressed by low-doses (Munoz *et al*., 2006; Hanahan *et al*., 2000; Mancuso *et al*., 2006; Bertolini *et al*., 2003), others have shown that the cytotoxic agent induced damage to the tumor vasculature can be repaired in the interval between two cycles of chemotherapy (Giovannini *et al*., 2010). The main purpose of metronomic chemotherapy is to eliminate or at least minimize this time interval thus limiting the extent of cell repair, further cell replication and alteration of the tumor microenvironment.

It has been observed that metronomic chemotherapy does not have a significant direct cytotoxic effect against tumor cells but rather is able to modify the tumor microenvironment. One of its prime effects is to delay or render ineffective the tumour's capacity to generate new blood vessels for the tumor stroma. It does this in various ways (Gonzalez-Billalabeitia *et al*., 2009; Miller *et al*., 2001; Pasquier *et al*., 2007; Laquente *et al*., 2007): (i) a selective induction of apoptosis and inhibition of proliferation and migration of activated endothelial cells (Bocci *et al*., 2002; Blansfield *et al*., 2008; Pasquier *et al*., 2010); (ii) modulating inhibitory (e.g., thrombospondin-1) and stimulatory factors of angiogenesis (Bocci *et al*., 2003; Pasquier *et al*., 2010; Park *et al*., 2010; Damber *et al*., 2006); (iii) inhibition of endothelial cell microtubules in vitro (Kerbel and Kamen, 2004); (iv) targeting Circulating Endothelial Progenitor Cells (CEPC) (Kerbel and Kamen, 2004; Mutsaers, 2007); (v) modulating the cell cycle through the upregulation of caveolin-1 and the downregulation of cyclin D1 (Bocci *et al*., 2012); (vi) reducing Treg cells that inhibit cell-mediated immunity against self cells (Burton *et al*., 2011) and stimulating the action of Dendritic cells (Tanaka *et al*., 2009a; 2009b).

These preclinical studies have led onto a series of clinical experimental trials in different types of cancer such as human breast cancer (Colleoni *et al*., 2002; 2006; Wong *et al*., 2010; Gonzalez-Billalabeitia *et al*., 2009; Bottini *et al*., 2006; Orlando *et al*., 2006; Dellapasqua *et al*., 2008; Garcia-Saenz *et al*., 2008), ovarian cancer (Jurado-Garcia *et al*., 2008; Garcia *et al*., 2008), prostate cancer (Glode, 2003; Lord *et al*., 2007; Fontana *et al*., 2009; 2010a; 2010b), cancer effecting the kidney (Krzyzanowska *et al*., 2007; Bellmunt *et al*., 2010), colon (Allegrini *et al*., 2008; 2012; Nannini *et al*., 2009), lung (Kato *et al*., 2004), adrenal medulla (Wortmann *et al*., 2010), nerves (Kong *et al*., 2006; Reardon *et al*., 2009; Stupp *et al*., 2005), in some lymphomas (Buckstein *et al*., 2006), melanoma (Bhatt *et al*., 2010) and others (Weerdt *et al*., 2001; Young *et al*., 2006; Steinbild *et al*., 2007; Briasoulis *et al*., 2009; Vogt *et al*., 2003).

Several drugs or their analogues were used for metronomic chemotherapy clinical schedules including cyclophosphamide (Penel *et al*., 2012), methotrexate (Colleoni *et al*., 2002; 2006; Wong *et al*., 2010; Gonzalez-Billalabeitia *et al*., 2009; Orlando *et al*., 2006; Garcia-Saenz *et al*., 2008), capecitabine (Dellapasqua *et al*., 2008), vinorelbine (Saloustros *et al*., 2011), irinotecan (Allegrini *et al*., 2008) and UFT (Allegrini *et al*., 2012).

Metronomic chemotherapy has been shown to have an important stabilizing effect on cancer (including chemotherapy-resistant disease) conferring prolonged clinical benefit and to have positive effects on the quality of life of patients with various types of cancer, without highgrade toxicity (Nelius *et al*., 2011). Moreover, low cost (Bocci *et al*., 2005) and oral administration (which reduces the need for hospitalization and enables patients to stay at home longer) are key characteristics of this schedule, offering important advantages in frail subgroups of patients (e.g., old patients) for whom new therapeutic options are greatly needed (Fontana *et al*., 2010b).

Based on the above mentioned studies, metronomic chemotherapy is increasingly being considered by veterinary oncologist as an anticancer therapeutic strategy. The first report of a metronomic chemotherapy protocol was presented as an abstract at the annual conference of the Veterinary Cancer Society a decade ago (Mutsaers, 2009). In that work, different types of tumors were treated with cyclophosphamide at 25 mg m^{-2} in combination with piroxicam 0.3 mg kg^{-1} daily. An objective response was achieved in two dogs after one month of therapy.

The first full paper was not published for another 6 years (Lana *et al*., 2007). It compared the outcome of nine dogs with splenic hemangiosarcoma treated with a traditional doxorubicin protocol dose-intense single-agent chemotherapy and nine dogs with the same disease treated with metronomic chemotherapy. The protocol consisted of piroxicam 0.3 mg kg^{-1} in combination with cyclophosphamide or etoposide at 12.5 to 25 mg m^{-2} to 50 mg m^{-2} administered daily for six months. Surprisingly, the median survival time and to an even larger extent, the disease-free interval proved better with

the metronomic protocol than with the traditional one, resulting in 178 days as compared to 133 and 178 days versus 126 respectively (Lana *et al*., 2007).

However, this study provided important information on toxicity. Toxicity did not exceed grade 2 according to the VCOG (VCOG, 2004) with the metronomic chemotherapy while gastroenteric toxicity was observed at grade 3 and 4 with doxorubicin. However, hemorrhagic cystitis was reported in two patients treated with cyclophosphamide.

A year later Elmslie *et al*. (2008) published a study of 85 dogs. Participating dogs had had a soft tissue sarcoma that had been incompletely excised, one group was treated with metronomic chemotherapy (30) the other group received no treatment (55). The protocol consisted of cyclophosphamide at 10 mg m^{-2} combined with piroxicam 0.3 mg kg^{-1} daily or on alternate days. The treated group had a disease-free interval almost double that of the untreated group (410 versus 211 days). Toxicity was reported as mild, about 40% of animals were reported as having grade 1 or 2 signs and only 1 subject developed hemorrhagic cystitis (grade 4).

The next paper on metronomic treatment was published in early 2012. Fifteen dogs with various tumors with distant metastases were treated with metronomic chemotherapy as first line treatment (Marchetti *et al*., 2012). The protocol consisted of daily administration of cyclophosphamide, 25 mg m^{-2} and celecoxib, 2 mg kg^{-1}. Six dogs obtained objective responses, including one complete remission. The average survival time was more than three months and toxicity almost absent. In this study, VEGF was measured before treatment and dogs who responded to the protocol showed a statistically significant lower blood concentration if compared to dogs who didn't respond.

In the same year, a study on 81 dogs with inoperable tumors, or tumors that had been incompletely removed or were chemoresistant with macroscopic evidence of distant metastases, was published (Tripp *et al*., 2011). These dogs were treated with lomustine at 2.84 mg m^{-2} daily given orally for an average of 98 days. In almost half the cases, lomustine was used in conjunction with an anti-inflammatory drug, in 29 dogs an NSAID was used. Results obtained using this protocol were comparable to those obtained by Marchetti *et al*. (2012), with a response rate of 36%. The non progression of the disease, observed in approximately 30% of cases, had a duration of about 137 days. Even the toxicity evaluation aligns with the studies cited above with mild gastrointestinal symptoms occurring in about a quarter of patients. In about 21% of cases there was an increase in ALT, probably because of liver toxicity induced by lomustine. Finally, thrombocytopenia was found in about one-fifth of the cases, however this was rarely higher than second degree toxicity. This predominantly occurred in patients with end-stage cancer, probably due to a cumulative toxicity.

A prospective study was designed to observe the efficacy of metronomic dosing of cyclophosphamide to reduce the number of circulating Treg cells and tumor Microvessel Density (MVD, microvessel density) in dogs with Soft Tissue Sarcoma (STS Soft Tissue sarcoma). 11 dogs were treated daily with 12.5 mg m^{-2} (5 cases) or 15 mg m^{-2} (6 cases) of cyclophosphamide per os while 21 healthy dogs were used as a control. The results showed significant efficacy in reducing the number of Treg lymphocytes circulating at both doses, while only the group treated with 15 mg m^{-2} showed a reduction in MVD (Burton *et al*., 2011).

In a recent study, chlorambucil was administered at a dose of 4 mg m^{-2} daily in dogs with spontaneous cancer (Leach *et al*., 2012). Of 36 cases, 58% recorded an objective response, with an overall mean survival time of 153 days. As in other trials, the observed toxicity did not exceed grade 2.

In a prospective study (Mitchell *et al*., 2012), 15 dogs with advanced malignancies were subjected to an experimental chemotherapy protocol that included a combination of metronomic cyclophosphamide (15 mg m^{-2}) and toceranib, a tyrosine kinase inhibitor recently approved for the treatment of canine mast cell tumors (London *et al*., 2003; 2009). The purpose of the study was not to observe the clinical response to treatment but rather to observe change in the number of circulating Treg cells and blood concentrations of interferon gamma (IFN-γ). The period of follow-up monitoring was short at 8 weeks, during this period 6 objective responses occurred, defined as stable disease and gastrointestinal and hematological toxicity no higher than grade 2.

In veterinary medicine, metronomic chemotherapy has several advantages compared to regime intense chemotherapy, especially in the context of veterinary oncology. There is minimal impact on the animal, cost is low and administration simple. Chemotherapy in low doses can be administered at home with minimal stress on the patient and minimal impact on the logistic organization of the owner. As cost of treatment is influential in choosing a therapeutic option, this type of chemotherapy, as compared with dose-intense protocols, has an unquestionable advantage. It has a low cost, approximately 1/10th of the cost of an intense scheme therapy.

Last, but not least, metronomic therapies have been shown to infrequently cause toxicity in veterinary patients (Lana *et al*., 2007; Tripp *et al*., 2011; Marchetti *et al*., 2012; Leach *et al*., 2012), except for an isolated case of hemorrhagic cystitis (Elmslie *et al*., 2008). This contributes to the cost-effectiveness of such a protocol as it rarely requires the use of medications to treat the side effects and it results in much less time in hospital, both events that contribute to the higher overall costs of a dose-intense protocol.

Given the encouraging results from various trials (Elmslie *et al*., 2008; Marchetti *et al*., 2012; Tripp *et al*., 2011), metronomic chemotherapy is offered as the treatment of choice for all patients with malignant tumors where owners are reluctant to embark on an aggressive therapy protocol, including surgery with a high American Society of Anesthesiologists ASA score.

It is also indicated in all patients with organ failure such as hepatic or renal insufficiency, in which the toxicity of chemotherapy may be fatal. In summary, metronomic chemotherapy achieves the main goals of veterinary oncology: Good quality of life of the patient, with an affordable cost/benefit ratio. It can be also offered as an alternative to dose intense chemotherapy in cases where the owners are not able to manage any side effects or where simple precautions required for elimination of drugs cannot be complied with.

Metronomic therapy could also present an attractive option in patients with an aggressive nature that would require sedation for each parenteral administration.

Finally, it is certainly proposed as being at the forefront of palliative care for patients at an advanced clinical stage, given that the objective in these cases is usually to stabilize disease with the least possible impact on the individual.

1.2. Candidate Drugs for Combinations with Metronomic Chemotherapy in Dogs

In human therapy in recent years, considerable interest has developed in drugs that could be easily used in combination with metronomic chemotherapy, such as Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) (Giorgi *et al*., 2012) and tyrosine kinase inhibitors (Kerbel, 2012).

The COX enzymes are mainly responsible for the conversion of arachidonic acid to Prostaglandins (PG). PGs have been shown to play a significant role in many disease processes including oncogenesis (Dore, 2011). Three isoforms of COX are known: COX-1, this is constitutive for the majority of cells and tissues and participates in the maintenance of homeostasis (Vane, 1971). COX-3 has been identified in the dog but its role is unknown (Chandrasekharan *et al*., 2002). COX-2 is normally present in a limited number of tissues, it is mainly expressed in pathological states such as in inflammatory reactions and in tumors upon stimulation by inflammatory mediators such as Interleukin-1 (IL-1), TNF-1 and lipopolysaccharides (Herschman, 2004; Masferrer *et al*., 1990). In humans, the contribution of PG to tumorigenesis (Greenhough *et al*., 2009) has been also described. PGE2 has been shown to exert its action by binding to a specific class of receptors on the cell surface, called EP. Through this binding, it influences a series of intracellular events that lead to tumor development. These include induction of cell proliferation and increased cell survival via inhibition of mechanisms of apoptosis. PGs also promote angiogenesis through the production of growth factors such as VEGF and bFGF. Cumulatively, this leads to increased invasion and metastatic capacity and suppression of the immune response (Greenhough *et al*., 2009; Gupta *et al*., 2007; Harris *et al*., 2002; Tsuji *et al*., 1998).

In dogs, several studies have shown increased expression of COX-2 in tumor tissues such as breast cancer (Souza *et al*., 2009; Dore *et al*., 2003; Heller *et al*., 2005; Queiroga *et al*., 2005; 2007), prostate cancer (L'Eplattenier *et al*., 2007; Mohammed *et al*., 2004; Sorenmo *et al*., 2004; Tremblay *et al*., 1999), transitional cell carcinoma (Khan *et al*., 2000; Knottenbelt *et al*., 2006; Lee *et al*., 2007), squamous cell carcinoma (Mohammed *et al*., 2004; Almeida *et al*., 2001) and many other forms of tumor (Boria *et al*., 2004; Borzacchiello *et al*., 2004; 2007; Impellizeri and Esplin, 2008; Khan *et al*., 2001; Kleiter *et al*., 2004; Mohammed *et al*., 2004; Mullins *et al*., 2004; Rossmeisl *et al*., 2009). NSAIDs have demonstrated antitumor efficacy in vitro (Brunelle *et al*., 2006; Pronovost *et al*., 2004; Wolfesberger *et al*., 2006) and they have been used in some clinical trials initially as a single agent (Schmidt *et al*., 2001; Knapp *et al*., 1994; Mohammed *et al*., 2002; Mutsaers *et al*., 2005; Sorenmo *et al*., 2004; Souza *et al*., 2009), revealing a response rate ranging from 17 to 33%. This rate increased to 47-83% if the objective was stable disease. These drugs were used in intensive chemotherapy regimens for oral squamous cell carcinomas and melanomas with a response rate of 25% (Boria *et al*., 2004).

Another class of drugs subject to much interest in the veterinary field in the last decade is Tyrosine Kinase Inhibitors (TKI).

The Tyrosine Kinase Receptors (TKR) are proteins that generally occur as monomers on the cell surface. They play a key role in the normal cellular signal transduction, regulating growth and cell differentiation. TKR interacts with Adenosine Triphosphate (ATP) by adding a phosphate group to its residues ("autophosphorylation") and onto other molecules to generate intracellular signals that influence proliferation and cell survival (London, 2004). This process generally begins in response to external signals generated by growth factors or other stimuli that trigger the cascade of tyrosine phosphorylation.

The TKR are aberrant in many tumors in dogs and humans. The anomalies include overexpression, activating mutations and autocrine activation through the co-expression of the receptor and the growth factor (Shchemelinin *et al*., 2006). This causes continuous stimulation of cellular signals that induce altered proliferation and cell survival, even in the absence of adequate stimulation (London, 2009).

The most studied TKR is certainly TKI or CD-117 in the dog. It is often mutated in mast cell tumors (Pryer *et al*., 2003) and Gastrointestinal Stromal Tumors (GIST) (Frost *et al*., 2003) in the dog.

Several other receptors such as VEGFR, PDGFR and FGFR (Dubreuil *et al*., 2009) are included in the TKR family.

The TKI drugs are small molecules capable of binding selectively to and inhibiting the RTKs. These act by reversibly or irreversibly blocking binding sites for ATP on kinase enzymes (Wanebo *et al*., 2006; Wakeling, 2005; Shchemelinin *et al*., 2006); in the absence of ATP binding, the kinase cannot work.

In veterinary medicine, few studies have been conducted investigating the efficacy of these anticancer drugs thus far. The use of imatinib (Isotani *et al*., 2008; Marconato *et al*., 2008), masitinib (Hahn *et al*., 2008) and toceranib (Pryer *et al*., 2003; London *et al*., 2009) for the treatment of mastocytomas has been tested in the dog, mainly investigating its inhibition of KIT. However toceranib, which has demonstrated a wider range of target TKs, has also been used in the treatment of other tumors, such as lymphoma, breast cancer, carcinoma of the bladder transitional cell, soft tissue sarcoma, melanoma, osteosarcoma, hemangiosarcoma, squamous cell carcinoma of the tongue, multiple myeloma, bronchial carcinoma, sebaceous carcinoma and anaplastic carcinoma (London *et al*., 2003).

Due to this property, but also others, toceranib is able to inhibit receptors in addition to KIT TK, such as VEGFR, PDGFR and Flt-3. That has sparked interest in the use of TKIs as an antiangiogenic agent.

Other studies have also shown an immunomodulatory effect for this class of drugs. In particular, a recent study of 15 dogs affected by cancer, demonstrated toceranib's ability to reduce the number of circulating Treg lymphocytes, thus suggesting an additional antitumor action for this molecule (Mitchell *et al*., 2012). This effect was already described in humans with sunitinib (Finke *et al*., 2008). Mitchell *et al*. (2012) tested toceranib as a single agent and subsequently it was combined with cyclophosphamide in a metronomic regime. No significant difference was observed in the absolute number of Treg lymphocytes before and after drug treatment. It was speculated that a synergistic effect in maintaining low levels of circulating Treg took place.

Another drug of current interest to the scientific community due to its anti-angiogenic properties in canine tumors is thalidomide. This drug has an immunomodulatory and antiangiogenetic effect (Kenyon *et al*., 1997). Its antiangiogenic effect on canine tumors has been demonstrated using canine osteosarcoma cells transplanted into athymic nude mice (Farese *et al*., 2004).

To our knowledge, to date the only clinical study on the efficacy of thalidomide in veterinary oncology has involved an unresectable case of head and neck squamous cell carcinoma in a cat (Marconato *et al*., 2012). Several unpublished studies on thalidomide are ongoing with interesting findings (Pierini, personal communication).

2. CONCLUSION

Cancer in dogs shares many features with human cancer, including histological appearance, tumor genetics, molecular targets, biological behavior and response to conventional therapies, so much so that the dog is considered a good model of human pathology (Paoloni and Khanna, 2007; Giorgi, 2012). The initiation and tumor progression in both species is influenced by similar factors including age, nutrition, sex, reproductive status and environmental exposure; for the latter in particular, the dog is considered a sentinel of environmental exposure because of its shorter life span (Bukowski *et al*., 1998; Marconato *et al*., 2009; Bettini *et al*., 2010). Furthermore, most, if not all, of the cancer associated genetic alterations that influence cancer progression in humans have been identified in canine cancer. Thus, the genome of the dog and human are similar enough to suggest that information learnt

about one species can be extrapolated and applied to the other (Ostrander *et al*., 2006; Lindblad-Toh *et al*., 2005). Many of the chemotherapy protocols used in veterinary medicine are based upon protocols used in human patients and they have similar treatment outcomes. For this reason, dogs serve as unique animal models for some human tumors, because they adequately mimic many of the features that define cancer in humans, including long periods of latency, genomic instability and an intact immune system (Gordon *et al*., 2009).

Thus, the metronomic chemotherapy experience in dogs could reveal innovative and unexplored schedules for humans. Veterinary oncology cases treated with metronomic schedules represent the unique opportunity to ethically investigate novel drugs or combination treatments that may be highly translatable to the human community.

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