Masitinib – a targeted therapy with applications in veterinary oncology and inflammatory diseases

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Abstract

Tyrosine kinase inhibitors (TKIs) are an innovative class of drug, which have recently become available to veterinary medicine. This targeted therapy inhibits enzymes involved in cellular signalling pathways that regulate key cell functions and cell survival. Over the last decade, TKIs have revolutionised the management of certain human cancers and are now making inroads into veterinary medicine, with canine mast cell tumour (MCT) being their first major success story. TKIs used in the veterinary setting, either having been borrowed from human medicine or developed especially to address unmet veterinary needs, include imatinib, masitinib and toceranib. Masitinib and toceranib have been approved for use in many parts of the world for the treatment of canine MCT. In many ways, masitinib may be considered the forerunner in this field, with respect to history of regulatory authorisation and commercialisation, developmental progress of phase III clinical studies, and also in the depth and breadth of clinical knowledge acquired in both dogs and cats. It will be masitinib therefore, with its completed phase III studies in canine oncology and immune-mediate indications, its long-term follow-up of MCT treatment (approximately 6 years), and development programme for numerous other canine and feline diseases that will serve as an example of what this remarkably versatile class of therapeutic drug promises to accomplish in veterinary medicine. We review the clinical development of masitinib in veterinary medicine to date, discuss safety-related issues and emerging new therapeutic directions.

Keywords: Masitinib, Tyrosine kinase inhibitor, Mast cell tumour, Atopic dermatitis, Cancer, Inflammatory disease, Protein kinase inhibitor

Review Methodology: We gathered information from publications and conference proceedings pertaining to masitinib's past and present clinical development programme and the references therein. Additional references were obtained by searching the database PubMed. Wherever necessary data from unpublished sources, including personal communications, toxicology reports or study progress reports, have been used. It should be noted that in certain instances the source material comes from conference proceedings and presentations reporting findings and observations from a limited number of case studies. Such material is included in this review as an account of different off-label experiences investigators in the field are sharing with the veterinary community. The importance of these communications, in our opinion, cannot be underestimated as it is from such humble beginnings that new therapeutic opportunities have first been identified and which are in fact serving as the impetuous for future clinical development today. However, such case studies cannot in themselves provide proof of efficacy and should not be interpreted as a list of indications for which masitinib has demonstrated efficacy. Another drawback of citing conference proceedings is that such communications are not always easy to locate. For this reason, we note that all those abstracts cited in this review are available on request from the developer of masitinib in the form a brochure 'Masitinib Scientific Data for Veterinary Medicine' – (contact@ab-science.com).

Introduction

About Masitinib

Targeted therapies represent a cutting-edge technology in the treatment of cancer through their ability to preferentially act on those cells or signalling pathways responsible for proliferation of tumours. Masitinib is an oral tyrosine kinase inhibitor (TKI) that targets a limited number of key kinases implicated in various cancers or diseases with inflammatory pathogenesis [1]. It works at a molecular level to modulate signalling pathways, which in turn can affect cells with a functional dependence on those pathways. Masitinib's foremost cellular target is the mast cell (through inhibition of essential growth and activation signalling pathways), which is capable of producing a wide variety of mediators including preformed granule-associated mediators, lipid-derived mediators and various cytokines. For example, mast cells can release large amounts of pro-inflammatory mediators with a consequence of triggering and sustaining an inflammatory response. Hence, down-regulation of mast cell activity can have an effect on many other downstream signalling pathways. Owing to its novel mechanism of action, masitinib can be of therapeutic benefit in treating a large number of oncological, dermatological, neurological and other immune-mediated conditions.

To date, masitinib has completed two pivotal phase III randomised controlled trials in veterinary medicine; the first in canine mast cell tumour (MCT) [2, 3] and the second in canine atopic dermatitis (CAD) [4]. Numerous phase I and II studies, supported by *in vitro* preclinical studies, help in making up a varied veterinary clinical development programme (Table 1). It is interesting to note that masitinib is being developed simultaneously in veterinary and human medicine. This approach recently helped in accelerating a development programme in human melanoma directly to a phase III randomised controlled clinical trial, partly through the knowledge gained from treatment of canine MCT. In human medicine, masitinib is under development for several oncological, inflammatory and neurological indications [5–12], with nine phase III studies currently underway or having received regulatory approval to be initiated (namely in the indications of: pancreatic cancer, gastro-intestinal stromal tumour (GIST), mastocytosis, asthma, melanoma, multiple sclerosis, rheumatoid arthritis, Alzheimer's disease and multiple myeloma).

Masitinib was the first approved targeted therapy in veterinary oncology, having been registered by the European Medicines Agency (EMA) in November 2008 for the treatment of non-resectable MCT grade II/III (EMA approval under the trade name Masivet®). Between Masivet's European launch in 2009 and the time of writing in January 2011, it is estimated that 1200 veterinarians have used the agent to treat approximately 4500 dogs. As of December 2010, masitinib has received conditional approval from the US Food and Drug Administration (FDA; under the trade name Kinavet®). Prior to its FDA approval, masitinib was selectively available in the USA as part of a personal importation programme for veterinarians, with 1302 recipients as of January 2011.

Using Masitinib

TKIs are convenient drugs, having the practical advantage of being administered orally in tablet form rather than by injection, which permits flexibility in dosing regimens and direct administration by the client at the point-of-care in their home. Masitinib has been rigorously tested in clinical trials to prove its efficacy and safety, with patient follow-up in the MCT trial having a duration of approximately 6 years to date. Common adverse effects associated with this therapeutic class of agents include diarrhoea and vomiting. However, such reactions are typically of mild-to-moderate intensity, last less than a few weeks and tend to have a transitory nature with improved tolerance possible over long-term treatment regimens [3, 7, 11]. Indeed, the masitinib phase III study of CAD showed better tolerance after approximately 3 months of treatment with a decrease in the frequency of adverse events, severe adverse events and non-fatal serious adverse events reported during the extension phase as compared with the first 12 weeks: (32% versus 59%), (7% versus 16%) and (3% versus 7%), respectively [4]. Furthermore, study discontinuation was similar between the masitinib and control groups during the extension phase (3.3 versus 3.2%, respectively); although, possible bias in withdrawal of intolerant dogs prior to the extension phase may be partly responsible for these observations. Nevertheless, the implication is that while masitinib is not completely free from such gastrointestinal signs, the majority are manageable with appropriate symptomatic treatments, with good tolerance probably beyond 3 months of treatment and during any long-term regimen. Additionally, all observed adverse reactions spontaneously resolved on cessation of treatment and there were no signs of withdrawal symptoms. This rapid recovery, free from withdrawal complications, is to be expected for masitinib as it is a 'functional inhibitor'. That is to say, on the molecular level masitinib acts as an ATP competitive inhibitor and by consequence its effect is highly dose dependent [1]. Combined with a short biological half-life, cessation of masitinib treatment effectively ends its influence on the body. Contraindications to the use of masitinib include dogs suffering from liver or renal function impairment and dogs with anaemia or neutropaenia. In addition, masitinib should not be administered to pregnant or lactating bitches, dogs less than 6 months of age or dogs less than 3.4 kg body weight.

Table 1 Masitinib is being developed for several oncology and inflammatory indications

Targets	Action	Therapeutic potential
KIT PDGFR FAK pathway	Inhibition of proto-oncogenic targets	MCT T-cell lymphoma Melanoma Splenic haemangiosarcoma
PDGFR Lyn/FAK	Potentiation of chemotherapeutic agents	Tumours treated with chemotherapies
Mast cells via KIT/Lyn	Inhibition of mast cell activation	Atopic dermatitis Arthritis Asthma Inflammatory bowel disease

This list of indications reflects the development programme of masitinib in veterinary medicine and should not be interpreted as a list of indications for which masitinib has demonstrated efficacy.

Masitinib's Pharmacological Profile

Major Targets of Masitinib

Masitinib potently and selectively targets KIT (also known as c-Kit, CD117, or mast/stem cell growth factor receptor) and platelet-derived growth factor receptors (PDGFR-α and -β), as well as Lyn, Fyn and Lck. At therapeutic doses, masitinib does not inhibit those tyrosine kinases or tyrosine kinase receptors attributed to possible toxicity [1]. This led Dubrueil *et al.* [1] to conclude that the 'highly selective nature of masitinib suggests that it will exhibit a better safety profile than other TKIs', while still delivering a sufficiently potent dose to be of therapeutic benefit. Stem cell factor, the ligand of the KIT receptor, is a critical growth factor for mast cells [13]; thus, masitinib's strong inhibitory effect on wild-type and juxtamembrane-mutated KIT receptors makes it an effective anti-mast cell agent. Additionally, masitinib was also shown to regulate the activation of mast cells through its targeting of Lyn and Fyn [1].

Safety of Masitinib and Management of Protein Loss Risk

The pivotal phase III study assessing masitinib in canine MCT reported an acceptable safety profile [3]. Adverse effects reported to occur at a significantly higher frequency in the masitinib treatment-arm compared with the placebo-arm were diarrhoea and vomiting, although these reactions were generally of mild-to-moderate intensity, relatively short-lived and medically manageable. However, increased blood urea nitrogen (BUN) and creatinine concentrations were also observed in those dogs with pre-existing renal abnormalities after masitinib treatment, prompting the advice that care should be taken when administering masitinib to dogs with impaired renal function [3]. This risk of renal disorder was further elucidated through a case study published by Sum *et al.*, which described the clinical signs, laboratory and histopathologic abnormalities, and clinical course of drug-induced minimal change nephropathy (MCN) in a dog after masitinib administration [14]. That article went on to speculate on possible mechanisms of action responsible for this condition. More recently, the phase III study of masitinib in the treatment of CAD has also reported an increased risk of protein loss [4]. In that study, protein loss was observed to occur almost uniquely during the first 3 months of treatment, with dogs recovering quickly and without relapse after discontinuation of treatment [4]. Thus, dogs being treated with masitinib must be regularly monitored by the veterinarian (bi-weekly during the first 3 months, then at least monthly) for adverse effects including possible protein loss syndrome. Indeed, once identified, the development of a protein loss management plan greatly mitigated any risk, with just one case reported following implementation. Briefly, the assessment of several biological markers of renal function led to a conclusion that albuminaemia was the best parameter through which to monitor the risk of masitinib-induced protein loss, using a threshold of 0.75 LLN (lower limit of normal blood albumin concentration). Thus, surveillance of albumin and proteinuria every other week for the first 3 months allows for the discontinuation of masitinib, while the dog is still clinically asymptomatic. Moreover, the reversibility of hypoalbuminaemia on treatment discontinuation supports a temporary rather than permanent interruption, with treatment resumed at a reduced dose for at least 3 months and close monitoring for signs of reoccurrence [4].

The Question of Cardiotoxicity

The increasing clinical application of TKIs has been accompanied by a growing concern that some of these therapies are associated with serious, albeit rare, cardiac toxicity. Of those TKIs that inhibit KIT, in addition to their main kinase targets (e.g. imatinib [Gleevec, Novartis], nilotinib [Tasigna, Novartis], sunitinib [Sutent, Pfizer] and dasatinib [Sprycel, Bristol-Myers Squibb]),

all have been linked with toxicity of the heart [15–20]. It is unlikely that cardiotoxicity associated with TKIs is a generic 'class effect' [15], but rather a consequence of these TKIs being multi-targeting and inadvertently inhibiting signalling pathways important for the correct function and/or survival of cardiomyocytes. While multi-targeting can enhance a drug's anticancer potency, this approach does appear to hold potential increased risk of cardiac dysfunction.

Masitinib has been designed specifically to optimise its selectivity against just a few key tyrosine kinases. The major targets of masitinib (KIT, PDGFR and Lyn) are not associated with cardiac toxicity [15]; also, none of those tyrosine kinases or tyrosine kinase receptors commonly attributed to TKI cardiac toxicity, are inhibited at therapeutic doses of masitinib [1]. For example, Abelson tyrosine kinase (ABL) is implicated in the cardiotoxicity of TKIs such as imatinib, dasatinib and nilotinib [15–18]. In contrast, masitinib is a weak inhibitor of ABL, with a relative selectivity for KIT versus ABL being 10-fold higher for masitinib than for imatinib (ABL IC_{50}/KIT IC_{50}=6.0 for masitinib versus 0.6 for imatinib) [1]. Thus, it is a reasonable assumption that masitinib will present a lower cardiotoxic risk than less specific KIT inhibitors.

Indeed, this quality of non- or very low cardiotoxicity has so far been evidenced through several *in vitro* and *in vivo* cardiotoxicity studies (AB Science, personal communication 2010). *In vitro*, drug-induced cytotoxicity and production of reactive oxygen species (ROS) were assessed using established models in toxicological and physiological test systems. Masitinib (up to 10 μM) neither displayed any toxic effect on proliferation/survival of human, rat or mouse cardiomyocytes, nor did it induce significant increase in cardiac production of ROS, as compared with other TKIs. Masitinib had no significant effect on hERG (human Ether-a-go-go Related Gene) channel stably expressed in Human Embryonic Kidney 293 cells at relevant therapeutic concentrations.

In dogs, *in vivo* studies have been conducted to evaluate the effect of masitinib on cardiovascular function and cardiac electrophysiology, heart weight and histology of the heart. These studies showed that masitinib (from 10 to 150 mg/kg/day) had no effect on heart rate or arterial pressure and did not modify any of the electrophysiological parameters. Results from chronic toxicology studies in dogs demonstrated that masitinib (ranging from 4 weeks at 150 mg/kg/day to 39 weeks at 30 mg/kg/day) had no clinically significant effect on any of the quantitative electrocardiographic parameters. Furthermore, masitinib was not associated with clinically significant changes in heart weight or histology at concentrations ranging from 5 to 150 mg/kg/day for up to 39 weeks. These findings are in general agreement with similar data acquired from mice and also *in vivo* cardiac tolerance data accumulated from the human clinical experience, in which there has been no evidence of cardiotoxicity to date (951 subjects as of 31 August 2010, consisting of: 95 healthy volunteer subjects, 40 patients with end-stage cancer, 441 patients with locally advanced or metastatic cancer and 375 patients with various non-oncology conditions). Only in rats and only after 26 weeks of exposure to the highly elevated dose of 100 mg/kg/day, far in excess of a clinically relevant dose, were potential signs of mild cardiotoxicity observed, including the development of minimal-to-slight myocardial degeneration/fibrosis. This latter result is not entirely unexpected as at such high doses, drugs of this class are no longer selective and will interact with most kinases with subsequent general multi-organ toxicity [21].

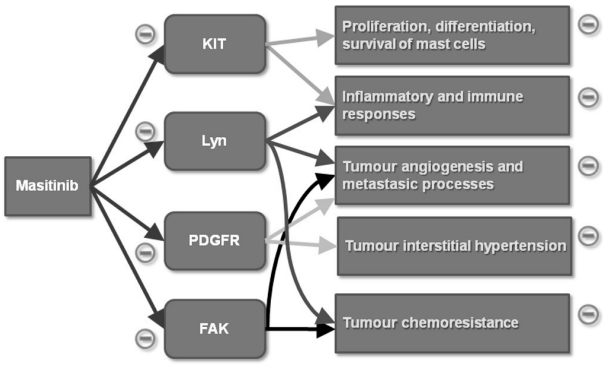

Figure 1 Main kinases targeted by masitinib and possible mechanisms of anticancer effect

Importantly, these data suggest that masitinib is not cardiotoxic at clinically relevant concentrations. Although these results and observations cannot completely rule out cardiac risks associated with masitinib treatment, the preclinical and clinical data already available are reassuring and do not provide any definitive contraindication for long-term use of masitinib with regard to cardiotoxicity.

Masitinib as an Anticancer Drug

Anticancer Mechanisms of Action

Other than the KIT tyrosine kinase receptor, masitinib also potently targets PDGFR-α/β, Lyn, Fyn [1] and to a lesser extent the focal adhesion kinase (FAK) pathway (Professor Patrice Dubreuil, personal communication 2011). Taken together, these targets of inhibition could explain the various mechanisms of action responsible for masitinib's anticancer properties (Fig. 1). Masitinib's strong inhibitory effect on wild-type and juxtamembrane-mutated KIT receptors results in cell cycle arrest and apoptosis of cell lines dependent on KIT signalling; for example, mast cells, melanocytes and interstitial cells of Cajal in the digestive tract all express KIT. Of these, mast cells are of particular interest, being widely implicated in a diverse range of diseases.

Stem cell factor, the ligand of the KIT receptor, is a critical growth factor for mast cells, essential to their survival, proliferation, differentiation, adhesion and degranulation processes [13]. Thus, masitinib exerts a direct

anti-proliferative and pro-apoptotic action on mast cells through its inhibition of KIT signalling. Although a topic of ongoing debate, there exists evidence indicating that recruitment of inflammatory cells, especially infiltration by mast cells, facilitates the growth and spread of cancers by producing molecules that enhance tumour invasiveness, with recent research linking mast cells to cancers as diverse as pancreatic cancer and multiple myeloma. For example, Haung *et al.* reported findings which strongly suggested that mast cells infiltrating into tumours produce a pro-tumour effect by remodelling the tumour's micro-environment [22]. Soucek *et al.* reported that the presence of mast cells was also required for the maintenance of established tumours in an animal model of pancreatic cancer [23]. Moreover, tumours could not be induced in mast-cell-deficient mice. This has led to the conclusion that mast cells can promote both neo-angiogenesis and tumour growth in pancreatic cancer [24]. Research by Vacca *et al.* concluded that mast cells contribute to the pathogenesis of multiple myeloma through release of angiogenic factors in their secretory granules, inducing angiogenesis and promoting tumour growth [25]. In addition, a study performed by Nakayama *et al.* claimed to demonstrate a clear role for mast cell-derived angiopoeitin 1 (Ang-1) in promoting angiogenesis in a mouse model of multiple myeloma, and provides evidence supporting a causal relationship between inflammation and tumour growth [26]. In a recent article reviewing the state-of-art in mast cell research, the question of mast cell involvement in tumour growth was summarised as follows, 'Much of the evidence linking tumour growth, or regression, to specific mast cell factors is circumstantial and controversial. Even in studies with W/W^v mast-cell-deficient mice, some groups report increased susceptibility to tumours, while others found diminished tumour growth. Nevertheless, the evidence in total makes a plausible case that the mast cell can regulate tumour growth and could be a potential target for adjuvant therapy in some but not all types of cancers' [27]. Thus, reduction of the mast cell burden may prove to be therapeutically beneficial in restraining the growth of numerous cancers, even those without a direct association with mast cell proliferation.

In addition to its KIT-related anti-proliferative properties, masitinib can also regulate the activation of mast cells through its targeting of Lyn and Fyn, key components of the transduction pathway leading to IgE-induced degranulation [28, 29]. This can be observed in the inhibition of the high-affinity IgE receptor (FcεRI)-mediated degranulation of human cord blood mast cells [1]. As a consequence of masitinib's down-regulation of mast cell activity, it reduces the array of mediators released, including VEGF, which in turn is likely to disrupt as yet unidentified downstream signalling pathways and angiogenesis processes. Masitinib may also reduce angiogenesis and enhance the chemotherapy sensitivity and drug availability at the tumour site through inhibition of PDGFR. Studies have suggested that PDGFR-β inhibition reduces tumour interstitial pressure and thus, increases the uptake of concomitantly administered drugs [30]. Inhibition of PDGFR kinase activity may also result in direct tumour cell growth arrest and apoptosis if PDGFR is constitutively activated for a particular tumour type, such as may be seen in some sarcomas.

In vivo and *in vitro* studies have demonstrated that masitinib can enhance the anti-proliferative effects of gemcitabine in human pancreatic cancer, as well as resensitise gemcitabine-resistant pancreatic tumour cell lines to gemcitabine when administered in combination with masitinib, a property not seen with other TKIs such as imatinib and dasatinib [8, 10]. The negative data obtained from imatinib and dasatinib, as well as the clinical failure of axitinib (Pfizer) in pancreatic cancer therapy, suggest that neither KIT-driven mast cell targeting nor inhibition of common kinase targets is sufficient to explain the difference in observed effect between masitinib and these agents. The working hypothesis currently proposed is that a protein/signalling pathway targeted by masitinib is important in the restoration of chemotherapy response, as opposed to inhibition of its main kinase targets or anti-mast cell properties. One possible candidate is FAK, a kinase that influences cell proliferation, survival and migration, and has been associated with tumour progression, metastasis and chemoresistance. Masitinib can block the FAK pathway in cells through the inhibition of FAK activation, without blocking its enzymatic activity (Professor Patrice Dubreuil, personal communication 2011). Additionally, it is thought that inhibition of Lyn kinase activity also has potential benefits in defence against metastasis and drug resistance [31, 32]. Research is ongoing to identify other currently unknown kinase or molecular targets of masitinib responsible for this observed effect.

MCT

MCT is the most common cutaneous malignant neoplasm in dogs, accounting for 16–21% of all skin tumours. Masitinib was specifically designed to target mast cells [1], working therefore directly at the origin of the tumour, in contradistinction to standard cytotoxic chemotherapies that inhibit replication of all cells, including healthy and useful ones.

Masitinib was approved for the treatment of canine MCT following the successful outcome of a pivotal trial evaluating outcomes of 202 dogs with grade II/III cutaneous MCT, recruited from 25 veterinary centres in the USA and France [3]. This study reported that masitinib significantly delayed time-to-progression (TTP), i.e. increased overall median TTP compared with placebo, with the effect being more pronounced when masitinib was used as first-line therapy, and regardless of whether the tumours expressed mutant or wild-type KIT (Table 2).

Table 2 Summary of phase III study results for masitinib treatment in MCT and long-term follow-up study [2, 3]

	Masitinib	Placebo	*P*-value
Best response rate (first 6 months)	80/160 (50%)	12/41 (29%)	0.020
Overall TTP for non-resectable tumours	173 days	75 days	0.001
TTP for non-resectable, non-mutated KIT tumours	140 days	75 days	0.027
TTP for non-resectable, mutated KIT tumours	241 days	83 days	0.002
12-month survival rate for non-resectable tumours	59/95 (62%)	9/25 (36%)	0.024
24-month survival rate for non-resectable tumours	33/83 (40%)	3/20 (15%)	0.040
Median overall survival for first-line, non-resectable	803 days	322 days	0.045

TTP, time-to-progression.

Indeed, 50% (80/160 dogs) of the overall population treated by masitinib experienced tumour size reduction by $\geq$50% of the initial measurements during the first 6 months of therapy, versus 12/41 dogs (29%) in the placebo group (P=0.02). Generally speaking, the objective of current anticancer treatment is tumour control, leading to prolonged survival, and if possible, complete cure. To further assess the long-term impact of masitinib on the survival of dogs with MCT, those dogs benefiting from treatment on the phase III study were enrolled in a compassionate-use programme. Findings from the follow-up study revealed that masitinib can achieve this therapeutic goal, again regardless of the MCT's KIT mutation status (Table 2), as evidenced by significantly increased survival rates at 12 and 24 months in masitinib-treated dogs with non-resectable tumours compared with those receiving placebo. Specifically, survival at 12 months was 62.1% for masitinib-treated dogs compared with 36% in the placebo arm (P=0.024). At 24 months, masitinib-treated dog survival was 39.8% compared with 15% in the placebo arm (P=0.04). A complete tumour response at 12 and 24 months was reported in 15 and 9%, respectively, of masitinib-treated dogs (observed cases) versus 0% complete responses with placebo. Furthermore, during this pivotal trial masitinib demonstrated an anti-metastatic potential, reaching statistical significance (P=0.006) in preventing the emergence of nodal/visceral metastases in dogs with recurrent or non-resectable grade II/III cutaneous MCT [33]. This finding is supported by data from a murine model, in which nude mice with subcutaneous grafts of tumoural Tel-Jak2 cell lines, a cell line that masitinib does not directly inhibit, showed improved survival with masitinib treatment compared with placebo (Professor Patrice Dubreuil, personal communication 2011). Analysis of data from the pivotal MCT trial also revealed that control of disease was highly correlated with long-term survival, indicating that tumour stabilisation also provides important clinical benefits. This is in contrast to the conventional approach of short-term chemotherapy, for which the aim is to achieve control of local tumour burden before excess collateral damage occurs in healthy tissue. Indeed, if this primary chemotherapeutic goal of local tumour control is not attained, then the survival time is worsened. The targeted nature of masitinib and its ability to stop tumour progression allows for a long-term therapeutic approach of tumour control, effectively making MCT a chronic rather than acute disease. In effect, masitinib introduces a new paradigm in oncology treatment for animals, with control of disease at 6 months being highly predictive of long-term survival, whereas short-term response at 6 weeks was not. This indicates that disease control over 6 months is a valuable treatment objective and a prognostic feature of masitinib therapy.

Masitinib's long-term treatment capabilities are further exemplified by three case studies, in which each dog received oral masitinib in monotherapy for over 4.5 years as part of the pivotal trial's extension phase [34]. The first and second cases presented as non-resectable grade II MCT with KIT mutation (exon 11 and exon 8, respectively). Complete response was achieved with masitinib as first-line treatment, which has persisted as of January 2011 for at least 60 and 56 months, respectively. The third case presented with non-resectable grade II MCT and no KIT mutation (wild-type). Masitinib administered as a third-line treatment induced a complete response, lasting for 58 months as of January 2011. Furthermore, masitinib was well tolerated over these extended durations of therapy.

Masitinib Off-Label Experiences

The off-label use of masitinib in Europe, applicable under the Cascade provisions, has revealed some interesting case studies that are now the focus of further clinical development [35]. Here we highlight notable examples of masitinib off-label use. It is noted that such case studies cannot in themselves provide proof of efficacy and should not be interpreted as a list of indications for which masitinib has demonstrated efficacy. They are significant, however, for providing valuable insights into new therapeutic opportunities and are indeed often a catalyst for further clinical development in both veterinary and human medicine.

For example, masitinib has been administered as maintenance chemotherapy of dogs with T-cell lymphoma [36, 37]. For some of these dogs, masitinib monotherapy of T-cell lymphoma was sufficient to maintain a complete or clinical remission. These clinical observations are supported by *in vitro* data demonstrating that the T-cell

lymphoma cell line OSW (Oswald) was particularly sensitive to masitinib, possibly because of inhibition of PDGFR [38]. Also, immunohistochemistry data from a biopsy of one dog that reported a complete response showed intense staining for phosphorylated PDGFR-β and discrete staining for PDGFR-β [37]. This is consistent with the literature in human mycosis fungoides (epitheliotropic T-cell lymphoma), which has shown positive immunohistochemistry reaction for both PDGF and PDGFR in the abnormal cells. In another case, a dog was diagnosed as having histologically confirmed neurofibrosarcoma with mast cell infiltration [39]. A strong relationship exists between the pathogenesis of neurofibrosarcoma and both mast cells and Schwann cells, therefore suggesting possible benefits from treatment with targeted KIT or PDGFR inhibitors, respectively [40, 41]. This tumour had recurred after two prior surgeries: chemotherapy with doxorubicin, and metronomic chemotherapy (endoxan/meloxicam). On initiation of masitinib monotherapy, an observed rapid tumour growth was quickly stabilised followed by a significant decrease in volume and a negligible mass after 10 weeks of masitinib treatment.

Finally, there have been reports on masitinib treatment of canine metastatic melanoma with survival times of at least 10 months, well in excess of the 1 month median survival time for stage IV melanoma with surgery (Professor Janos Butinar, personal communication 2010). In one case study, the dog presented with a histologically proven metastatic melanoma that was unresponsive to chemotherapy. After introduction of masitinib, a complete response was achieved within 2 months of treatment. This dog was alive at least 4 months after the initial resection, before being lost to follow-up. These observations suggest that masitinib might represent a therapeutic option for dogs with melanoma, a hypothesis supported by *in vitro* findings that show masitinib can sensitise canine melanoma tumour cell lines to doxorubicin, including doxorubicin-resistant (17CM98) melanoma cells [38, 42].

Masitinib as a Potentiator of Chemotherapies

There is evidence that masitinib can exert an anticancer action that extends beyond inhibition of its main tyrosine kinase targets. *In vivo* and *in vitro* studies have shown that masitinib can enhance the anti-proliferative effects of gemcitabine in human pancreatic cancer, even in gemcitabine-resistant cell lines [8, 10]. Such chemosensitisation may allow lower concentrations of chemotherapeutic agent to be administered, thereby reducing toxicity risk, or may increase the efficacy of the chemotherapeutic agent administered at standard doses. The precise mechanism or mechanisms whereby masitinib acts as a chemosensitiser are not yet clear and additional investigations are being conducted. Of course, the present lack of full mechanistic understanding is only a minor barrier to implementation if the therapeutic benefit of a novel approach can be proven in practice. To this end, masitinib's potential as a chemosensitiser in canine combination chemotherapies was further investigated [38, 43]. It was reported by Thamm *et al.*, that these *in vitro* data establish proof-of-concept that masitinib strongly sensitised histiocytic sarcoma cells to vinblastine; osteosarcoma and mammary carcinoma cells to gemcitabine; and to a lesser degree, several cell lines were sensitised to doxorubicin [43]. Thus, barring possible additive toxicity issues, masitinib could potentially be used as a chemosensitiser, with plausible applications being a masitinib/doxorubicin combination for the treatment of B-cell lymphoma, haemangiosarcoma, melanoma and bladder carcinoma; a masitinib/vinblastine combination for the treatment of histiocytic sarcoma; and a masitinib/gemcitabine combination for the treatment of osteosarcoma and mammary carcinoma. It was also commented that although the experimental conditions did not necessarily reflect those to be used in the clinical setting (masitinib was used at 5 and 10 μM over a 72-h incubation time), in itself this should not preclude clinical significance, as similar high concentrations were necessary in the related human pancreatic cancer *in vitro* study with clinical benefit manifested at lower, tolerable concentrations in an *in vivo* mouse model and subsequent phase II human study [8, 10].

Following on from these *in vitro* observations, phase I studies in dogs with advanced tumours documented that masitinib administered in combination with carboplatin or doxorubicin had acceptable and manageable safety profiles, without added toxicity [43, 44]. Moreover, encouraging anti-tumour activity was observed in both combination studies with a number of dogs achieving partial or complete responses; although care should be taken in the interpretation of such observations given the absence of a control group and small population size typical of such phase I studies. These results have, however, led to the implementation of several phase II studies to further investigate masitinib's potential to sensitise various canine cancer cell lines to cytotoxic agents (see Table 1).

Masitinib for Inflammatory Diseases

The Role of Mast Cells in Inflammation

Mast cells are characterised by their heterogeneity, not only regarding tissue location and structure but also at functional and histochemical levels. Mast cell activation is followed by the controlled release (degranulation) of a variety of mediators that are essential for the defence of the organism against invading pathogens [45]. However, in the case of over-activation the uncontrolled hypersecretion of these mediators can be harmful to the patient. The large variety of mediators produced by mast cells can be categorised broadly as: preformed granule-associated

Table 3 Summary of phase III study results for masitinib treatment in CAD after 12 weeks of masitinib treatment[1] [4]

	Masitinib	Control	*P*-value
CADESI-02 relative change from baseline[2]	$-46\% \pm 3.0$	$-29\% \pm 4.1$	<0.001
CADESI-02 response rate, $\geq 50\%$	86/141 (61.0%)	27/76 (35.5%)	<0.001
Pruritus relative change from baseline	$-22\% \pm 4.5$	$-25\% \pm 6.3$	0.737
Pruritus relative change (severely pruritic)[3]	$-46\% \pm 5.1$	$-29\% \pm 6.5$	0.045
Pruritus response rate (severely pruritic)[4]	20/51 (39.2%)	8/35 (22.9%)	0.040
Pruritus and CADESI-02 response rate	64/139 (46.0%)	22/75 (29.3%)	0.022

[1]Analyses according to the modified intent-to-treat population, observed cases dataset, with the exception of pruritus response rate (severely pruritic), which is according to the modified intent-to-treat population, missing data as failure dataset.
[2]Least square means $\pm$ standard error given for observed cases dataset with an ANCOVA (repeated analysis of covariance model) adjusted on baseline value. All analyses are done with same model.
[3]Analysis for subpopulation of dogs with severe pruritus at baseline.
[4]Pruritus response was expressed as the proportion of dogs achieving the *a priori* threshold of $\geq 20\%$ improvement, with a maximum value of 100 mm, at week 12. The implication of these dual criteria on pruritus response is that responders from the severe baseline pruritus subpopulation will experience an improvement of $\geq 40\%$. CADESI-02, Canine Atopic Dermatitis Extent and Severity Index score version 2.

mediators (e.g. histamines); lipid-derived mediators (e.g. prostaglandins); and various cytokines (e.g. interleukins and tumour necrosis factor alpha). These mediators can, alone or in synergy with macrophage-derived and T-cell-derived cytokines, generate a complex inflammatory response and induce the recruitment and activation of inflammatory cells to the site of degranulation.

As previously mentioned, the main cellular target of masitinib is the mast cell and consequently masitinib can provide therapeutic benefit in immune-mediated diseases with mast cell involvement.

Canine Atopic Dermatitis

The treatment of generalised atopic dermatitis remains a challenge, especially in severe or refractory cases. Mast cells are known to produce a variety of inflammatory mediators that are in part responsible for the complex inflammatory cascade associated with allergic disease. As such, mast cells represent an attractive, hitherto untapped, therapeutic target for atopic dermatitis management [46]. To this end, a phase III randomised controlled trial has recently been completed in which dogs with confirmed diagnosis of CAD received oral masitinib (12.5 mg/kg/day) or placebo-control for 12 weeks with concomitant flea treatment and a restrictive list of permitted antibiotic or antifungal treatments. Dogs presenting with CAD that were exclusively seasonal, food-related or caused by flea allergy, were excluded. Findings from a study population of 306 dogs, one of the largest CAD cohorts tested to date, provided strong evidence that daily administration of masitinib achieved significant reduction in the signs of CAD and could therefore be an effective treatment option (Table 3) [4]. A reduction in primary endpoint (CADESI-02 score) of $\geq 50\%$ at week 12 was observed in 61% of masitinib-treated dogs compared with 35% of control dogs ($P < 0.001$), according to the intent-to-treat, observed cases dataset. Furthermore, in the severely pruritic dog subpopulation, 65% of clients assessed masitinib efficacy as good/excellent compared with 35% of clients from the control population ($P=0.05$). Similarly, 63% of investigators assessed masitinib's overall efficacy as good/excellent compared with 35% for controls ($P < 0.001$). Overall, a positive response was observed for treatment-naïve dogs, dogs resistant to cyclosporin and/or corticosteroids and dogs with severe pruritus, the latter two groups representing populations with high unmet medical need.

Feline Applications

Mast cells are involved in a number of feline-related oncological and inflammatory diseases, such as asthma [47], inflammatory bowel disease [48] and MCTs [49]. Currently, masitinib is only officially authorised for use in dogs; however, researchers are actively developing the knowledge for feline therapy with pharmacokinetic and safety studies of masitinib in cats having already been published [50, 51]. Off-label use of masitinib has again revealed some interesting case studies. These include encouraging results in asthmatic cats. One cat presenting with corticosteroid-intolerance and another with corticosteroid-resistance have both been treated with masitinib for over 14 months and have shown an improved quality of life (Dr Ludivine Kinon, personal communication 2010). Such reports prompted further clinical study including a randomised, placebo-controlled study of oral masitinib (50 mg/day) for treatment of experimental feline asthma, in which masitinib treatment produced positive responses including a significant reduction in eosinophilic airway inflammation after 4 weeks [52]. At this dose a high occurrence of moderate-to-severe proteinuria was observed in the masitinib-treated cats. However, another study evaluating the safety of masitinib in healthy cats did not observe any occurrence of clinically relevant proteinuria at a dose of 50 mg every other day for 4 weeks [51]. That safety study concluded that masitinib was tolerated in the majority of cats, although further long-term administration and pharmacokinetic studies are needed to assess the use of masitinib in cats.

Oncology-related cases in cats have also been reported [35]. One feline subject presenting with MCT experienced rapid improvement in clinical status after 7 days of masitinib treatment and partial response in two subcutaneous nodules. Another subject had a metastasised cutaneous melanoma for which the primary tumour had been resected. After 6 months of daily masitinib treatment, the metastasis had totally disappeared and lymph nodes were stable without need for further resection. Following 14 months of treatment the tumour was controlled with no signs of relapse. Finally, an *in vitro* study of feline vaccine associated sarcomas cell lines showed masitinib has potential as an anti-neoplastic agent against this disease [53].

Summary

Perhaps the most compelling aspect of the therapeutic use of masitinib from a veterinarian's viewpoint is the possible diversity of its applications. Already documented to be a valuable anticancer therapeutic for MCT treatment of dogs, masitinib also shows real promise as effective treatment of CAD and a valuable alternative treatment option for dogs that are severely pruritic or refractory to current therapies. Considering also the potential to treat a wide range of other cancers currently being evaluated by veterinary clinical investigators, either as a single agent or in combination with existing therapies, and additional anti-inflammatory uses, masitinib is likely to become an increasingly important tool in the veterinarian's therapeutic armamentarium.

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